Research article

Association between GPER gene polymorphisms and GPER expression levels with cancer predisposition and progression

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A R T I C L E  I N F O

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A B S T R A C T

Estrogen is a female sex steroid hormone that plays a significant role in physiological functions. Evidence suggests that estrogen-signaling pathways are closely linked to cancer development and progression. The novel G protein-coupled estrogen receptor (GPER or GPR30) has been shown to influence cancer predisposition and progression, although results of related studies remain equivocal. Thus, this meta-analysis aimed to estimate the relationship between GPER gene polymorphisms and GPER expression levels, with cancer predisposition and progression. The pooled results showed that two GPER polymorphisms, rs3808350 and rs3808351, were significantly associated with cancer predisposition, especially in the Asian population, but no significant association was detected for rs11544331. In parallel, we also found that cancer aggressiveness and progression correlated with rs3808351 and GPER expression in cancerous tissues. Altogether, our findings suggest that GPER plays a pivotal role in cancer pathogenesis and progression. We suggest that rs3808350 and rs3808351 may be used as a prospective biomarker for cancer screening; while rs3808351 and GPER expression can be used to examine the prognosis of patients with cancer. Further biological studies are warranted to confirm our findings.

1. Introduction

Estradiol (E2) is a major form of estrogen and displays pleiotropic steroid function that play regulatory roles in many physiological processes [1, 2]. Biosynthesis of E2 is determined by the conversion of testosterone by a rate-limiting enzyme, aromatase (CYP19A1) [1, 2, 3, 4, 5, 6]. E2-mediated effects are modulated through both genomic and non-genomic pathways by the nuclear and membrane estrogen receptor (ER), respectively [2]. Recent reports have suggested a pivotal role of E2 in both the development and malignant progression of multiple cancers [7]. Several meta-analysis have demonstrated that cancer risk is associated with the polymorphism of ER-alpha (ERα) [8], but not ER-beta (ERβ) [9]. However, the role of membrane ERs, such as the G protein-coupled estrogen receptor (GPER), with cancer pathogenesis remains elusive.

GPER has been identified as a novel ER, and is a seven-transmembrane domain protein that is structurally distinguished from the classical ERα and ERβ [10]. GPER mediates rapid E2-induced non-genomic signaling events, resulting in long-term transcriptional changes and a broad range of response among a large variety of cell types [10, 11]. Such evidence was supported by the expression of GPER in various human tissues, including lung, heart, brain, liver, skeletal muscle, and lymphoid tissues [12]. Additionally, E2 exerts ten times higher binding capacity to GPER than ERα [13], implying a critical role of GPER in regulating normal physiological functions.

GPER overexpression has been reported in several hormone-dependent malignancies, including cancers of the breast, ovaries, and endometrium [10]. The upregulation of GPER is also evident in semi-noma and lung cancer [10, 14]. Additionally, GPER overexpression has also been associated with poor treatment outcomes such as lowered efficacy of primary endocrine treatment in breast cancer patients [15] and poor-prognosis of endometrial cancers, uterine carcinosarcoma, and endometriosis [16]. The finding indicates that GPER expressed in ERα/β-negative breast cancer could induce the expression of connective tissue growth factor (CTGF) [17], and thus binding of E2 to GPER for cell proliferation and migration. Hence, several studies have been proposed to identify novel GPER ligands with specific antiproliferative effects against estrogen-based malignancies [18, 19].

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Single nucleotide polymorphisms (SNPs) are variations in the genomic sequence that could potentially result in modifications of gene expression level as well as protein structure, level, and function [17]. The expression level of GPER mRNA is possibly affected by its polymorphism [20]. Although several SNPs have been identified in the GPER gene, only three were reported to have higher biological relevance with human neoplasms, which are rs3808350, rs3808351, and rs11544331 [10]. However, the role of GPER polymorphism in cancer remains inconclusive as shown by different results in various studies [10, 13, 16, 17]. Therefore, this meta-analysis was conducted in order to understand the role of GPER with cancer predisposition and progression.

2. Methods

2.1. Literature search and data extraction

A meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [21]. A literature search was conducted in MEDLINE and EMBASE using keywords such as “GPER/GPR30”, “polymorphisms”, “immunohistochemistry”, “expression/level”, and “cancer”, singularly and in combination. The literature search was updated until July, 2020. The inclusion criteria of studies were as follows: (1) evaluating the association between GPER rs3808350, rs11544331, and rs3808351 polymorphisms and cancer predisposition, (2) conducted with a case-control design, and (3) evaluating GPER expression level (immunohistochemistry) and cancer progression. Data were extracted as follows: (1) name of the first author, (2) year of publication, (3) type of cancer, (4) the number of cases and controls, (5) number of genotypes in cases and controls, (6) number of haplotypes of rs3808350/rs3808351/rs11544331 in cases and controls, and (7) number of patients with GPER+/− or high/low.

2.2. Statistical analysis

Meta-analysis for each gene polymorphism was performed for two or more studies, as previously described [3, 4, 5, 6, 22, 23, 24, 25]. Genotypic frequency of GPER gene polymorphism was tested for deviation from the Hardy–Weinberg equilibrium (HWE) in the control subjects if HWE was not reported. The genetic association was examined using different genetic models, including allelic (a vs. A), recessive (aa vs. Aa + AA), dominant (aa + Aa vs. AA), over dominant (Aa vs. aa + AA), homozygous (aa vs. AA), and heterozygous (Aa vs. AA) models [5, 22, 23, 24, 25, 26, 27, 28, 29, 30]. The associations between GPER gene polymorphisms and GPER expression levels with cancer predisposition and progression were calculated by the pooled odds ratio (OR) and 95% confidence interval (CI). Heterogeneity among studies was evaluated using Q test and I² statistic. A significant Q-statistic (p < 0.10) indicated heterogeneity across studies. The I² values indicated no (0–24.9%), low (25–49.9%), moderate (50–74.9%), or high (75–100%) heterogeneity. The random-effect model (REM) was used if heterogeneity existed; otherwise, the fixed-effect model (FEM) was used [31, 32, 33, 34, 35, 36, 37]. Subgroup analysis was conducted by stratifying based on ethnicity, type of cancer, and localization of GPER expression. In addition, we also evaluated the association between rs3808350 and tumor size, as well as the involvement of haplotypes rs3808350/rs3808351/rs11544331 with cancer predisposition. Potential publication bias was assessed by Beggs funnel plots and Egger’s regression test. Beggs funnel plot was applied if the pooled effect size consisted of 10 or more studies. The Newcastle Ottawa Scale (NOS) was adopted to assess the quality of the case-control study, with a score of 8–9 for all included studies, indicating a low risk of bias (Supplementary Table 1). A sensitivity analysis was performed by sequentially omitting each study one at a time, and the results remained unchanged (data not shown), implying the robustness and stability of the findings. A quantified result of p < 0.05 was indicative of statistical significance.

3. Results

3.1. Relationship between GPER gene polymorphisms and cancer

For GPER gene polymorphisms, a total of 142 articles were screened, among which 11 were reviewed. Six studies were excluded due to not relating to cancer or GPER rs3808350, rs11544331, and rs3808351 polymorphisms. Five studies were then included in this meta-analysis [10, 13, 16, 17, 38]. From 5 studies, Chevalier et al. [10] and Giess et al. [17] recruited testicular and breast cancer patients, respectively, while Kasap et al. [16] and Hong et al. [13] enrolled patients with uterine leiomyoma and adenomyosis/uterine leiomyoma/another precancerous lesion of uterine-cervix, respectively. The last included study recruited gynecomastia patients [38], and although it should be noted that some reports have classified the condition as a non-malignant male breast disorder [39], gynecomastia has shown strong association with GPER rs3808350, exhibiting a nearly 10-fold increased risk of breast cancer in men [40]. A total of 1,288 (case: 601, control 687), 5,565 (case: 729, control: 4,836), and 1,294 (case: 610; control: 684) subjects for GPER rs3808350, rs11544331, and rs3808351 polymorphisms, respectively, were further analyzed. All studies complied with the HWE except for the study from Chevalier et al. (for rs11544331 and rs3808351) [10]. Details of the retrieved studies are shown in Table 1.

The pooled result of the analyses is shown in Table 2. Overall, there was no significant association between GPER rs3808350, rs11544331, and rs3808351 polymorphisms with cancer predisposition in all inheritance models, even when the studies evaluating gynecomastia or/and study deviated from HWE were excluded (Table 2). However, subgroup analyses stratified by ethnicity revealed a significant association between rs3808350 (G vs. A, OR = 1.38, 95%CI = 1.06–1.79, p = 0.015; GG vs. AG + AA, OR = 2.20, 95%CI = 1.42–3.43, p = 0.000 or OR = 2.11, 95% CI = 1.19–3.74, p = 0.010; GG vs. AA, OR = 1.83, 95%CI = 1.10–3.04, p = 0.019; AG vs. AA, OR = 0.51, 95%CI = 0.28–0.95, p = 0.033; Table 2) and rs3808351 (A vs. G, OR = 0.51, 95%CI = 0.34–0.75, p = 0.000; AA vs. GA + GG, OR = 0.34, 95%CI = 0.14–0.78, p = 0.011; AA + GA vs. GG, OR = 0.48, 95%CI = 0.29–0.81, p = 0.006; AA vs. GG, OR = 0.28, 95%CI = 0.11–0.69, p = 0.005; GA vs. GG, OR = 0.56, 95%CI = 0.32–0.98, p = 0.043; Table 2) with cancer predisposition. Ethnicity did not associate with predisposition of cancer for rs11544331 (data not shown). In addition, no association was also observed in any haplotypes of rs3808350/rs3808351/rs11544331 with cancer predisposition (Table 3).

In addition to the association of GPER polymorphism with cancer predisposition, we also evaluated the association between rs3808351 and tumor size (Table 4). The analysis showed that rs3808351 (AA + GA vs. GG, OR = 0.46, 95%CI = 0.28–0.76, p = 0.002; GA vs. GG, OR = 0.46, 95%CI = 0.27–0.79, p = 0.004; Table 5) was associated with smaller tumor size.

3.2. Relationship between GPER expression levels and cancer progression

A total of 204 articles were first screened to evaluate the association between GPER expression levels with cancer progression. After reviewing the title, abstract, and removing duplications, 151 articles were excluded, and 53 articles were then further evaluated. Among them, 33 articles were subsequently removed either because the data cannot be extracted, or the studies did not provide immunohistochemistry results. Finally, 20 articles were included in this meta-analysis [41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60]. The characteristics of the included studies are shown in Table 6.

The meta-analysis results regarding pooled GPER expression levels and cancer progression are shown in Table 7. In brief, no associations were found between GPER expression levels with tumor size, stage, nor
grade. Subgroup analysis by ethnicity and cancer type were also performed, yielding similar findings, with the exception of a significant association between GPER expression with higher tumor stage in the Asian population (OR = 2.22, 95% CI = 1.12-4.41, p = 0.022, Table 7). No association was also observed when the analysis was performed based on the localization of GPER (data not shown).

### 3.3. Publication biases

Publication biases were examined by Begg's funnel plots and Egger's regression tests. Overall, funnel plots were symmetrical (data not shown) and p-values of Egger's regression test greater than 0.05, suggesting that publication biases did not likely influence the results.

### 4. Discussion

To date, this study is the first to summarize the association between GPER gene polymorphisms and GPER expression levels with cancer. The pooled meta-analyses results demonstrated that GPER rs3808350 and rs3808351, but not rs11544331, were significantly associated with cancer predisposition, specifically in the Asian population. Patients harbouring the A allele of rs3808351 exhibited a lower risk of developing cancer and displayed smaller tumor size. Moreover, GPER expression levels in cancerous tissues were correlated with higher tumor stage in the Asian population.

Our finding reinforces previous reports that A allele carriers of rs3808350 and rs3808351 exhibit protective effects against uterine leiomyoma and gynecomastia risks in the Turkish population [17, 38]. Similar to our findings, Giess et al. [17] observed that AA and AG genotypes of rs3808351 were correlated with lower tumor stage and grade. Although we did not observe a significant association between rs11544331 and cancer risk, it has been suggested that rs11544331 (P16L) can alter the conformational structure and localization of GPER, resulting in defective GPER function and the aggravated migration of carcinoma cells [61]. We also found no significant relationship between haplotypes of rs3808350/rs3808351/rs11544331 with cancer predisposition, possibly because our analysis was pooled from two studies reporting different cancer type/disease. Considering the potential functional significance of rs3808350 and rs3808351, further studies should try to estimate the relationship between rs3808350 and rs3808351 with cancer in a larger population and other ethnicities to test whether our findings are statistically robust.

Because rs3808350 and rs3808351 are located in the 5’ region of the GPER gene (rs3808350 (-642) is located in the 5’-regulatory region, while rs3808351 (-124) is located in the 5’-untranslated region and containing the gene promoter) [10], these polymorphisms may influence the transcription level of GPER. However, no related studies are currently available. Since our results showed that GPER expression in cancerous tissues correlate with the aggressiveness of malignancies and that the A allele of rs3808351 exhibits protective effects against tumor progression in the Asian population, it is reasonable to speculate that the G allele of rs3808351 may be associated with the upregulation of GPER transcription. However, only one study has reported the functional role of GPER polymorphisms in relation to post-transcriptional expression. The study reported that only rs10235056 was significantly correlated with GPER mRNA expression [20]. Therefore, further studies are still required to reveal the exact molecular mechanism underlying our significant findings.

Although in general we did not find any relationship between expression level and localization of GPER with cancer progression, other studies have reported that GPER overexpression is strongly associated with lower survival rates in several cancer types [43, 54, 55, 59, 60, 62, 63]. Contrasting, some studies demonstrated that loss of GPER protein corresponds with low GPER mRNA and poorer prognosis of endometrial and breast cancer patient [50, 64], possibly due to GPER promoter hypermethylation [64]. Moreover, it seems that the localization of GPER in the plasma membrane is responsible for cancer aggressiveness [63]. Thus, in order to evaluate the prognostic value of GPER in cancer patients, GPER protein level, localization, and promoter hypermethylation must be examined simultaneously.

Despite being the first meta-analysis in the field, several limitations of this study should be noted. First, only a limited number of studies were included for meta-analysis of GPER gene polymorphisms and cancer. Consequently, further studies are still warranted to test our findings with a larger sample size. Second, because the etiologies of cancer are complex, other genetic and environmental factors need to be addressed and may influence the relationship between GPER gene polymorphism, GPER level, and its localization in different cancer types. Hence, publication...
Table 2. Meta-analysis for the association between GPER polymorphisms and cancer.

| SNP          | Genetic model | Group         | No. of studies | Test of association | Stat. Model | Test of heterogeneity | Publication bias p-value (Egger's test) |
|--------------|---------------|---------------|----------------|---------------------|-------------|-----------------------|----------------------------------------|
|              |               |               |                | OR                  | 95% CI      | p-value               |                                         |
|              |               |               |                | p-value             |             |                       |                                         |
|              |               |               |                | I^2 (%)             |             |                       |                                         |
| rs3808350    | G vs. A       | Overall       | 5              | 1.02                | [0.72; 1.45]| 0.888                 | 0.003                                  | 74.50                                  | 0.815                                  |
|              |               | Overall*      | 4              | 0.91                | [0.64; 1.29] | 0.604                 | 0.027                                  | 67.14                                  | 0.943                                  |
|              |               | Asian         | 3              | 1.38                | [1.06; 1.79] | 0.015                 | 0.548                                  | 0                                       | 0.357                                  |
|              |               | Asian*        | 2              | 1.22                | [0.86; 1.74] | 0.252                 | 0.595                                  | 0 NA                                   |
|              |               | Caucasian     | 2              | 0.74                | [0.44; 1.24] | 0.268                 | 0.025                                  | 79.89                                  | NA                                     |
|              | GG vs. AG + AA| Overall       | 5              | 1.20                | [0.59; 2.45] | 0.602                 | 0.001                                  | 77.16                                  | 0.840                                  |
|              |               | Overall*      | 4              | 0.99                | [0.43; 2.31] | 0.996                 | 0.003                                  | 77.71                                  | 0.837                                  |
|              |               | Asian         | 3              | 2.20                | [1.42; 3.43] | 0.000                 | 0.902                                  | 0                                       | 0.057                                  |
|              |               | Asian*        | 2              | 2.11                | [1.19; 3.74] | 0.010                 | 0.700                                  | 0 NA                                   |
|              |               | Caucasian     | 2              | 0.47                | [0.12; 1.81] | 0.273                 | 0.036                                  | 77.15                                  | NA                                     |
|              | GG vs. AG     | Overall       | 5              | 0.90                | [0.71; 1.13] | 0.379                 | 0.114                                  | 46.18                                  | 0.584                                  |
|              |               | Overall*      | 4              | 0.81                | [0.63; 1.05] | 0.121                 | 0.237                                  | 29.12                                  | 0.403                                  |
|              |               | Asian         | 3              | 1.06                | [0.72; 1.57] | 0.753                 | 0.221                                  | 33.66                                  | 0.359                                  |
|              |               | Asian*        | 2              | 0.79                | [0.46; 1.38] | 0.424                 | 0.342                                  | 0 NA                                   |
|              |               | Caucasian     | 2              | 0.77                | [0.44; 1.34] | 0.359                 | 0.068                                  | 69.93                                  | NA                                     |
|              | GG vs. AA     | Overall       | 5              | 0.95                | [0.44; 2.04] | 0.913                 | 0.003                                  | 74.06                                  | 0.583                                  |
|              |               | Overall*      | 4              | 0.73                | [0.32; 1.68] | 0.468                 | 0.021                                  | 69.04                                  | 0.558                                  |
|              |               | Asian         | 3              | 1.83                | [1.10; 3.04] | 0.019                 | 0.355                                  | 3.36                                   | 0.246                                  |
|              |               | Asian*        | 2              | 1.43                | [0.72; 2.86] | 0.302                 | 0.314                                  | 1 NA                                   |
|              |               | Caucasian     | 2              | 0.42                | [0.08; 2.05] | 0.287                 | 0.018                                  | 82.03                                  | NA                                     |
| rs1154431    | T vs. C       | Overall       | 5              | 0.91                | [0.76; 1.08] | 0.299                 | 0.204                                  | 32.48                                  | 0.283                                  |
|              |               | Overall**     | 3              | 0.80                | [0.63; 1.01] | 0.064                 | 0.462                                  | 0                                       | 0.389                                  |
|              | TT vs. CT + CC| Overall       | 4              | 0.76                | [0.49; 1.19] | 0.244                 | 0.234                                  | 29.66                                  | 0.662                                  |
|              |               | Overall**     | 2              | 0.77                | [0.48; 1.24] | 0.295                 | 0.966                                  | 0                                       | 0.265                                  |
|              | TT vs. CT vs. CC| Overall     | 5              | 0.93                | [0.75; 1.16] | 0.555                 | 0.205                                  | 32.41                                  | 0.502                                  |
|              |               | Overall**     | 3              | 0.76                | [0.56; 1.03] | 0.080                 | 0.398                                  | 0                                       | 0.543                                  |
|              | TT vs. CC     | Overall       | 4              | 0.68                | [0.42; 1.09] | 0.114                 | 0.214                                  | 32.91                                  | 0.522                                  |
|              |               | Overall**     | 2              | 0.66                | [0.40; 1.11] | 0.122                 | 0.748                                  | 0                                       | 0.723                                  |
|              | CT vs. CC     | Overall       | 5              | 0.97                | [0.78; 1.22] | 0.854                 | 0.252                                  | 25.31                                  | 0.606                                  |
|              |               | Overall**     | 3              | 0.78                | [0.56; 1.08] | 0.136                 | 0.418                                  | 0                                       | 0.532                                  |
| rs3808351    | A vs. G       | Overall       | 5              | 1.07                | [0.61; 1.87] | 0.809                 | 0.000                                  | 89.43                                  | 0.657                                  |
|              |               | Overall**     | 3              | 0.68                | [0.41; 1.12] | 0.135                 | 0.032                                  | 70.73                                  | 0.481                                  |
|              | AA vs. GA + GG| Overall       | 5              | 1.27                | [0.49; 3.29] | 0.618                 | 0.000                                  | 82.84                                  | 0.817                                  |
|              |               | Overall**     | 3              | 0.60                | [0.37; 0.97] | 0.040                 | 0.103                                  | 55.95                                  | 0.903                                  |
|              | AA + GA vs. GG| Overall       | 5              | 1.16                | [0.56; 2.38] | 0.680                 | 0.000                                  | 87.44                                  | 0.882                                  |
|              |               | Overall**     | 3              | 0.66                | [0.36; 1.21] | 0.182                 | 0.054                                  | 65.68                                  | 0.219                                  |
|              | AA vs. GG     | Overall       | 5              | 1.56                | [0.44; 5.51] | 0.484                 | 0.000                                  | 88.31                                  | 0.798                                  |
|              |               | Overall**     | 3              | 0.54                | [0.19; 1.57] | 0.263                 | 0.069                                  | 62.47                                  | 0.995                                  |
|              |               | Asian         | 3              | 1.12                | [0.12; 9.73] | 0.916                 | 0.000                                  | 89.02                                  | 0.988                                  |
|              |               | Asian*        | 2              | 0.28                | [0.11; 0.69] | 0.005                 | 0.210                                  | 36.18                                  | NA                                     |
|              |               | Caucasian     | 2              | 2.42                | [0.28; 20.91] | 0.419                 | 0.000                                  | 93.40                                  | NA                                     |
| GA vs. GG    | Overall       | 5              | 1.15                | [0.61; 2.19] | 0.653                 | 0.000                                  | 82.10                                  | 0.728                                  | (continued on next page)
bias might affect the accuracy of our pooled studies. Notwithstanding, detailed functional analyses are still needed to uncover the exact molecular mechanisms of the observed significant association between GPER and cancer.

It is notable that rs3808350 and rs3808351 have the potential to be used as a prospective biomarker for cancer, with potential use of rs3808351 in particular as a prognostic marker for cancer progression, particularly in Asians. Thus, future studies should address the possibility

| SNP | Genetic model | Group | No. of studies | Test of association | Stat. Model | Test of heterogeneity | Publication bias p-value (Egger's test) |
|-----|---------------|-------|----------------|---------------------|-------------|-----------------------|--------------------------------------|
|     |               |       |                | OR                  | 95% CI      | p-value               | p-value | I² (%)                  |
| Overall** | 3 | 0.73 | [0.40; 1.32] | 0.307 | Random | 0.081 | 60.15 | 0.001 |
| Asian | 3 | 0.78 | [0.33; 1.81] | 0.564 | Random | 0.020 | 74.22 | 0.402 |
| Asian* | 2 | 0.56 | [0.32; 0.98] | 0.043 | Fixed | 0.243 | 26.59 | NA |
| Caucasian | 2 | 1.93 | [0.59; 6.31] | 0.271 | Random | 0.000 | 90.87 | NA |

*analysis by excluding Korkmaz et al (2014); **analysis by excluding Korkmaz et al (2014) and Chevalier et al (2014); CI, confidence interval; OR, odds ratio; Stat. model, statistical model. Bold values indicate statistically significant differences between cases and control, p < 0.05.

Table 3. Characteristics of individual studies and meta-analysis for the association between rs3808350/rs3808351/rs11544331 haplotypes and cancer risk.

| No | Author (year) | Haplotypes | Case Events Total | Control Events Total | OR (95% CI) [Random] | p-value |
|----|---------------|------------|------------------|----------------------|-----------------------|---------|
| 1  | Korkmaz et al (2014) | AGC | 70 | 109 | 87 | 104 | 0.55 (0.23–1.34) | 0.193 |
| 2  | Kasap et al (2016) | AGC | 60 | 111 | 45 | 78 | 1.00 (0.58–1.73) | 0.990 |
| 1  | Korkmaz et al (2014) | AGT | 9 | 109 | 12 | 104 | 1.00 (0.58–1.73) | 0.990 |
| 2  | Kasap et al (2016) | AGT | 30 | 111 | 18 | 78 | 0.59 (0.29–1.19) | 0.143 |
| 1  | Korkmaz et al (2014) | AAC | 25 | 109 | 28 | 104 | 1.87 (0.35–0.89) | 0.458 |
| 2  | Kasap et al (2016) | AAC | 11 | 111 | 17 | 78 | 1.37 (0.35–0.89) | 0.458 |
| 1  | Korkmaz et al (2014) | GGC | 31 | 109 | 34 | 104 | 0.86 (0.49–1.50) | 0.598 |
| 2  | Kasap et al (2016) | GGC | 44 | 111 | 10 | 78 | 1.13 (0.50–2.56) | 0.755 |
| 1  | Korkmaz et al (2014) | GGT | 8 | 109 | 12 | 104 | 1.64 (0.76–3.54) | 0.205 |
| 2  | Kasap et al (2016) | GGT | 25 | 111 | 17 | 78 | 1.64 (0.76–3.54) | 0.205 |
| 1  | Korkmaz et al (2014) | AAT | 11 | 109 | 6 | 104 | 1.13 (0.50–2.56) | 0.755 |
| 2  | Kasap et al (2016) | AAT | 14 | 111 | 12 | 78 | 1.13 (0.50–2.56) | 0.755 |
| 1  | Korkmaz et al (2014) | GAC | 39 | 109 | 20 | 104 | 0.53 (0.23–1.23) | 0.142 |
| 2  | Kasap et al (2016) | GAC | 18 | 111 | 12 | 78 | 0.53 (0.23–1.23) | 0.142 |
| 1  | Korkmaz et al (2014) | GAT | 25 | 109 | 9 | 104 | 0.46 (0.27; 0.79) | 0.004 |
| 2  | Kasap et al (2016) | GAT | 25 | 111 | 28 | 78 | 0.46 (0.27; 0.79) | 0.004 |

CI, confidence interval; OR, odds ratio.

Table 4. Characteristics of individual studies for the association between rs3808351 and tumor size.

| No | Author (year) | Sample size | SNP | Definition of allele | *p HWE | Genotype distribution |
|----|---------------|-------------|-----|----------------------|--------|----------------------|
|    |               |             |     |                      |        |                      |
|    |               |             |     |                      |        | ≥ T2 | < T2 | ≥ T2 | < T2 | GG | GA | AA | GG | GA | AA |
| 1  | Chevalier et al (2014) | 56 | 56 | rs3808351 | A | 0.086 | 2 | 9 | 6 | 8 | 32 | 12 |
| 2  | Giess et al (2010) | 104 | 246 | | | 0.9729 | 67 | 30 | 7 | 61 | 64 | 17 |

Alt., alternative allele; Ref., reference allele; SNP, Single nucleotide polymorphism. *p for Hardy–Weinberg equilibrium test in controls.

Table 5. Meta-analysis for the association between rs3808351 and tumor size.

| SNP | Genetic model | No. of studies | Test of association | Stat. Model | Test of heterogeneity | Publication bias p-value (Egger's test) |
|-----|---------------|----------------|---------------------|-------------|-----------------------|--------------------------------------|
|     |               |                | OR                  | 95% CI      | p-value               | p-value | I² (%) |
| rs3808351 | A vs. G | 2 | 0.79 | [0.29; 2.09] | 0.637 | Random | 0.028 | 79.25 | NA |
| rs3808351 | AA vs. GA + GG | 2 | 0.84 | [0.40; 1.74] | 0.643 | Fixed | 0.107 | 61.37 | NA |
| rs3808351 | AA + GA vs. GG | 2 | 0.46 | [0.28; 0.76] | 0.002 | Fixed | 0.180 | 44.33 | NA |
| rs3808351 | AA vs. GG | 2 | 0.53 | [0.23; 1.23] | 0.142 | Random | 0.111 | 60.47 | NA |
| rs3808351 | GA vs. GG | 2 | 0.46 | [0.27; 0.79] | 0.004 | Fixed | 0.292 | 9.78 | NA |

CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism; Stat. model, statistical model. Bold values indicate statistically significant differences between ≥ T2 and < T2.
Table 6. Characteristics of individual studies for the association between GPER expression levels and cancer progression.

| No | Author (year) | Cancer type | Tumor size ≥2 cm | Tumor stage ≥2 | Tumor grade ≥2 |
|----|---------------|-------------|------------------|----------------|----------------|
|    |               |             | GPER+/High Total | GPER-/Low Total | GPER+/High Total | GPER-/Low Total |
| 1  | Aidad et al (2014) | Breast cancer | – | – | – | – | 11 | 33 | 6 | 18 |
| 2  | Aquino et al (2018)a | Salivary Gland Tumors | – | – | – | – | 16 | 26 | 1 | 5 |
| 3  | Friese et al (2017)a | Cervical cancer | – | – | – | 96 | 114 | 35 | 42 | 102 | 111 | 38 | 41 |
| 4  | Heublein (2011)-1 | Ovarian Granulosa Cell Tumors | – | – | – | 1 | 8 | 2 | 7 | – | – | – |
| 5  | Ignatov et al (2011) | Breast cancer | – | – | – | 1 | 3 | 2 | 12 | – | – | – |
| 6  | Ignatov et al (2011)-2 | Ovarian Granulosa Cell Tumors | – | – | – | 1 | 3 | 2 | 12 | – | – | – |
| 7  | Ino et al (2019)* | Uterine cervical adenocarcinoma | – | – | – | 9 | 19 | 1 | 34 | – | – | – |
| 8  | Martin et al (2018)a* | Breast cancer | – | – | – | 36 | 50 | 77 | 100 | 48 | 73 | 149 | 176 |
| 9  | Samartzis et al (2014)a | Breast cancer | – | – | – | 1 | 3 | 2 | 12 | – | – | – |
| 10 | Krakstad et al (2012)* | Endometrial cancer | – | – | – | 68 | 333 | 79 | 141 | – | – | – |
| 11 | Liu et al (2019)a | NSCLC | – | – | – | 63 | 120 | 30 | 30 | – | – | – |
| 12 | Liu et al (2019)b | NSCLC | – | – | – | 39 | 78 | 32 | 72 | – | – | – |
| 13 | Luo et al (2011) | Breast cancer | – | – | – | 138 | 198 | 10 | 30 | 53 | 66 | 23 | 30 |
| 14 | Martin et al (2018)b* | Breast cancer | 124 | 327 | 372 | 910 | 132 | 327 | 351 | 910 | 263 | 327 | 775 | 910 |
| 15 | Samartzis et al (2014)b | Breast cancer | – | – | – | 99 | 189 | 48 | 50 | 77 | 100 | 48 | 73 | 149 | 176 |
| 16 | Smith et al (2009)* | Ovarian cancer | – | – | – | 39 | 52 | 37 | 82 | – | – | – |
| 17 | Steiman et al (2013) | Breast cancer | – | – | – | 21 | 27 | 14 | 21 | – | – | – |
| 18 | Tian et al (2018)b* | Gastric cancer | 8 | 26 | 18 | 58 | 17 | 26 | 40 | 58 | – | – | – |
| 19 | Ye et al (2019)* | Breast cancer | – | – | – | 46 | 74 | 127 | 175 | 62 | 73 | 149 | 176 |
| 20 | Yu et al (2014) | Breast cancer | – | – | – | 48 | 66 | 13 | 30 | 53 | 66 | 23 | 30 |

Bold values indicate statistically significant p < 0.05.

Table 7. Meta-analysis for the association between GPER expression levels and cancer progression.

| Group | No. of studies | OR (95% CI) [Random] | p-value |
|-------|----------------|-----------------------|---------|
| Tumor size ≥2 cm | | | |
| Overall (GPER+/-) | 2 | 0.80 (0.58–1.10) | 0.168 |
| Overall (GPER high/low) | 4 | 0.88 (0.55–1.39) | 0.575 |
| Breast cancer (GPER+/-) | 3 | 0.87 (0.51–1.46) | 0.590 |
| Breast cancer (GPER high/low) | 2 | 0.80 (0.58–1.10) | 0.168 |
| Tumor stage ≥2 | | | |
| Overall (GPER+/-) | 11 | 1.18 (0.85–1.64) | 0.326 |
| Overall (GPER high/low) | 6 | 0.87 (0.58–1.31) | 0.497 |
| Asian (GPER+/-) | 3 | 2.22 (1.12–4.41) | 0.022 |
| Asian (GPER high/low) | 3 | 1.65 (0.38–7.21) | 0.505 |
| Caucasian (GPER+/-) | 8 | 0.86 (0.72–1.04) | 0.120 |
| Caucasian (GPER high/low) | 3 | 0.85 (0.58–1.20) | 0.345 |
| Breast cancer (GPER+/-) | 4 | 1.15 (0.69–1.92) | 0.595 |
| Breast cancer (GPER high/low) | 4 | 0.80 (0.59–1.09) | 0.153 |
| Ovarian cancer (GPER+/-) | 3 | 0.78 (0.38–1.61) | 0.505 |
| Tumor grade ≥2 | | | |
| Overall (GPER+/-) | 12 | 1.22 (0.68–2.20) | 0.507 |
| Overall (GPER high/low) | 5 | 0.54 (0.25–1.17) | 0.117 |
| Caucasian (GPER+/-) | 10 | 0.94 (0.56–1.60) | 0.829 |
| Caucasian (GPER high/low) | 4 | 0.69 (0.31–1.55) | 0.368 |
| Breast cancer (GPER+/-) | 5 | 1.06 (0.44–2.54) | 0.894 |
| Ovarian cancer (GPER+/-) | 2 | 0.49 (0.05–5.12) | 0.549 |
of GPER polymorphisms can be used as an early detection marker for malignancies in clinical settings. Altogether, our findings indicate that GPER plays a crucial role in cancer pathogenesis and progression.

Declarations

Author contribution statement

Zulfikar Syambani Ulhaq: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Gita Vita Soraya, William Ka Fai Tse: Analyzed and interpreted the data; Wrote the paper.

Alvi Milliana: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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References

[1] Z.S. Ulhaq, Brain aromatase modulates cardiac functions in embryonic zebrafish, Int. J. Vet. Sci. Med. 7 (2019) 31–34.
[2] Z.S. Ulhaq, M. Kishida, Brain aromatase modulates serotonergic neuron by regulating serotonin levels in zebrafish embryos and larvae, Front. Endocrinol. 9 (2018) 230.
[3] Z.S. Ulhaq, C.P. Garcia, Estrogen receptor beta (ESR2) gene polymorphism and susceptibility to dementia, Acta Neurol. Belg. (2020) 1–3.
[4] Z.S. Ulhaq, The association between genetic polymorphisms in estrogen receptor genes and the risk of ovarian cancer: a meta-analysis, Turk. J. Ophthalol. 50 (2020) 216–220.
[5] Z.S. Ulhaq, Estrogen receptor-30 gene polymorphisms are associated with Parkinson’s disease: an updated meta-analysis, Egypt. J. Med. Genet. 21 (2020) 14.
[6] Z.S. Ulhaq, Gene polymorphisms associated with bilateral cataract, Int. J. Retina. 3 (2020).
[7] Z.S. Ulhaq, Vitamin D and its receptor polymorphisms are associated with glaucoma, J. Fr. Ophtalmol. (2020) 1009–1019.
[8] Z.S. Ulhaq, G.V. Soraya, Budu, L.R. Wulandari, The role of IL-6-174 G/C polymorphism and intracranial IL-6 levels in the pathogenesis of ocular diseases: a systematic review and meta-analysis, Clin. Exp. Optom. 3 (2020) 143–151.
[9] Z.S. Ulhaq, C.P. Garcia, Estrogen receptor beta (ESR2) gene polymorphism and susceptibility to dementia, Acta Neurol. Belg. (2020) 1–3.
[10] Z.S. Ulhaq, Comment on the assessment of ‘Association of interleukin-6 gene polymorphisms and glaucoma: systematic review and meta-analysis, Eur. J. Ophthalomol. (2020) 1120672120962049.
[11] Z.S. Ulhaq, G.V. Soraya, Aqueous humor interleukin-6 levels in primary open-angle glaucoma (POAG): a systematic review and meta-analysis, Arch. Soc. Espanola Oftalmol. 95 (2020) 315–321.
[12] Z.S. Ulhaq, Chemokine IL-8 level in aqueous humor of open-angle glaucoma: a meta-analysis, Acta Soc. Espanola Oftalmol. 95 (2020) 114–119.
[13] G.V. Soraya, Z.S. Ulhaq, Crucial laboratory parameters in COVID-19 diagnosis and prognosis: an updated meta-analysis, Med. Clin. 155 (2020) 143–151.
[14] Z.S. Ulhaq, G.V. Soraya, Interleukin-6 as a potential biomarker of COVID-19 progression, Med. Maladies Infect. 50 (2020) 362–383.
[15] G.V. Soraya, Z.S. Ulhaq, Interleukin-6 levels in children developing SARS-CoV-2 infection, Pediatr. Neonatol. 61 (2020) 253–254.
[16] Z.S. Ulhaq, G.V. Soraya, F.A. Fauziah, Recurrent positive SARS-CoV-2 RNA tests in recovered and discharged patients, Rev. Clin. Exp. (2020).
[17] Z.S. Ulhaq, G.V. Soraya, The prevalence of ophthalmic manifestations in COVID-19 and the diagnostic value of ocular tissue/bioid, Graefes Arch. Exp. Ophthalomol. Allbrecht Von Graefes Arch. Klin. Exp. Ophthalomol. 258 (2020) 1351–1352.
[18] H.A. Korkmaz, T. Edglinli, E. Eren, K. Demir, E.D.P. Cakir, S.K. Celik, B. Ozkan, GPER30 gene polymorphisms are associated with gynecoma in risk factors, Horm. Res. Paediatr. 83 (2015) 177–182.
[19] C.B. Nieweboer, A.E. Schorrier, Gynaecomastia and breast cancer in men, BMJ 336 (2008) 709–715.
[20] L.A. Britton, J.D. Carreon, G.L. Gierach, K.A. McGlynn, G. Gridley, Etiological factors for male breast cancer in the U.S. Veterans Affairs medical care system database, Breast Cancer Res. Treat. 119 (2010) 39–47.
[21] K. Friese, B. Kost, A. Vattal, F. Marme, C. Kuhn, S. Mahner, C. Dammeketz, U. Jerschke, S. Heubel, The G-protein-coupled estrogen receptor (GPER/GPR30) may serve as a prognostic marker in early-stage cervical cancer, J. Canc. Res. Oncol. 144 (2018) 1–13.
[22] S. Tian, N. Zhan, R. Li, W. Dong, Downregulation of G-Protein-Coupled estrogen receptor (GPER) is associated with reduced prognosis in patients with gastric cancer, Med. Sci. Monit. Int. Med. J. Exp. Clin. Res. 25 (2019) 3115–3126.
[23] S. Ye, Y. Xu, J. Li, Z. Zheng, P. Sun, T. Wang, Prognostic role of GPER/GPR30 in triple-negative breast cancer is associated with menstrual status, Endocrin. Connect. 8 (2019) 661–671.
[24] S.G. Martin, M.N. Lebot, B. Sukkarm, G. Ball, A.R. Green, E.A. Rakha, I.O. Ellis, J.S. Storr, Low expression of G-Protein-coupled oestrogen receptor 1 (GPER) is associated with adverse survival of breast cancer patients, Oncotarget 9 (2018) 25946–25956.
