Recurrent Mutations in BRCA1, BRCA2, RAD51C, PALB2 and CHEK2 in Polish Patients with Ovarian Cancer

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Simple Summary: In Poland, ovarian cancer is the fourth leading cause of death from cancer among women. Several founder mutations in the BRCA1, BRCA2, PALB2, RAD51C, and CHEK2 genes are associated with breast and ovarian cancer. The aim of the study was to analyze the frequency and magnitude of association of 21 recurrent founder germline mutations in the above genes with ovarian cancer risk among unselected patients in Poland. The ovarian cancer risk was associated with mutations in BRCA1, BRCA2, RAD51C, and PALB2 but not in the CHEK2 gene. Excluding CHEK2, pathogenic mutations in the other 18 alleles were present in 12.5% of cases and 0.6% of healthy controls. A mutation was found in 25.8% of familial cases vs. 9.9% of non-familial cases. We recommend that in Poland all women with ovarian cancer and first-degree female relatives should be tested for the panel of 18 founder mutations in BRCA1, BRCA2, PALB2, and RAD51C.

Abstract: The aim of the study was to analyze the frequency and magnitude of association of 21 recurrent founder germline mutations in BRCA1, BRCA2, PALB2, RAD51C, and CHEK2 genes with ovarian cancer risk among unselected patients in Poland. We genotyped 21 recurrent germline mutations in BRCA1 (9 mutations), BRCA2 (4 mutations), RAD51C (3 mutations), PALB2 (2 mutations), and CHEK2 (3 mutations) among 2270 Polish ovarian cancer patients and 1743 healthy controls, and assessed the odds ratios (OR) for developing ovarian cancer for each gene. Mutations were detected in 369 out of...
2095 (17.6%) unselected ovarian cancer cases and 117 out of 1743 (6.7%) unaffected controls. The ovarian cancer risk was associated with mutations in BRCA1 (OR = 40.79, 95% CI: 18.67–114.78; \( p = 0.29 \times 10^{-15} \)), in BRCA2 (OR = 25.98; 95% CI: 1.55–434.8; \( p = 0.001 \)), in RAD51C (OR = 6.28; 95% CI 1.77–39.9; \( p = 0.02 \)), and in PALB2 (OR 3.34; 95% CI: 1.06–14.68; \( p = 0.06 \)). There was no association found for CHEK2. We found that pathogenic mutations in BRCA1, BRCA2, RAD51C or PALB2 are responsible for 12.5% of unselected cases of ovarian cancer. We recommend that all women with ovarian cancer in Poland and first-degree female relatives should be tested for this panel of 18 mutations.

Keywords: ovarian cancer; recurrent mutations; BRCA1; BRCA2; RAD51C; PALB2; CHEK2; cancer risk

1. Introduction

In Poland, ovarian cancer is the second most common gynecological cancer and the fourth leading cause of death from cancer among women, with 1.5% as the average lifetime risk for developing ovarian cancer [1,2]. In 2017, there were 3775 new cases diagnosed and 2670 reported deaths [3]. Several factors have been shown to reduce the risk of ovarian cancer, including oral contraceptive use [4], especially observed in the last few decades [5], high parity [6], and breast-feeding [7]. Ovarian cancer often presents at a late stage (70% in FIGO stage III or IV) [8] due to the lack of effective screening programs and because early-stage disease is typically asymptomatic [9].

A family history of ovarian cancer is a strong risk factor and several predisposing genes have been identified [10]. Approximately one-fifth of ovarian cancers have been associated with a mutation in one of these genes. The most important genetic causes of ovarian cancer are mutations in BRCA1 and BRCA2 genes, which account for up to 15% of all cases [11–14]. BRCA1 and BRCA2 encode proteins involved in recombinational repair of damaged DNA [11] and it has been found that mutations in other genes involved in the repair of DNA damage by homologous recombination, including e.g., RAD51C, RAD51D, BRIP1, PALB2, and CHEK2 may be associated with ovarian cancer risk [15–21].

In Poland, founder mutations in BRCA1, BRCA2, PALB2, CHEK2, and RAD51C have been associated with familial breast and ovarian cancer. Founder mutations in BRCA1 (nine alleles) and BRCA2 (four alleles) are frequent among Polish breast and ovarian cancer patients [12–14,22–24]. The other known founder mutations were identified in PALB2 (two alleles), CHEK2 (four allele) and recently RAD51C (three alleles, data not published) genes [15,18,21,25–27]. It has been shown that population-based genetic testing in defined populations, independent of personal or family history of cancer may be a useful approach for determining cancer susceptibility [28]. Founder mutations can be detected rapidly and at relatively low cost in specific ethnic-groups. Until now in Poland, this approach has been applied for testing of founder BRCA1/2, PALB2, and CHEK2 mutations in association with breast cancer risk [23,24,29,30]. The prevalence and association of the founder mutations with ovarian cancer risk in the Polish population was limited to analysis of BRCA1/2 in relatively small groups of cases [12–14,25,31], and was not performed for other genes.

The aim of the study was to analyze the prevalence and association of recurrent founder germline mutations in BRCA1, BRCA2, RAD51C, PALB2, and CHEK2 genes with ovarian cancer risk among unselected patients in the Polish population. In addition, we estimated the hazard ratio for all-cause survival among mutation carriers in comparison to non-carriers.

2. Results
2.1. Association of BRCA1, BRCA2, RAD51C, PALB2, and CHEK2 Mutations with Ovarian Cancer Risk

Genotyping of 21 mutations in BRCA1, BRCA2, RAD51C, PALB2, and CHEK2 genes was performed for all 2270 ovarian cancer patients and 1743 healthy controls. For 175 ovarian
cancer patients, genotyping was incomplete and therefore these cases were excluded, leaving finally 2095 ovarian cancer patients in further analyses.

The highest frequency of tested mutations in ovarian cancer patients was detected for the \( BRCA1 \) gene. One of 9 mutations in \( BRCA1 \) was detected in 220 out of 2095 (10.5%) ovarian cancer cases and in 5 out of 1743 controls (0.29%), and was associated with odds ratio 40.8 (95% CI: 18.7–114.8; \( p = 0.29 \times 10^{-15} \)). One of 4 tested \( BRCA2 \) mutations was found in 15 out of 2095 (0.72%) unselected ovarian cancer patients but in none of 1743 controls, and was associated with odds ratio 26.0 (95% CI: 1.55–434.8; \( p = 0.001 \)).

One of 3 mutations in \( RAD51C \) was seen in 15 out of 2095 (0.72%) ovarian cancer cases and in 2 out of 1743 controls (0.11%) (OR = 6.28; 95% CI 1.77–39.9; \( p = 0.02 \)).

One of 2 mutations in \( PALB2 \) was detected in 12 out of 2095 (0.57%) cases and in 3 of 1743 controls (0.17%) (OR = 3.34; 95% CI 1.06–14.68; \( p = 0.06 \)).

The three mutations in the \( CHEK2 \) gene were analyzed separately with respect to the consequences for proteins. Two protein truncating mutations (c.1100delC, c.444+1G>A) were detected in 17 cases (0.81%) and 10 controls (0.57%). The missense mutation (c.470T>C) was found in 90 cases (4.3%) and 97 controls (5.57%). Neither of these differences was statistically significant.

The distributions of the tested mutations in \( BRCA1 \), \( BRCA2 \), \( PALB2 \), and \( RAD51C \) genes and odds ratios (ORs) for risk of ovarian cancer are shown in Table 1.

### Table 1

| Mutation | Cases, n = 2095 (%) | Controls, n = 1743 (%) |
|----------|---------------------|-----------------------|
| **BRCA1** |                     |                       |
| 5382insC (c.5266dupC) | 124 (5.92) | 3 (0.17) |
| 300T>G (c.181T>G) | 48 (2.29) | 1 (0.06) |
| 4153delA (c.4035delA) | 10 (0.48) | 0 (0.00) |
| 1806C>T (c.1687C>T) | 6 (0.29) | 0 (0.00) |
| 185delAG (c.68_69delAG) | 8 (0.38) | 0 (0.00) |
| 3819del5 (c.3700_3704del5) | 14 (0.69) | 1 (0.06) |
| 3875del4 (c.3756_3759delGTCT) | 3 (0.14) | 0 (0.00) |
| 530C>T (c.5251C>T) | 1 (0.05) | 0 (0.00) |
| 794delT (c.794_795delCT) | 6 (0.29) | 0 (0.00) |
| Any BRCA1 mutation | 220 (10.50) | 5 (0.29) |
| OR 40.79 (95% CI: 18.67–114.78), \( p = 0.29 \times 10^{-15} \) | | |
| **BRCA2** |                     |                       |
| 4075delGT (c.3847_3848delGT) | 3 (0.14) | 0 (0.00) |
| 8138del5 (c.7913_7917delTTCCT) | 3 (0.14) | 0 (0.00) |
| 886delGT (c.658_659del) | 4 (0.19) | 0 (0.00) |
| 6174delT (c.5946delCT) | 5 (0.24) | 0 (0.00) |
| Any BRCA2 mutation | 15 (0.72) | 0 (0.00) |
| OR 25.98 (95% CI: 1.55–434.8), \( p = 0.001 \) | | |
| **RAD51C** |                     |                       |
| c.905-2_905-1delAG | 2 (0.10) | 2 (0.11) |
| c.577C>T | 10 (0.48) | 0 (0.00) |
| c.502A>T | 3 (0.14) | 0 (0.00) |
| Any RAD51C mutation | 15 (0.72) | 2 (0.11) |
| OR 6.28 (95% CI: 1.77–39.87), \( p = 0.02 \) | | |
| **PALB2** |                     |                       |
| c.172_175delTTGT | 4 (0.19) | 2 (0.11) |
| c.509_510delIGA | 8 (0.38) | 1 (0.06) |
| Any PALB2 mutation | 12 (0.57) | 3 (0.17) |
| OR 3.34 (95% CI: 1.06–14.68), \( p = 0.06 \) | | |
| **CHEK2** |                     |                       |
| c.1100delC | 6 (0.29) | 4 (0.23) |
| c.444+1G>A | 11 (0.53) | 6 (0.34) |
| Both protein truncating CHEK2 mutation | 17 (0.81) | 10 (0.57) |
| OR 1.42 (95% CI: 0.66–3.22), \( p = 0.38 \) | | |
| missense mutation, c.470T>C | 90 (4.30) | 97 (5.57) |
| OR 0.76 (95% CI: 0.57–1.02), \( p = 0.07 \) | |
Excluding CHEK2, a pathogenic mutation was present in 262 of 2095 cases of ovarian cancer (12.5%). A mutation was found in 25.8% of familial cases and in 9.9% of non-familial cases. The mutations were detected in 103 (39.3%) of women diagnosed under age 50, in 102 (38.9%) of women diagnosed between age 50 and 60, and in 57 (21.8%) of women diagnosed above the age of 60. In regard to the histopathology of ovarian cancer, the mutations were found in 14.0% of serous cases, 5.2% of endometrioid cases, 3.6% of mucinous, 1.6% of clear cell cases, and 6.0% of cases with other or unspecified histology.

An analysis of the presence of mutations in BRCA1, BRCA2, RAD51C, PALB2, and CHEK2 with the age of onset of ovarian cancer revealed a lower age of ovarian cancer diagnosis in BRCA1 mutation carriers than in non-carriers of BRCA1 (51.6 vs. 57.6, \( p = 2.97 \times 10^{-11} \)). This was not found in carriers of mutations in BRCA2, RAD51C, PALB2 or CHEK2. There was no statistically significant association of mutations with age of onset among patients with a positive family history of ovarian cancer.

### 2.2. All-Cause Survival

Information on all-cause survival was available from 1924 cases and 1084 deaths were recorded (56.3%). The 5-year survival for all patients was 47.2% and the 10-year survival was 26.1%. The 5- and 10-year survival of mutation carriers, except of CHEK2 protein truncating, was in general longer than for non-carriers (Table 2). Among carriers of CHEK2 protein truncating mutations, 50% of patients survived 5 years from ovarian cancer diagnosis, but all died within 10 years.

| Survival | 5-Year Survival Rate (%) | 10-Year Survival Rate (%) |
|----------|--------------------------|---------------------------|
| BRCA1    | 50.53%                   | 27.04%                    |
| BRCA2    | 54.55%                   | 40.00%                    |
| RAD51C   | 58.33%                   | 40.00%                    |
| PALB2    | 54.55%                   | 33.33%                    |
| CHEK2, c.1100delC and c.444+1G>A | 50.00% | *              |
| CHEK2, c.470T>C | 54.17% | 34.48% | |
| Non-carriers | 46.82% | 25.71% | |

* All carriers died.

The estimated hazard ratio for all-cause survival among mutation carriers was not statistically significant for any of the tested genes (Table 3).

### 2.3. Discussion

The goal of our study was to estimate the prevalence of 21 founder/recurrent germline mutations among unselected ovarian cancer patients in Poland, and to assess the odds ratios (ORs) for developing ovarian cancer. Mutations were detected in 369 out of 2095 (17.6%) unselected ovarian cancer cases and 117 out of 1743 (6.7%) unaffected controls. BRCA1 mutations were much more frequent than BRCA2 mutations (10.5% versus 0.72%, respectively). These mutations were associated with odds ratios of 40.79 (95% CI: 18.67–114.78; \( p = 0.29 \times 10^{-15} \)) and 25.98 (95% CI: 1.55–434.8; \( p = 0.001 \), for BRCA1 and
BRCA2, respectively. Previous studies of Polish ovarian cancer patients were much smaller, ranging from 134 to 364 cases [12–14,25,31]. Full sequencing of all exons of BRCA1 and BRCA2 suggests that the founder/recurrent mutations tested in the current study constitute about 65% of all mutations in Poland [14,25]. The results of our study are concordant with previously reported analyses which show a high prevalence of BRCA1/2 mutations among ovarian cancer patients with dominant founder/recurrent population-specific mutations located in BRCA1.

Mutations in RAD51C, PALB2, and CHEK2 genes were previously analyzed in ovarian cancers [15,19,21,26]. In our study we show and confirm that in Poland mutations in RAD51C and PALB2 are associated with ovarian cancer risk.

Mutations in the RAD51C gene were reported to predispose to ovarian cancer, as well as to breast cancer but only in families with ovarian cancer cases [15–17,25,32,33]. In the meta-analysis encompassing approximately 24,000 ovarian cancer patients from 53 studies and more than 100,000 controls, mutations in RAD51C were associated with a 5-fold elevated risk of ovarian cancer (OR = 5.59; 95%CI: 4.42–7.07; p < 0.0001) [16]. In our study one of three tested recurrent RAD51C germline mutations was detected in 15 out of 2095 (0.72%) unselected ovarian cancer patients giving the odds ratio of 6.28 (95% CI: 1.77–39.87; p = 0.02).

Germline mutations in PALB2 were reported to correlate with an increased risk of ovarian cancer [18,19,34,35]. In Poland there is little data about association of PALB2 mutations with ovarian cancer. In 2 studies of 333 unselected and 339 unrelated ovarian cancer cases, the PALB2 mutations were detected in 0.6% of patients [25,26]. In our analysis recurrent PALB2 mutations were detected in 12 out of 2095 (0.57%) unselected ovarian cancer cases giving the odds ratio of 3.34, but the difference was of borderline statistical significance (95% CI: 1.06–14.6; p = 0.06). This is concordant with results of analyses performed on other populations which showed 3–4 times higher frequency of PALB2 mutations in ovarian cancer patients than in controls [18,19,34,35]. Comparison of prevalence of PALB2 mutations between studies suggests that recurrent mutations are more common in Poland than in other populations.

CHEK2 mutations are known to increase the risk of prostate and breast cancer [29,36–38]. With regards to ovarian cancer, the results are inconsistent [21,25,27,37,39]. In our study there was no significant association of tested CHEK2 mutations, including 2 protein truncation and 1 missense mutation, with ovarian cancer risk.

In our analysis one of the BRCA1, BRCA2, RAD51C, and PALB2 mutations was found in 25.8% of familial cancer cases and in 9.9% of non-familial cases, similar to what has been reported previously [18,33,40,41].

We did not have access to specific causes of death and we estimated the hazard ratios for all-cause survival among carriers of mutations in BRCA1, BRCA2, RAD51C, PALB2, and CHEK2 genes in comparison with women who carried no mutation. There was no significant association of mutation status in any of the tested genes with overall all-cause survival among carriers at 5 or 10 years. Several studies, including a large meta-analysis encompassing almost 8000 ovarian cancer cases, have reported that BRCA1 and BRCA2 carriers have better survival than non-carriers [13,42–46], but the survival advantage diminishes with time from diagnosis.

We show significant correlation of mutations in BRCA1, BRCA2, and RAD51C with ovarian cancer risk. The association was also detected for PALB2 mutations, but it was of borderline statistical significance. It will require analyses on larger sample sizes to provide precise estimates for PALB2 for clinical counselling [18,19,34].

The results of this study based on analysis of selected founder mutations in ovarian cancer patients may have important implications for the population. Panel testing offers a cheaper alternative to methods based on whole gene DNA-sequencing and therefore may be applicable to testing of the female population at large. Testing women for the panel of population-specific recurrent/founder mutations may be a valuable approach for therapy decisions in ovarian cancer patients (i.e., platinum or PARP inhibitors in BRCA1/2 carriers).
or for preventing hereditary ovarian cancer. Women who are found to carry a mutation in one of these genes would be candidates for preventive salpingo-oophorectomy which has been shown to be an effective means of cancer control.

4. Materials and Methods

4.1. Study Groups

The study group consisted of 2270 unselected ovarian cancer patients diagnosed between 1998 and 2019 at the International Hereditary Cancer Center (IHCC) in Szczecin and elsewhere in Poland (Kraków, Rzeszów, Opole, Zielona Góra, Poznań). The clinical information included age at diagnosis, tumor histology, and family history of breast and ovarian cancers. Data on vital status were collected from the Ministry of Digital Affairs in July 2019. The characteristics of ovarian cancer patients are presented in Table 4.

Table 4. Characteristics of ovarian cancer patients.

| Feature                              | n = 2270 |
|--------------------------------------|----------|
| Age of diagnosis (mean, range)       | 57 (19–87) |
| Histological type of cancer          |          |
| Serous                               | 1133 (49.9%) |
| Clear-cell                           | 70 (3.1%) |
| Endometrioid                         | 286 (12.6%) |
| Mucinous                             | 147 (6.5%) |
| Other or undefined/mixed             | 38 (1.7%) |
| Unknown                              | 596 (26.3%) |
| Family history of ovarian cancer     |          |
| Yes                                  | 334 (14.7%) |
| No                                   | 1633 (71.9%) |
| Unknown                              | 303 (13.3%) |
| Family history of BC                 |          |
| Yes                                  | 372 (16.4%) |
| No                                   | 1615 (71.1%) |
| Unknown                              | 283 (12.5%) |
| Mean follow up in years (range)      | 10.1 (0.1–21.0) |
| Death (all causes)                   |          |
| Yes                                  | 1179 (51.9%) |
| No                                   | 895 (39.4%) |
| Unknown                              | 196 (8.6%) |

The control group consisted of 1743 healthy women selected from the registry of the International Hereditary Cancer Center in Szczecin. They were recruited between 2002–2019 as a part of the population-based study of the approximately 2 million inhabitants of the West Pomerania region in Poland, designed to identify familial aggregations of cancer syndromes, or they independently came to the International Hereditary Cancer Center for genetic counseling. For all individuals we collected demographic and clinical data as well as information about family history of cancer. The mean age of controls at the time of recruitment was 55.8 years (range 18–90 years).

Healthy controls were followed since time of recruitment, and the mean follow up was 26.1 months (0–213 months).

4.2. Sample Preparation and Molecular Analyses

A blood sample was obtained from all cases and controls and DNA was isolated using a previously described method [47]. DNA samples were stored at 4 °C prior to genotyping. The analysis encompassed genotyping of 21 selected recurrent/founder germline mutations localized in five genes: BRCA1 (9 mutations), BRCA2 (4 mutations), RAD51C (3 mutations), PALB2 (2 mutations), and CHEK2 (3 mutations) (Table 5).

All mutations, with the exception of c.5266dupC in BRCA1, were genotyped by Real-Time PCR using customized TaqMan Assays on LightCycler Real-Time PCR 480 System
(Real-Time PCR System, Roche Diagnostics, Indianapolis, IN, USA) following the standard protocol. The reaction mix for analysis of each sample included GoTaq® Probe qPCR Master Mix (Promega, Madison, WI, USA), TaqMan Genotyping Assays × 40 or TaqMan Genotyping Assays × 80 (Applied Biosystems, Foster City, CA, USA), and deionized water (Promega, Madison, WI, USA). Samples were analyzed on 384-well plates. On each analyzed plate were included 3 control samples: positive and negative for tested mutations, and a water-blind control. The genotyping was performed using LightCycler 480 Instrument and data were analyzed using LightCycler 480 Basic Software Version 1.5 (Roche Diagnostics, Indianapolis, IN, USA). Analysis for c.5266dupC mutation in BRCA1 was performed using allele-specific amplification PCR (ASA-PCR) as described previously [33]. The sequences of primers and probes used for genotyping are available on request.

All study participants provided written informed consent. The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin.

Table 5. Recurrent mutations tested in BRCA1, BRCA2, RAD51C, PALB2, and CHEK2 genes.

| Gene     | cDNA                                                                 | Protein Change        | Molecular Consequence |
|----------|----------------------------------------------------------------------|-----------------------|-----------------------|
| BRCA1    | 5382insC (c.5266dupC)                                               | p.Gln1756fs           | frameshift            |
|          | 300'T>G (c.181T>G)                                                   | p.Cys61Gly            | missense              |
|          | 4153delA (c.4035delA)                                               | p.Glu1346fs           | frameshift            |
|          | 1806C>T (c.1687C>T)                                                 | p.Gln563Ter           | nonsense              |
|          | 185delAG (c.68_69delAG)                                             | p.Glu23fs             | frameshift            |
|          | 3819del5 (c.3700_3704del5)                                          | p.Val1234fs           | frameshift            |
|          | 3875del4 (c.3756_3759delGTCT)                                       | p.Ser1253fs           | frameshift            |
|          | 5370C>T (c.5251C>T)                                                 | p.Arg1751Ter          | nonsense              |
|          | 794delT (c.794_795delCT)                                            | p.Ser265Cysfs         | frameshift            |
| BRCA2    | 4075delGT (c.3847_3848delGT)                                         | p.Val1283fs           | frameshift            |
|          | 8138del5 (c.7913_7917delTTCCT)                                       | p.Ala2637_Phe2638insTer| frameshift            |
|          | 886delGT (c.658_659del)                                             | p.Val220fs            | frameshift            |
|          | 6174delT (c.5946delT)                                               | p.Ser1982fs           | frameshift            |
| RAD51C   | c.905-2_905-1delAG                                                   | p.Glu303TrpfsX41      | skipping of exon 7    |
|          | c.57C>T                                                             | p.Arg193Ter           | nonsense              |
|          | c.502A>T                                                            | p.Arg168Ter           | nonsense              |
| PALB2    | c.172_175delTTGT                                                   | p.Gln60fs             | frameshift            |
|          | c.509_510delGA                                                     | p.Arg170fs            | frameshift            |
| CHEK2    | c.1100delC                                                          | p.Thr367fs            | frameshift            |
|          | c.444+1G>A                                                          | p.E149fsX6            | skipping of exon 2    |
|          | c.470T>C                                                            | p.Ile157Thr           | missense              |

4.3. Statistical Analysis

To analyze the association of tested mutation with ovarian cancer risk, odds ratios were estimated separately for each gene, BRCA1, BRCA2, RAD51C, PALB2, and CHEK2. The odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) were calculated using a logistic regression model.

In addition, the Mann–Whitney or T-test was used for comparison of the age of ovarian cancer onset between patients with and without mutation. This analysis was performed separately in patients with positive and negative family history of ovarian cancer.

To estimate overall survival, all ovarian cancer patients were followed from the date of diagnosis until death from any cause. Analysis was performed for each gene separately (BRCA1, BRCA2, RAD51C, PALB2, and CHEK2) using Cox proportional hazards. In addition, the 5- and 10-year survival among the BRCA1, BRCA2, RAD51C, PALB2, and CHEK2 mutation carriers was calculated.

All analyses were done in R Project for Statistical Computing (version 3.5.2) and p-values less than 0.05 were recognized as statistically significant.
5. Conclusions

We found that a pathogenic mutation in BRCA1, BRCA2, RAD51C or PALB2 was found in 12.51% of unselected cases of ovarian cancer in Poland. We recommend that all women with ovarian cancer in Poland and first-degree female relatives be tested for this panel of 18 mutations.

Author Contributions: Conceptualization, A.J.; methodology, A.L., P.B. (Piotr Baszuk), and A.J.; software, A.L. and P.B. (Piotr Baszuk); validation, A.L.; formal analysis, A.L., P.B. (Piotr Baszuk), and A.J.; investigation, A.L., K.B., K.P., and W.K.; resources, J.M., J.G., J.T.-S., M.S., M.J., P.B. (Paweł Blecharz), T.K., M.S.-N., R.M., C.C., T.D., T.H., A.T.-G., T.B., J.L., and A.J.; data curation, A.L. and A.J.; writing—Original draft preparation, A.L. and A.J.; writing—Review and editing, A.L., J.M., J.G., J.T.-S., M.S., M.J., P.B. (Paweł Blecharz), T.K., M.S.-N., R.M., K.B., K.P., W.K., C.C., T.D., T.H., A.T.-G., T.B., P.B. (Piotr Baszuk), S.A.N., J.L., and A.J.; visualization, A.L., S.A.N. and A.J.; supervision, A.J.; project administration, A.L. and A.J.; funding acquisition, A.L., J.L. and A.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of Science and Higher Education “Regional Initiative of Excellence” in years 2019–2022, Grant No 002/RID/2018/19.

Institutional Review Board Statement: The study was approved by the Ethics Committee of Pomeranian Medical University in Szczecin (protocol code KB-0012/153/16 (BN-001/33/04), 20 December 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We would like to thank Nina Peruga for helping with data collection and molecular analyses.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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