Betaglycan Gene (TGFBR3) Polymorphism Is Associated with Increased Risk of Endometrial Cancer

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Abstract: We investigated single nucleotide polymorphism (SNP) of the betaglycan gene (TGFBR3) encoding the TGFβ co-receptor in endometrial cancer (EC) and its association with betaglycan expression. The study group included 153 women diagnosed with EC and 248 cancer-free controls. SNP genotyping and gene expression were analyzed using TaqMan probes. Three out of the eight SNPs tested, i.e., rs12566180 (CT; OR = 2.22; 95% CI = 1.15–4.30; p = 0.0177), rs6680463 (GC; OR = 2.34; 95% CI = 1.20–4.53; p = 0.0120) and rs2296621 (TT; OR = 6.40; 95% CI = 1.18–34.84; p = 0.0317) were found to be significantly associated with increased risk of EC (adjusted to age, body mass index, menarche and parity). Among the analyzed SNPs, only rs2296621 demonstrated the impact on the increased cancer aggressiveness evaluated by the WHO grading system (G3 vs. G1/2, GT—OR = 4.04; 95% CI = 1.56–10.51; p = 0.0026; T—OR = 2.38; 95% CI = 1.16–4.85; p = 0.0151). Linkage disequilibrium (LD) analysis revealed high LD (r2 ≥ 0.8) in two haploblocks, constructed by rs2770186/rs12141128 and rs12566180/rs6680463, respectively. In the case of C/C haplotype (OR = 4.82; 95% CI = 1.54–15.07; p = 0.0116—Bonferroni corrected) and T/G haplotype (OR = 3.25; 95% CI = 1.29–8.15; p = 0.0328—Bonferroni corrected) in haplotype rs12566180/rs6680463, significantly higher frequency was observed in patients with EC as compared to the control group. The genotype-phenotype studies showed that SNPs of the TGFBR3 gene associated with an increased risk of EC, i.e., rs12566180 and rs2296621 may affect betaglycan expression at the transcriptomic level (rs12566180—CC vs. TT, p < 0.01; rs2296621—GG vs. TT, p < 0.001, GT vs. TT, p < 0.05). Functional consequences of evaluated TGFBR3 gene SNPs were supported by RegulomeDB search. In conclusion, polymorphism of the TGFBR3 gene may be associated with an increased EC occurrence, as well as may be the molecular mechanism responsible for observed betaglycan down-regulation in EC patients.

Keywords: SNP; TGFβ; TGFBR3; betaglycan; endometrial cancer

1. Introduction

Endometrial cancer (EC) is one of the leading female cancer-related causes of death with around 382,069 new cases and 89,929 deaths worldwide each year. Significantly higher incidence rate is observed in developed countries in contrast to less-developed ones with the world morbidity around...
8.4/100,000 of female population [1]. In Poland, its incidence rate takes the third position among cancers and occurs predominantly in women in their menopausal and post-menopausal age (30.2/100,000). However, EC occurrence in Poland has risen rapidly in the last three decades, although the mortality trend is stable, as it is observed in global population [2]. In the future, the incidence of endometrial cancer is expected to increase due to the gradual aging of the population.

According to the clinico-pathological features and different pathogenesis, endometrial cancer is commonly classified into type I—endometrioid and type II—non-endometrioid. Type I is the most diagnosed type of endometrial cancer (75–90%) and develops from glandular cells in the endometrium lining. Endometrioid tumors are represented predominantly by endometrial adenocarcinomas, which are estrogen-dependent and tend to be low grade with favorable prognosis. Non-endometrioid cancers typically include papillary serous or clear cell carcinomas, in general, histological subtypes characterized by more aggressive phenotypes with poor outcome [3,4]. Endometrial cancer is mostly diagnosed in the early stages (FIGO I and II) as it is observed in 75% of patients. In this stage, 5-year overall survival is 74–91%, whereas for more advanced stages, 5-year overall survival rates are 57–66% and 20–26%, for FIGO III and FIGO IV, respectively [5]. Molecular classification distinguished based on a large scale, comprehensive genetic analysis of EC according to The Cancer Genome Atlas includes four subgroups, i.e., DNA polymerase epsilon ultramutated (POLE), microsatellite instability hypermutated (MSI), copy-number low and copy-number high subgroup. Each of categories is characterized by distinct clinical, pathological and molecular alterations. The POLE subgroup displays polymerase epsilon mutations in exonuclease domain, which results in a remarkable high mutation rate (232 × 10^6 mutations per Mb). The MSI subgroup is related to deficiencies in a DNA mismatch repair system leading to common mutations of ARID5B, PTEN, PIK3CA and PIK3R1 genes. The copy-number low subgroup is described also as microsatellite stable and corresponds to more than half of low-grade endometrioid tumors, whereas copy-number high subgroup reflects to serous histopathology [6–8]. Moreover, up to 5% of ECs are described as familial ones, due to the loss-of-function or expression alterations of DNA mismatch repair genes, i.e., (MLH1, MSH2, MSH6 or PMS2). The most frequent form of inherited EC is associated with Lynch syndrome, which increases the risk of developing EC to 25–60% [9,10].

According to the molecular findings, impaired TGFβ signaling has been reported in ECs [11–13]. The canonical signal, mediated via TGFβ factors, occurs through TGFβ membrane receptors type I (TGFβRI) and type II (TGFβRII), which possess serine/threonine kinase activity. Among huge number of TGFβ factors, three classical TGFβ isoforms, i.e., TGFβ1, TGFβ2 and TGFβ3, were identified. Dimeric TGFβ factors bind to the TGFβRII receptor, which in turn activates TGFβRI receptor. The activated TGFβRII/TGFβRI complex trans-phosphorylates cytoplasmic effectors, i.e., Smad2/3 proteins, forming a heterocomplex with the Smad4 protein, are translocated to the nucleus, where together with other transcription factors, regulate gene expression [14]. Appropriate signaling in TGFβ pathway requires the presence of co-receptors termed TGFβ receptors type III (TGFβRIII). TGFβ co-receptors are deprived of any known enzymatic activity; however, they are anchored in the cell membrane, and they are responsible for TGFβ ligand presentation to their canonical TGFβ receptors. The plethora of TGFβ ligands and receptors results in the regulation of many cellular processes, such as growth and proliferation, survival, apoptosis, cells adhesion, remodeling of extracellular matrix, angiogenesis and embryonic development [15].

The first identified TGFβ co-receptor was betaglycan [16]. Betaglycan gene (TGFBR3), located on chromosome 1, encodes a transmembrane proteoglycan. Literature data and our previous studies indicate the contribution of betaglycan loss to the development and progression of cancers originated from different tissue types, i.e., breast, endometrium, ovary, prostate, lung, bladder, liver, pancreas, kidney, and neuroblastoma [17–28]. Down-regulation of betaglycan expression seems to be engaged in the impaired TGFβ signaling initiated by the TGFβ2 isoform to which it displays the highest affinity.

Until now, there is no efficient and rapid molecular method suitable for neither early diagnosis nor prediction of EC risk. This became a basis for betaglycan (TGFBR3) gene single nucleotide
polymorphism (SNP) investigations. In the current study, we examined eight SNPs within the TGFBR3 gene and their association with primary EC and their clinico-pathological variables, as well as potential impact on betaglycan expression.

2. Materials and Methods

2.1. Study Population

In the study, we enrolled only Caucasian women born and living in Poland. The case-control study involved 153 women who underwent surgery of EC and 248 healthy individuals which served as cancer-free controls. Biological material (cancer group—endometrial tissue samples and peripheral blood; cancer-free control—peripheral blood) was collected in the II Department of Gynecology, Lublin Medical University, Lublin, Poland and in the Department of Gynecological Oncology, Medical University of Lodz, Lodz, Poland, between 2012–2017. Inclusion criteria for case group, included women with diagnosed primary endometrial adenocarcinomas, who had not received neither hormonal therapy, radiation therapy nor chemotherapy prior surgery; whereas control group was recruited from non-related women during periodic health check-ups, who have never been diagnosed with endometrial cancer or other tumors.

Cancer tissue specimens, after the surgery, were divided into two portions; one was fixed in buffered formalin (pH 7.4) for routine histological assessment while the other was immediately placed at −70 °C. Clinical stage was assigned based on surgico-pathological findings according to the revised FIGO staging, while WHO classification was applied to determine the histological type and grade. Table 1 presents socio-demographic and clinical characteristics of the patients and examined samples. All studied cancer samples were classified as endometrioid cancers (type I)—endometrial adenocarcinomas.

2.2. Ethical Approval

The study was conducted in accordance with the ethical principles of the 1975 Helsinki Declaration and its later amendments. The local Independent Committees of Bioethics of Lublin Medical University, Medical University of Lodz and University of Lodz approved the tissues collections and study protocols. All methods in the study were performed in accordance with above-mentioned bioethical permissions. All participating subject gave written, informed consent prior to enrolment.

2.3. Lifestyle Risk Factors

Study participants were interviewed during the examination about socio-demographic, health related information and reproductive history (parity and menarche). Body mass index (BMI) was calculated as current weight in kilograms divided by square of height expressed in meters. Any missing survey data were subsequently completed using patient’s query.

2.4. Genomic DNA Isolation

Genomic DNA was extracted from peripheral blood collected in the presence of anti-coagulant (EDTA) using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) and stored at −70 °C. The quality and quantity of DNA was estimated spectrophotometrically with BioPhotometer plus (Eppendorf, Hamburg, Germany). DNA samples were characterized with A260 nm/A280 nm ratio, which was in the range of 1.8–2.0.

2.5. SNP Selection and Genotyping

Eight SNPs in the TGFBR3 gene were selected according to NCBI SNPs database: rs883873, rs2770186, rs12141128, rs12566180, rs6680463, rs1805110, rs1805113, rs2296621. All SNPs were supposed to have minor allele frequency (MAF) ≥5% and localization assigned as 5′ regulatory region, intron or exon of the TGFBR3 gene, which is located on chromosome 1. Characteristics of studied SNPs are presented in Table 2.
Table 1. Socio-demographic and clinical characteristics of investigated subjects.

| Socio-Demographic Characteristics | Cases (n = 153) | Controls (n = 248) | p       |
|-----------------------------------|----------------|-------------------|---------|
| AGE (years) a                     | 62.1 ± 8.6     | 63.4 ± 9.0        | 0.1896  |
| min                               | 35             | 35                |         |
| max                               | 85             | 85                |         |
| 35–44 b                           | 2 (1.3)        | 10 (4.0)          |         |
| 45–54 b                           | 25 (16.3)      | 36 (14.5)         |         |
| 55–64 b                           | 75 (49.0)      | 84 (33.9)         |         |
| 65–74 b                           | 38 (24.8)      | 78 (31.5)         |         |
| 74–85 b                           | 13 (8.5)       | 40 (16.1)         | 0.009   |
| BMI (kg/m²) a                     | 28.5 ± 6.6     | 24.9 ± 4.0        | <0.001  |
| obesity (BMI > 30 kg/m²) b        | 56 (36.6)      | 11 (4.4)          | <0.001  |
| Menarche (years) a                | 13.8 ± 2.0     | 11.9 ± 1.6        | <0.001  |
| Parity (childbirths) a            | 1.9 ± 1.4      | 1.1 ± 0.9         | <0.001  |

| Clinico-Pathological Characteristics |                      |
|--------------------------------------|----------------------|
| Histological Diagnosis               | endometrial adenocarcinoma |
| Tumor Stage c                        |                      |
| I b                                  | 89 (58.2)            |
| II b                                 | 38 (24.8)            |
| III b                                | 18 (11.8)            |
| IV b                                 | 8 (5.2)              |
| Histological Grade d                 |                      |
| G1 b                                 | 37 (24.2)            |
| G2 b                                 | 94 (61.4)            |
| G3 b                                 | 22 (14.4)            |
| Depth OF Myometrial Invasion         |                      |
| <1/2 b                               | 82 (53.6)            |
| >1/2 b                               | 71 (46.4)            |
| Vascular Space Invasion              |                      |
| not present b                        | 85 (55.6)            |
| present b                            | 23 (15.0)            |
| data not available b                 | 45 (29.4)            |

BMI—body mass index, a Mean ± SD. b Number of subjects (percent total). c International Federation of Gynecology and Obstetrics staging system (FIGO). d World Health Organization grading system.

Table 2. Characteristics of studied polymorphisms.

| rs Number | Polymorphism         | Localization     | Maf   |
|-----------|----------------------|------------------|-------|
| rs883873  | g.92380302A > G      | 5’ regulatory region | 0.1394|
| rs2770186 | g.92378843T > C      | 5’ regulatory region | 0.4730|
| rs12141128| g.92373747A > G      | 5’ regulatory region | 0.4736|
| rs12566180| c.−114 + 2392C > T   | intron            | 0.4209|
| rs6680463 | c.−114 + 7008C > G   | intron            | 0.4687|
| rs1805110 | c.44C > T (p.Ser15Phe)| exon             | 0.1859|
| rs1805113 | c.2025T > C (p.Phe675=)| exon           | 0.2798|
| rs2296621 | c.2285 – 99G > T     | intron            | 0.1050|

Real-Time PCR method with TaqMan Genotyping Assays (Thermo Fisher Scientific, Waltham, MA, USA) was applied for SNPs genotyping. The characteristics and sequences of used TaqMan probes are shown in Supplementary Table S1. PCR amplifications were conducted in a total volume of 10 µL and consisted of 5 µL (2X) of TaqMan Genotyping Master Mix buffer (Thermo Fisher Scientific, Waltham, MA, USA), 0.25 µL (40X) TaqMan Genotyping Assay (Thermo Fisher Scientific, Waltham, MA, USA) and 10 ng of template DNA. Thermal conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of sequential incubations at 95 °C for 15 s and at 60 °C for
1 min and final endpoint measurement of fluorescence. Real-Time PCR amplifications and allelic discrimination were performed using Mastercycler® ep realplex (Eppendorf, Hamburg, Germany).

2.6. Expression of the TGFBR3 Gene

Total RNA was extracted from frozen endometrial tissues using PureLink RNA Mini kit (Thermo Fisher Scientific, Waltham, MA, USA) according to dedicated protocol. The amount and quantity of isolated RNA was assessed spectrophotometrically with BioPhotometer plus (Eppendorf, Hamburg, Germany) based on A260 nm/A280 nm ratio, which was in the range 1.8–2.0. Total RNA (1 µg) was transcribed using RevertAid™ H Minus First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer’s recommendation. cDNA synthesis was performed in Thermocycler 2720 (Applied Biosystems, Foster City, CA, USA) with the following incubations: 10 min at 25 °C, 120 min at 37 °C and 5 min at 85 °C. The obtained cDNA was stored at −70 °C. Real time PCR was performed using TaqMan probes (Thermo Fisher Scientific, Waltham, MA, USA) in line with the manufacturer’s protocol on Mastercycler® Epgradient S Realplex (Eppendorf, Hamburg, Germany) in the presence of TaqMan Gene Expression Master Mix Thermo Fisher Scientific, Waltham, MA, USA). GAPDH served as a reference gene. The catalogue numbers of probes were Hs00234259_m1 for TGFBR3 and Hs99999905_m1 for GAPDH. The relative expression level was normalized to GAPDH and was calculated using the following equation: $2^{-\Delta Ct} \times 1000$.

2.7. Statistical Analysis

The genotype frequency was tested for agreement with Hardy–Weinberg equilibrium (HWE) and assessed by chi-square goodness-of-fit test. Case-control differences in genotype and allelic distribution were analyzed using Pearson’s χ² (chi-square) or Fisher’s exact tests against the homozygote of the common allele as the reference group (OR = 1.00). Dominant and recessive genetic models were also implemented in the analysis. Variants of homozygotes and heterozygotes were combined to evaluate the dominant effect. SNPs distribution and their association with the clinico-pathological parameters were evaluated by multiple logistic regression. Genotype and allelic associations with endometrial cancer risk were expressed as odds ratio (ORs) and 95% confidence interval (95% CI) in crude and multivariate model including age, BMI, parity, and age at menarche.

Linkage disequilibrium (LD) and haplotypes distribution analysis were performed using the powerful online platform SHEsis (http://analysis.bio-x.cn/myAnalysis.php) [29]. Haplotypes with frequency less than 0.03 were excluded from the analysis. Bonferroni correction was applied for multiple comparisons of SNPs haplotypes.

To assess inter-group differences of socio-demographic parameters (age, BMI, parity and menarche), as well as the TGFBR3 gene expression levels between respective genotypes of the analyzed SNPs, the first Shapiro–Wilks test was applied to determine the normality of obtained data. Following, the statistical significance of difference was evaluated using either Student’s t-test, for normally distributed data, or Mann–Whitney test, for non-normally distributed data. $p < 0.05$ in a two-tailed test was considered statistically significant. A statistical analysis of obtained data was conducted using GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla, CA, USA) and PQStat version 1.6.8 (PQStat Software, Poland).

2.8. Bioinformatic Analysis

The functional consequences of significant SNPs were examined in RegulomeDB, which is a public database dedicated for noncoding SNP and annotates SNPs with known and putative regulatory elements in non-coding regions of human genome, such as regulatory DNA elements including regions of DNAase hypersensitivity, binding sites of transcription factors, and promoter regions that have been biochemically characterized to regulate transcription. RegulomeDB annotations are based on an integration of data from ENCODE project and other published literature, combined together
by self-developed score system ranging from 1–6. A higher rank corresponds to a less functional significance [30].

3. Results

3.1. SNPs Association with EC

Eight single nucleotide polymorphisms in the TGFBR3 gene and their association with EC risk and invasiveness were evaluated. Three of analyzed polymorphisms, i.e., rs883873 (g.92380302A > G), rs2770186 (g.92378843T > C) and rs12141128 (g.92373747A > G) are located in 5′ regulatory region, whereas the other five polymorphisms, i.e., rs12566180 (c.–114 + 2392C > T), rs6680463 (c.–114 + 7008C > G), rs18051110 (p.Ser15Phe), rs1805113 (p.Phe675=) and rs2296621 (c.2285 – 99G > T) are located downstream start codon of the TGFBR3 gene.

Based on a comparison of 153 women diagnosed with endometrial cancer and 248 healthy controls, we found significant differences in the distribution of the three studied SNPs (Table 3) adjusted to the following covariates, i.e., age, BMI, menarche and parity. Significant differences between endometrial cancer patients and control women were noted in body mass index (BMI; \( p < 0.001 \)), menarche (\( p < 0.001 \)) and parity (\( p < 0.001 \)). In addition, the subjects’ age showed significant differences between case and control group when analyzed in 10 years subgroups (\( p = 0.009 \)) (Table 1). Accordingly, age, BMI, menarche and parity were selected as main covariates in further analysis. All studied SNPs were in Hardy–Weinberg equilibrium (HWE) except polymorphic site rs1805110 (p.Ser15Phe), which was excluded from further analysis. The obtained results indicate that the polymorphisms of the highest importance for the increased endometrial cancer risk are rs12566180 (c.–114 + 2392C > T), rs6680463 (c.–114 + 7008C > G) and rs2296621 (c.2285 – 99G > T). SNPs, rs12566180 (c.–114 + 2392C > T) and rs6680463 (c.–114 + 7008C > G) were found to be more frequent as heterozygous variants in the study group as compared to the controls with respective frequencies 55.6% vs. 44.0% (\( p = 0.0177 \)) and 58.2% vs. 45.2% (\( p = 0.0120 \)), increasing the risk of endometrial cancer about 2.3 times. In turn, the polymorphic site rs2296621 (c.2285 – 99G > T) was found to be 6.4-fold more frequent in the case of study group compared to the controls (4.6% vs. 0.8%, \( p = 0.0317 \)) as homozygous variant TT (Table 3).

Analysis of significantly altered SNPs in the cancer group in the context of clinico-pathological parameters revealed that in the case of rs2296621 (c.2285 – 99G > T) the genotype GT and allele T are associated with an increased histological grade according to the WHO grading system (G3 vs. G1/2). The GT genotype was observed in 54.5% of high-graded tumors (G3) compared to 23.7% of less-graded (G1/2) (OR = 4.04; 95% CI = 1.56–10.51; \( p = 0.0026 \)). Respectively, allele T carriers demonstrated G3 tumors more frequent than G1/2, i.e., 31.8% vs. 16.4% (OR = 2.38; 95% CI = 1.16–4.85; \( p = 0.0151 \)) (Supplementary Table S2).

Linkage disequilibrium (LD) analysis revealed that among eight studied SNPs in the TGFBR3 gene, four of them were in high LD (\( r^2 \geq 0.8 \)) (Figure 1) and were arranged in two haploblocks constructed by rs2770186/rs12141128 and rs12566180/rs6680463. The frequency of haplotypes rs12566180/rs6680463 C/C and T/G were significantly higher in endometrial cancer patients as compared to healthy controls: for C/C haplotype 0.038 vs. 0.008 (OR = 4.82; 95% CI = 1.54–15.07; \( p = 0.0116 \)—Bonferroni corrected) and for T/G haplotype 0.045 vs. 0.014 (OR = 3.25; 95% CI = 1.29–8.15; \( p = 0.0328 \)—Bonferroni corrected), respectively (Table 4).
Table 3. Genotype distribution and allelic frequencies of investigated SNPs in the TGFB3 gene for women with diagnosed endometrial cancer and healthy controls.

| SNP Genotype/Allele | Cancer (n = 123) | Control (n = 248) | OR \(^a\) | 95% CI | p Value | OR \(^b\) | 95% CI | p Value | HWE \(^c\) |
|---------------------|------------------|------------------|----------|--------|---------|----------|--------|---------|-----------|
|                     | Number | Frequency (%) | Number | Frequency (%) | | | | | | |
| rs883873 (g.92380302A > G) | | | | | | | | | | |
| AA                  | 107    | 69.9            | 200    | 80.6            | 1.00 (ref.) | 1.00 (ref.) | | | | 0.3110 |
| AG                  | 45     | 29.4            | 47     | 19.0            | 1.79        | 1.12–2.87 | 0.0149 | 1.26 | 0.68–2.33 | 0.4607 |
| GG                  | 1      | 0.7             | 1      | 0.4             | 1.87        | 0.12–30.18 | 1     | -   | -        | -         |
|                      |        |                 |        | 6.049; \(p = 0.0486\) | | | | | | |
| rs2770186 (g.92380302A > G) | | | | | | | | | | |
| TT                  | 22     | 14.4            | 46     | 18.5            | 1.00 (ref.) | 1.00 (ref.) | | | | 0.8572 |
| TC                  | 89     | 58.2            | 120    | 48.4            | 1.55        | 0.87–2.76 | 0.1345 | 1.54 | 0.74–3.22 | 0.2469 |
| CC                  | 42     | 27.5            | 82     | 33.1            | 1.07        | 0.57–2.01 | 0.8231 | 1.09 | 0.49–2.41 | 0.8286 |
|                      |        |                 |        | 3.672; \(p = 0.1595\) | | | | | | |
| rs2770186 (g.92380302A > G) | | | | | | | | | | |
| AA                  | 107    | 69.9            | 200    | 80.6            | 1.00 (ref.) | 1.00 (ref.) | | | | 0.3110 |
| AG                  | 45     | 29.4            | 47     | 19.0            | 1.79        | 1.12–2.87 | 0.0149 | 1.26 | 0.68–2.33 | 0.4607 |
| GG                  | 1      | 0.7             | 1      | 0.4             | 1.87        | 0.12–30.18 | 1     | -   | -        | -         |
|                      |        |                 |        | 6.049; \(p = 0.0486\) | | | | | | |
| rs2770186 (g.92380302A > G) | | | | | | | | | | |
| AT                  | 131    | 43.5            | 212    | 42.7            | 1.03        | 0.77–1.37 | 0.8415 | 1.02 | 0.70–1.50 | 0.9100 |
| CT                  | 173    | 56.5            | 284    | 57.3            | 0.97        | 0.73–1.29 | 0.8415 | 0.98 | 0.67–1.44 | 0.9100 |
Table 3. Cont.

| SNP Genotype/Allele | Cancer (n = 123) | Control (n = 248) | OR  | 95% CI       | p Value | OR  | 95% CI       | p Value | HWE  |
|---------------------|------------------|-------------------|-----|--------------|---------|-----|--------------|---------|------|
|                     | Number           | Frequency (%)     | Number | Frequency (%) |         |     |              |         |      |
| rs12141128 (g.92373747A > G) |                   |                   |       |              |         |     |              |         |      |
| AA                  | 23               | 15.0              | 49    | 19.8         | 1.00    |     |              |         |      |
| AG                  | 88               | 57.5              | 118   | 47.6         | 1.59    | 0.90–2.80 | 0.1082 | 1.65 | 0.80–3.38 | 0.1742 | 0.6113 |
| GG                  | 42               | 27.5              | 81    | 32.7         | 1.10    | 0.59–2.05 | 0.7518 | 1.14 | 0.52–2.47 | 0.7418 |         |
| χ² = 3.833; p = 0.1471 |                   |                   |       |              |         |     |              |         |      |
| AG or GG vs. AA     | 130              | 199               | 1.39  | 0.81–2.39 | 0.2318 | 1.43 | 0.72–2.83 | 0.3018 |      |
| AG or AA vs. GG     | 111              | 167               | 1.28  | 0.82–2.00 | 0.2713 | 1.27 | 0.72–2.23 | 0.4023 |      |
| A                   | 134              | 216               | 43.5  | 1.01        | 0.76–1.35 | 1.0000 | 1.00 | 0.68–1.46 | 0.9920 |      |
| G                   | 172              | 280               | 56.5  | 0.99        | 0.74–1.32 | 1.0000 | 1.00 | 0.69–1.46 | 0.9920 |      |
| rs12566180 (c.−114 + 2392C > T) |                   |                   |       |              |         |     |              |         |      |
| CC                  | 30               | 19.6              | 68    | 27.4         | 1.00    |     |              |         |      |
| CT                  | 85               | 55.6              | 109   | 44.0         | 1.77    | 1.06–2.96 | 0.0393 | 2.22 | 1.15–4.30 | 0.0177 | 0.0570 |
| TT                  | 38               | 24.8              | 71    | 28.6         | 1.21    | 0.68–2.17 | 0.5169 | 1.18 | 0.58–2.41 | 0.6484 |         |
| χ² = 5.497; p = 0.0640 |                   |                   |       |              |         |     |              |         |      |
| CT or TT vs. CC     | 123              | 180               | 1.55  | 0.95–2.52 | 0.0769 | 1.73 | 0.94–3.17 | 0.0775 |      |
| CT or CC vs. TT     | 115              | 177               | 1.21  | 0.77–1.92 | 0.4062 | 1.42 | 0.80–2.51 | 0.2256 |      |
| C                   | 145              | 245               | 49.4  | 0.92        | 0.69–1.23 | 0.5777 | 0.95 | 0.67–1.35 | 0.7761 |      |
| T                   | 161              | 251               | 50.6  | 1.08        | 0.81–1.44 | 0.5777 | 1.05 | 0.74–1.50 | 0.7761 |      |
| rs6680463 (c.−114 + 7008C > G) |                   |                   |       |              |         |     |              |         |      |
| GG                  | 29               | 19.0              | 68    | 27.4         | 1.00    |     |              |         |      |
| GC                  | 89               | 58.2              | 112   | 45.2         | 1.86    | 1.11–3.12 | 0.0174 | 2.34 | 1.20–4.53 | 0.0120 | 0.1275 |
| CC                  | 35               | 22.9              | 68    | 27.4         | 1.21    | 0.67–2.19 | 0.5376 | 1.09 | 0.53–2.24 | 0.8243 |         |
| χ² = 6.758; p = 0.0341 |                   |                   |       |              |         |     |              |         |      |
| GC or CC vs. GG     | 124              | 180               | 1.61  | 0.99–2.64 | 0.0544 | 1.74 | 0.95–3.20 | 0.0749 |      |
| GC or GG vs. CC     | 118              | 180               | 1.27  | 0.80–2.04 | 0.3125 | 1.60 | 0.90–2.86 | 0.1114 |      |
| G                   | 147              | 248               | 50.0  | 0.92        | 0.70–1.23 | 0.5902 | 0.99 | 0.69–1.41 | 0.9470 |      |
| C                   | 159              | 248               | 50.0  | 1.08        | 0.81–1.44 | 0.5902 | 1.01 | 0.71–1.45 | 0.9470 |      |
### Table 3. Cont.

| SNP Genotype/Allele | Cancer (n = 123) | Control (n = 248) | OR \(^a\) | 95% CI | p Value | OR \(^b\) | 95% CI | p Value | HWE \(^c\) |
|---------------------|------------------|------------------|--------|--------|--------|--------|--------|--------|---------|
| Number | Frequency (%) | Number | Frequency (%) | | | | | | |
| rs1805110 c.44C > T | | | | | | | | | |
| (p.Ser15Phe) | | | | | | | | | |
| CC | 0 | 0.0 | 0 | 0.0 | - | - | - | - | - | - | - | - | - |
| CT | 112 | 73.2 | 212 | 85.5 | 0.46 | 0.28–0.77 | 0.0024 | 0.79 | 0.42–1.50 | 0.4771 | <0.001 |
| TT | 41 | 26.8 | 36 | 14.5 | 2.16 | 1.30–3.56 | 0.0024 | 1.26 | 0.66–2.39 | 0.4771 | |
| \(\chi^2 = 9.199; p = 0.0100\) | | | | | | | | | | |
| CT or TT vs. CC | 153 | 248 | - | - | - | - | - | - | - | - | - | - | - |
| CT or CC vs. TT | 112 | 212 | 0.46 | 0.28–0.77 | 0.0024 | 0.79 | 0.42–1.50 | 0.4771 | |
| C | 112 | 36.6 | 212 | 42.7 | 0.77 | 0.58–1.04 | 0.0853 | 0.79 | 0.42–1.50 | 0.4771 | |
| T | 194 | 63.4 | 284 | 57.3 | 1.29 | 0.96–1.73 | 0.0853 | 1.26 | 0.66–2.39 | 0.4771 | |
| rs1805113 c.2025T > C | | | | | | | | | |
| (p.Phe675=) | | | | | | | | | |
| TT | 56 | 36.6 | 87 | 35.1 | 1.00 | (ref.) | 1.00 | (ref.) | 1.00 | (ref.) | 1.00 | (ref.) | 1.00 | (ref.) | 0.5127 |
| TC | 68 | 44.4 | 123 | 49.6 | 0.86 | 0.55–1.34 | 0.5055 | 0.62 | 0.34–1.12 | 0.4771 | 0.6117 |
| CC | 29 | 19.0 | 38 | 15.3 | 1.19 | 0.66–2.14 | 0.5707 | 0.93 | 0.44–1.96 | 0.8513 | |
| \(\chi^2 = 1.336; p = 0.5127\) | | | | | | | | | | |
| TC or CC vs. TT | 97 | 161 | 0.94 | 0.62–1.42 | 0.7518 | 0.70 | 0.40–1.21 | 0.2001 | |
| TC or TT vs. CC | 124 | 210 | 0.77 | 0.45–1.32 | 0.3438 | 0.81 | 0.42–1.57 | 0.5405 | |
| T | 180 | 58.8 | 297 | 59.9 | 0.96 | 0.72–1.28 | 0.7642 | 1.10 | 0.77–1.58 | 0.6083 | |
| C | 126 | 41.2 | 199 | 40.1 | 1.04 | 0.78–1.40 | 0.7642 | 0.91 | 0.63–1.31 | 0.6083 | |
| rs2296621 c.2285−99G > T | | | | | | | | | |
| (p.Phe675=) | | | | | | | | | |
| GG | 103 | 67.3 | 178 | 71.8 | 1.00 | (ref.) | 1.00 | (ref.) | 1.00 | (ref.) | 1.00 | (ref.) | 1.00 | (ref.) | 0.0988 |
| GT | 43 | 28.1 | 68 | 27.4 | 1.09 | 0.70–1.72 | 0.6985 | 0.87 | 0.49–1.53 | 0.6226 | |
| TT | 7 | 4.6 | 2 | 0.8 | 6.05 | 1.23–29.66 | 0.0178 | 6.40 | 1.18–34.94 | 0.0317 | |
| \(\chi^2 = 6.272; p = 0.0435\) | | | | | | | | | | |
| GT or TT vs. GG | 50 | 70 | 1.23 | 0.80–1.91 | 0.3428 | 1.04 | 0.60–1.80 | 0.8951 | |
| GT or GG vs. TT | 146 | 246 | 0.17 | 0.03–0.83 | 0.0178 | 0.15 | 0.03–0.80 | 0.0267 | |
| G | 249 | 81.4 | 424 | 85.5 | 0.74 | 0.51–1.09 | 0.1237 | 0.80 | 0.50–1.30 | 0.3703 | |
| T | 57 | 18.6 | 72 | 14.5 | 1.35 | 0.92–1.97 | 0.1237 | 1.24 | 0.77–2.00 | 0.3703 | |

\(^a\) Crude. \(^b\) Adjusted for age, BMI, parity and menarche. \(^c\) Hardy–Weinberg equilibrium test for controls. \(^d\) Testing dominant genetic model. \(^e\) Testing recessive genetic model; \(p < 0.05\) along with corresponding ORs are in bold.
For genotype TT (p < 0.001) demonstrated a statistically lower TGFBR3 mRNA level with regard to the wild-type carriers.

3.2. Association of the TGFBR3 Gene SNPs and Betaglycan mRNA Expression—Genotype-Phenotype Analysis

Figure 2 shows the mRNA expression level of the TGFBR3 gene in 50 EC patients in relation to the genotypes of eight studied SNPs of the TGFBR3 gene. Six SNPs of the TGFBR3 gene were found to modulate its expression. The significant down-regulation of TGFBR3 mRNA was observed in the case of homozygous variant of rs2770186 for genotype CC (p < 0.05), rs12141128 for genotype GG (p < 0.05), rs1805110 for phenotype variant Phe/Phe (p < 0.01) and rs2296621 for genotype TT (p < 0.05) as compared to heterozygous variants. Furthermore, rs883873 polymorphism for genotype AG (p < 0.05), rs12566180 for genotype TT (p < 0.01) and rs2296621 for genotype TT (p < 0.001) demonstrated a statistically lower TGFBR3 mRNA level with regard to the wild-type carriers.
Figure 2. Impact of single-nucleotide polymorphisms related to the TGFBR3 gene on betaglycan mRNA expression in women with EC. Data are shown as scatter dot plots, horizontal lines represent median, whereas whiskers correspond to interquartile range. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. 
4. Discussion

Transforming growth factors $\beta$ isoforms, i.e., TGF$\beta$1, TGF$\beta$2 and TGF$\beta$3, belong to a large superfamily of cytokines, which were identified due to their important role in normal development and homeostasis. TGF$\beta$ pathway controls many opposed processes, which is known as the pleiotropic effect on cell and tissue physiology. TGF$\beta$ cascade is responsible for both suppression or induction of cell proliferation and apoptosis, as well as regulates autophagy, cell dormancy and senescence. Deregulation of TGF$\beta$ signaling, both at induction step and downstream signaling contributes to developmental anomalies and diseases, in particular fibrosis and cancer, which is associated with overexpression of TGF$\beta$ isoforms [31,32]. Moreover, in cancer cells, disturbed signal mediation in TGF$\beta$ pathway triggers its role from a tumor suppressor, early in neoplastic transformation, to a cancer-promoting and metastatic agent in advanced clinical stages of the disease [33].

In cancer cells, alteration of TGF$\beta$ signaling, which plays the pleiotropic role during carcinogenesis, may be influenced by gene polymorphism. Knowledge of the potential role of polymorphism of the TGF$\beta$R3 gene encoding betaglycan and its relation to development of EC is elusive. Risk of endometrial cancer development is highly associated with different lifestyle and socio-demographic factors including obesity, onset of menarche, reproductive history, ethnicity and patient’s age [34–40]. Overweight, young age at menarche or nulliparity cause prolonged exposure to estrogens, which possess high proliferative potential, in particular to uterus lining. In the case of obesity, unopposed estrogen stimulation is the result of reduction of progesterone synthesis and higher levels of circulating estrogens. During pregnancy, the estrogen exposure is balanced by the shift toward progesterone signaling. For this reason, nulliparous women have a higher risk of developing endometrial cancer due to extended estrogen stimuli. Moreover, the increased number of births shows a protective effect on endometrial cancer occurrence [40–42]. Our study has demonstrated that rs12566180 (c.-114 + 2392C > T), rs6680463 (c.-114 + 7008C > G) and rs2296621 (c.2285 – 99G > T) polymorphisms of the TGF$\beta$R3 gene are associated with increased risk of EC, both as crude or adjusted for socio-demographic risk factors, such as age, body mass index (BMI), menarche and parity. None of the patients had received EC related hormonal therapy prior to surgery; however, the patients’ records concerning hormonal replacement therapy as well as Lynch syndrome history were not available. In spite of the fact, that Lynch syndrome significantly increases the risk of EC to 25–60%, its impact on development of EC can be excluded as its occurrence ranges between 0.5% and 4.6% of all EC cases [9,10].

The rs12566180 (c.-114 + 2392C > T) and rs2296621 (c.2285 – 99G > T) polymorphisms significantly altered in endometrial cancer are located within intronic regions of the TGF$\beta$R3 gene, which may indicate their potential impact on transcription and stability of the primary transcript. Obtained results suggest that studied SNPs are involved in the observed betaglycan down-regulation in endometrial cancer; however, the only one of them, i.e., rs2296621 (c.2285 – 99G > T), seems to be related to pronounced tumor aggressiveness. Bioinformatic analysis using RegulomeDB showed that rs12566180 (c.-114 + 2392C > T) and rs6680463 (c.-114 + 7008C > G) have a score of 4, whereas rs2296621 (c.2285 – 99G > T) has a score of 2 [30]. Taking into account SNPs case-control study, haplotype analysis and genotype-phenotype findings, the observed results indicate that rs12566180 (c.-114 + 2392C > T), rs6680463 (c.-114 + 7008C > G) and rs2296621 (c.2285 – 99G > T) could be regarded as potential markers for EC. However, further studies are required.

According to the literature data, polymorphism of the TGF$\beta$R3 gene is considered as a mechanism responsible for betaglycan down-regulation of HBV-infection related hepatocellular carcinoma and ovarian cancer [17,43–45]. Bae et al. [17] evaluated six SNPs, i.e., rs1805110 (p.Ser15Phe), rs2810904 (p.Ala72=), rs2306888 (p.Ser173=), rs1805113 (p.Phe675=), rs284878 (p.Thr749=) and newly identified SNP p.Thr711=, in the TGF$\beta$R3 gene in a group consisting of 67 patients with hepatocellular carcinoma. In their study, rs1805110 (p.Ser15Phe) polymorphism was found to be present at a high frequency, i.e., in about 98.5% of examined cancer samples; however, the lack of case-control comparison made it impossible to confirm the association between rs1805110 (p.Ser15Phe) SNP occurrence and betaglycan down-regulation [17]. Similar results were presented by Xin et al. [45]. Among 16 different SNPs in
genes encoding components of TGFβ pathway, i.e., TGFβ1, TGFβR1/2 and betaglycan, significantly changed frequency of rs1805110 polymorphic site was found to be associated with incidence of HBV-related hepatocellular carcinoma (T allele, OR = 1.33; 95% CI = 1.09–1.63; p = 0.005) for male Chinese patients of Han ethnicity. According to the study by Kim et al. [43] rs1805113 (Phe676Phe) in exon 13 and rs1805117 in 3′-UTR (p = 0.009 and p = 0.008, respectively) polymorphisms were significantly associated with HBV clearance. In addition, Cox relative hazards analysis revealed that the GGTCAA haplotype of rs2306888, rs1805112, rs1805113, rs284878, rs1805117 and rs1804506 polymorphisms showed a significant association with the age of HCC occurrence among chronic HBV patients (relative hazard = 1.38; p = 0.007). In turn, the study presented by Charbonneau et al. [44] showed that in the case of mucinous invasive epithelial ovarian cancer (EOC), significantly altered distribution of polymorphisms rs12129174 (c.384 + 1320G > A) and rs4658265 (c.247 – 16378G > A), located in the introns of the TGFBR3 gene, was moderately correlated with patients’ survival (rs12129174—HR = 1.61; 95% CI = 1.18–2.19; p = 0.0038; rs4658265—HR = 1.56; 95% CI = 1.20–2.05; p = 0.0012).

Furthermore, besides above-mentioned relationship between SNPs of the TGFBR3 gene and neoplastic transformation, the importance of polymorphism in betaglycan encoding gene was reported for other non-cancerous diseases, i.e., premature ovarian failure (POF), testicular dysgenesis, sickle cell anemia, pulmonary emphysema and primary open angle glaucoma [46–51]. It is suggested that the TGFBR3 gene polymorphism may play a potential role in determining bone mineral density, as well as optic disc area parameters [52–54].

Interestingly, in our study, six out of eight analyzed SNPs of the TGFBR3 gene were found to have an impact on betaglycan expression in EC. Altered betaglycan expression may be responsible for impaired TGFβ signaling initiated by TGFβ isoforms and simultaneous redirection of this signal to Smad-independent pathways. TGFBR3 gene downregulation has been stated in the case of different cancers, and the observed decline in the expression of the TGFBR3 gene appears to be correlated with cancer progression, when tumor cells demonstrate an increase invasiveness and metastatic potential [17–23,25–28].

As previously described by different research groups, TGFβ signaling induced by TGFβ isoforms may be engaged in the induction of epithelial-mesenchymal transition (EMT). EMT is a biological process characterized by the reorganization of the epithelial tissue structure and is manifested by the acquisition of the mesenchymal phenotype resulting in the loss of polarity and adhesion by cells together with ability to migration and invasion. This process plays a vital role during physiological events, i.e., embryogenesis, organogenesis and morphogenesis of different tissues, wound healing, as well as inflammation. In cancer progression, EMT is responsible for the development of drug resistance and metastasis due to the increased cancer cell motility [55–57].

In summary, our study has demonstrated for the first time the role of the TGFBR3 gene polymorphism and its association with the increased risk of EC development. Moreover, we have shown that the TGFBR3 gene SNPs may modulate betaglycan expression at the transcriptomic level. Our findings contribute to a better understanding of the importance of gene polymorphism in the TGFβ signaling, especially at the level of signal initiation through TGFβ2 isoform mediated exclusively by betaglycan. Along with our previous findings concerning the significance of allelic loss in the TGFBR3 gene, where LOH was reported in 52% of examined cancer samples, SNPs may be an additional mechanism responsible for betaglycan deregulation in EC [28,58]. What is more, obtained results strongly support the view of individual variability among EC patients and suggest the necessity of developing personalized diagnostic and/or therapeutic approach in the treatment of endometrial cancer.

Supplementary Materials: Table S1: Characteristics and sequences of TaqMan probes used for genotyping of TGFBR3 gene; Table S2: The association between significantly altered SNPs, i.e., rs12566180, rs6680463 and rs2296621 polymorphisms and clinico-pathological parameters of studied cancer samples.

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