Association of FGFR2 rs2981582, SIRT1 rs12778366, STAT3 rs744166 gene polymorphisms with pituitary adenoma

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Received August 22, 2016; Accepted January 13, 2017

DOI: 10.3892/ol.2017.5840

Abstract. The aim of the present study was to determine the association between sirtuin 1 (SIRT1), fibroblast growth factor receptor 2 (FGFR2) and signal transducer and activator of transcription 3 (STAT3) polymorphisms, and pituitary adenoma (PA) development, invasiveness, hormonal activity and recurrence. The present study included 143 patients with a diagnosis of PA. The reference group involved 808 healthy subjects. The genotyping of SIRT1 rs12778366, FGFR2 rs2981582 and STAT3 rs744166 was performed using the quantitative polymerase chain reaction method. The SIRT1 rs12778366 polymorphism analysis in the overall group revealed differences in the genotype distribution between patients with PA and control group subjects. The rs12778366 T/C genotype was observed to be different in non-invasive, non-recurrent and inactive PA subgroups compared with the control group, while the C/C genotype was observed to be different in invasive, recurrent and active PA subgroups compared with the control group. STAT3 rs744166 polymorphism analysis in the overall group revealed differences in the genotype distribution between patients with PA and the control groups. The rs744166 G/G genotype was observed to be different in invasive, non-recurrent and active PA subgroups compared with the control group, while the rs744166 A/A genotype was observed to be different in the active PA subgroup compared with the control group, and was also different in terms of invasiveness and recurrence in PA subgroups. The present study demonstrated that SIRT1 rs12778366 is associated with pituitary adenoma development while STAT3 rs744166 is associated with PA invasiveness, hormonal activity and recurrence.

Introduction

Pituitary adenomas (PAs), located in a bone cavity termed the sella turcica, are one of the most common types of intracranial neoplasms, with reported estimated prevalence rates ranging between 14.4 and 22.5% in pooled autopsy and radiological series, respectively (1). Although the majority of PAs are benign, it is not uncommon for them to grow large and extend locally into the surrounding structures, invading the sphenoid bone inferiorly, the cavernous sinus laterally (2-7.8) and/or compressing the optic chiasm, if the direction of expansion is suprasellar, thus resulting in neurological complications, including headache and visual impairment (9-17). Certain types of PA are extremely invasive and may cause extensive destruction of the skull base (18). Investigation of tumour invasiveness is required, as this affects the management and prognosis of PA (19). The aim of the present study was to identify possible genes involved in PA tumourigenesis, which may serve as potential diagnostic and prognostic molecular markers. The present study selected 3 genes, sirtuin 1 (SIRT1), fibroblast growth factor receptor 2 (FGFR2) and signal transducer and activator of transcription 3 (STAT3), which are associated with different types of cancer, but are connected in pathogenic processes (20-23).

SIRT1 is a nicotinamide adenine dinucleotide-dependent histone deacetylase (HDAC) (24), which serves an important role in maintaining the balance between cell death and survival through targeting the Ku70-B-cell lymphoma-like protein 4 pathway (25), p53 (26,27) and forkhead box O3 (28), among others. A significant increase in the level of SIRT1 in hepatocellular carcinoma (29), breast cancer (30), prostate cancer (31), ovarian cancer (32), gastric cancer (33), colon cancer (34), glioblastoma (35) and lymphoma (36) was previously suggested to be associated with the development and invasion of these tumours. Furthermore, the rs12778366 polymorphism of the SIRT1 gene was found to be associated with breast cancer (37).

FGFR2 is a member of the FGFR family of tyrosine kinase receptors and participates in the process of tumourigenesis by inducing mitogenic and survival signals, and promoting invasiveness and angiogenesis (38). If cancer cells overexpress an FGFR with altered ligand-binding specificity, FGFs, secreted from neighbouring cells, stimulate the cancer cells, creating a paracrine loop (38). FGFR2 was previously revealed to be

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Key words: pituitary adenoma, gene polymorphism, fibroblast growth factor receptor 2 rs2981582, sirtuin 1 rs12778366, signal transducer and activator of transcription 3 rs744166
overexpressed in bladder (39) and lung cancer (40). Additionally, the importance of the FGF2 rs2981582 gene polymorphism was investigated in breast (41–50) and prostate cancer (51).

STAT3 is activated in tumour cells and numerous immune cells of the tumour microenvironment, and is associated with tumour cell proliferation, invasion and angiogenesis (52–54). The effect of STAT3 was previously studied in the tumour development of colorectal adenocarcinoma (55), hepatocellular carcinoma (56), multiple myeloma (57), glioblastoma (58), prostate cancer (59), and head and neck cancer (60). The STAT3 rs744166 polymorphism was also evaluated in gastric (61,62), colon (63) and lung cancer (64).

These findings support the hypothesised role of SIRT1, FGF2 and STAT3 as tumour promoters. However, an association between SIRT1, FGF2 and STAT3 polymorphisms, and PA development, invasiveness, PA activity and recurrence has not yet been reported. The aim of the present study was to determine these associations.

Materials and methods

Patients and selection. Permission to undertake the present study was obtained from the Biomedical Research Ethics Committee of Lithuanian Health Sciences University (Kaunas, Lithuania). The study was conducted in the Departments of Ophthalmology and Neurosurgery, Lithuanian Health Sciences University Hospital (Kaunas, Lithuania).

The participants comprised of 143 patients with a diagnosis of PA. The reference group involved 808 healthy subjects. The reference group was created by taking into consideration the distribution of age and gender in the PA group. Therefore, the median patient age of the control group and the PA group did not differ significantly (P<0.05). Demographic data of the study subjects are presented in Table I.

The inclusion criteria were as follows: Determined and confirmed PA via magnetic resonance imaging (MRI); general good condition of the patient; consent of the patient to take part in the study; age ≥18 years; and no other brain tumours or tumours with other localizations.

All PAs were analysed based on MRI findings. The pre-operative MRI investigations were performed with 1.5T MRI scanners (Siemens MAGNETOM Avanto: Siemens AG, Munich, Germany; 1.5 T Philips ACHIEVA: Philips Healthcare, DA Best, The Netherlands) using a head coil and a standard pituitary scanning protocol, obtaining T1-weighted (T1W) sagittal and coronal and T2W/turbo spin echo coronal pre-contrast images, and T1W coronal and sagittal gadolinium-enhanced MR images with the intravenous agent gadodiamide (Omnisncc; GE Healthcare Life Sciences, Chalfont, UK). The retrospective analysis of MRI data was conducted by an experienced radiologist. The suprasellar extension and sphenoid sinus invasion by PAs were classified according to Wilson-Hardy classification (Hardy classification, modified by Wilson) (19). The degree of suprasellar and parasellar extension was graded as stages A-E. The degree of sellar floor erosion was graded between I and IV. Grade III, localized sellar destruction, and grade IV, diffuse destruction, were considered to be invasive PAs. The Knosp classification system (4) was used to quantify invasion of the cavernous sinus, in which only grades 3 and 4 define true invasion of the tumour into the cavernous sinus: Grade 0, no cavernous sinus involvement; grades 1 and 2, the tumour pushes into the medial wall of the cavernous sinus, but does not go beyond a hypothetical line extending between the centres of the two segments of the internal carotid artery (grade 1) or it goes beyond such a line, but without passing a line tangential to the lateral margins of the artery itself (grade 2); grade 3, the tumour extends laterally to the internal carotid artery within the cavernous sinus; and grade 4, total encasement of the intracavernous carotid artery.

DNA extraction and genotyping. The DNA extraction and analysis of the gene polymorphisms of SIRT1 rs12778366, FGF2 rs2981582 and STAT3 rs744166 were performed at the Laboratory of Ophthalmology at the Institute of Neuroscience of the Lithuanian University of Health Sciences (Kaunas, Lithuania). DNA was extracted from 200 μl venous blood (white blood cells) using a DNA purification kit based on the magnetic beads method (MagJET Genomic DNA kit; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to manufacturer's instructions.

The genotyping of SIRT1 rs12778366, FGF2 rs2981582 and STAT3 rs744166 was performed using the quantitative polymerase chain reaction (qPCR) method with a Rotor-Gene Q Real-Time PCR Quantification system (Qiagen, Inc., Valencia, CA, USA). All 3 single-nucleotide polymorphisms were determined using TaqMan® Genotyping assays (Applied Biosystems; Thermo Fisher Scientific, Inc.), C_1340370_10 (rs12778366), C_2917302_10 (rs2981582) and C_3140282_10 (rs744166), according to the manufacturer's protocols.

The Allelic Discrimination program (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used during the qPCR. The assay was then continued following the manufacturer protocols. The Allelic Discrimination program was completed, and the genotyping results were received. The program determined the individual genotypes according to the fluorescence intensity rate from different detectors: Molecular marker labeled with VIC fluorescent dye was chosen for the X axis and a molecular marker labeled with FAM fluorescent dye was selected for the Y axis. These dy-labeled probes were included in the TaqMan® Genotyping assays.

Statistical analysis. Statistical analysis was performed using SPSS 20.0 software (IBM SPSS, Armonk, NY, USA).

Table I. Demographic characteristics of patients with PA and reference group subjects.

| Group      | n  | Min/max/median age, years | Females, n (%) |
|------------|----|-------------------------|---------------|
| PA         | 143| 19/87/52.5              | 88 (65.67)    |
| Control    | 808| 20/90/58                | 510 (63.12)   |
| P-value    | -  | 0.793*                  | 0.882         |

*P-value for comparison of the median age between the PA and control groups. PA, pituitary adenoma; min, minimum; max, maximum.
The data are presented as absolute numbers with percentages in brackets, and as median with minimum/maximum values. The frequencies of genotypes are presented as percentages.

Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of rs1277836, rs2981582 and rs744166 using the $\chi^2$ test in all groups. The distribution of analysed SIRT1 genotypes and allele frequencies in the control and PA groups did not match the Hardy-Weinberg equilibrium. The genotype T/C was significantly less frequent (P<0.001) in the PA group compared with the control group. C/C genotype was significantly more frequent (P<0.001) in the PA group compared with the control group. STAT3 rs744166 G/G genotype was significantly less frequent (P=0.003) in the PA group compared with the control group. PA, pituitary adenoma; SIRT1, sirtuin 1; FGFR2, fibroblast growth factor receptor 2; STAT3, signal transducer and activator of transcription 3; HWE, Hardy-Weinberg equilibrium.

### Results

**Genotype distribution in the PA patients and the control group.**

The genotyping of SIRT1 rs1277836, FGFR2 rs2981582 and STAT3 rs744166 was performed in the PA group and the control group subjects (Table II).

The distribution of analysed SIRT1 genotypes and allele frequencies in the control and PA groups did not match the Hardy-Weinberg equilibrium. The SIRT1 rs1277836 polymorphism analysis in the overall group revealed differences in the genotype distribution between patients with PA and control group subjects (P<0.001). The genotype T/C was significantly less frequent in the PA group compared with the healthy controls (P<0.001) and the genotype C/C was significantly more frequent in the PA group compared with the healthy control group (18.9 vs. 2.5%, respectively; P<0.001) (Table II).

The distribution of analysed FGFR2 genotypes and allele frequencies in the control and PA groups did not match the
Hardy-Weinberg equilibrium. Statistical analysis did not reveal significant genotype (G/G, G/A and A/A) distribution differences between the control and PA groups: 41.6 vs. 39.2%, 53.1 vs. 58.7%, and 5.3 vs. 2.1%, respectively (P=0.174) (Table II).

The distribution of the analysed STAT3 rs744166 genotypes and allele frequencies did not match the Hardy-Weinberg equilibrium in the control group, but it did in the group of patients with PA. STAT3 rs744166 polymorphism analysis in the overall group revealed differences in the genotype distribution between the patients with PA and the control group (P=0.012). The genotype G/G was less frequent in the PA group compared with the healthy controls (9.1% vs. 19.1%, respectively; P=0.003) (Table II).

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**Table III. Frequency of single nucleotide polymorphisms in patients with PA and control group according to gender.**

| Gene marker | Control group, n (%) | PA group, n (%) |
|-------------|----------------------|----------------|
|             | Females | Males | P-value | Females | Males | P-value |
|             | HWE     |        |         | HWE     |        |         |
| **SIRT1 rs12778366** | | | | | | |
| Genotype    |         |         |         |         |         |         |
| T/T         | 407 (79.8) | 240 (80.5) | 0.811 | 71 (80.7) | 45 (81.8) | 0.866 |
| T/C         | 89 (17.5) | 52 (17.4) | 1.000 | 0 (0.0) | 0 (0.0) | 1.00 |
| C/C         | 14 (2.7) | 6 (2.0) | 0.518 | 17 (19.3) | 10 (18.2) | 0.866 |
| Total       | 510 (100.0) | 298 (100.0) | | 88 (100.0) | 55 (100.0) | |
| Allele      |         |         |         |         |         |         |
| T           | 903 (88.5) | 532 (89.3) | | 142 (80.7) | 90 (81.8) | |
| C           | 117 (11.5) | 64 (10.7) | | 34 (19.3) | 20 (18.2) | |
| **FGFR2 rs2981582** | | | | | | |
| Genotype    |         |         |         |         |         |         |
| G/G         | 218 (42.7) | 118 (39.6) | 0.675 | 39 (44.3) | 17 (30.9) | 0.197 |
| G/A         | 265 (52.0) | 164 (55.0) | 0.398 | 48 (54.5) | 36 (65.5) | 0.224 |
| A/A         | 27 (5.3) | 16 (5.4) | 1.0 | 1 (1.1) | 2 (3.6) | 0.559 |
| Total       | 510 (100) | 298 (100) | | 88 (100) | 55 (100) | |
| Allele      |         |         |         |         |         |         |
| G           | 701 (68.7) | 400 (67.1) | | 126 (71.6) | 70 (63.6) | |
| A           | 319 (31.3) | 196 (32.9) | | 50 (28.4) | 40 (36.4) | |
| **STAT3 rs744166** | | | | | | |
| Genotype    |         |         |         |         |         |         |
| G/G         | 104 (20.4) | 50 (16.8) | 0.378 | 7 (8.0) | 6 (10.9) | 0.815 |
| G/A         | 229 (44.9) | 134 (45.0) | 0.986 | 43 (48.9) | 25 (45.5) | 0.733 |
| A/A         | 177 (34.7) | 114 (38.3) | 0.312 | 38 (43.2) | 24 (43.6) | 1.00 |
| Total       | 510 (100) | 298 (100) | | 88 (100) | 55 (100) | |
| Allele      |         |         |         |         |         |         |
| G           | 437 (42.8) | 234 (39.3) | | 57 (32.4) | 37 (33.6) | |
| A           | 583 (57.2) | 362 (60.7) | | 119 (67.6) | 73 (66.4) | |

* SIRT1 rs12778366 T/C genotype is significantly less frequent (P<0.001) in PA females compared with control females. * SIRT1 rs12778366 T/C genotype is significantly less frequent (P<0.001) in PA males compared with control males. * SIRT1 rs12778366 C/C genotype is significantly more frequent (P<0.001) in PA females compared with control females. * SIRT1 rs12778366 C/C genotype is significantly more frequent (P<0.001) in PA males compared with control males. * STAT3 rs744166 G/G genotype is significantly less frequent (P=0.004) in PA females compared with control females. PA, pituitary adenoma; SIRT1, sirtuin 1; FGFR2, fibroblast growth factor receptor 2; STAT3, signal transducer and activator of transcription 3; HWE, Hardy-Weinberg equilibrium.

Genotype distribution in the PA patients and the control group by gender. All 3 SNPs were analysed in the PA and control groups according to gender (Table III). SIRT1 rs12778366 polymorphism analysis did not reveal any statistically significant differences between females and males with PA in genotype (T/T, T/C and C/C) distribution (80.7, 0 and 19.3% vs. 81.8, 0 and 18.2%, respectively; Table III). Comparing SIRT1 rs12778366 genotype distribution in healthy females and females with PA, significant differences were revealed. The T/C genotype was less frequently present in females with PA compared with the healthy control females (0 vs. 17.5%, respectively; P<0.001) and C/C was more frequent in PA females compared with healthy females (19.3 vs. 2.7%,
respectively; P<0.001). The T/T genotype did not exhibit any significant differences when healthy females and females with PA were compared. When analysing genotype distribution in males, T/C genotype distribution showed statistically significant difference between males with PA and healthy males (0 vs. 17.4%, respectively; P<0.001) and the C/C genotype was more frequent in males with PA compared with the control group (18.2 vs. 2.0%, respectively; P<0.001) (Table III).

**Table IV. Binomial logistic regression analysis in patients with pituitary adenoma and the control group.**

| Gene       | Model     | Genotype | OR (95% CI)     | P-value | AIC     |
|------------|-----------|----------|-----------------|---------|---------|
| SIRT1 rs12778366 | Co-dominant | T/T      | 1.000           |         | 720.516 |
|            |           | T/C      | 0 (0.000)       | 0.995   |         |
|            |           | C/C      | 7.530 (4.087-13.873) | <0.001 |         |
|            | Recessive | T/T+C    | 1.000           |         | 780.895 |
|            |           | C/C      | 9.171 (4.982-16.881) | <0.001 |         |
|            | Additive  | -        | 1.584 (1.187-2.115) | 0.002   | 780.214 |
| STAT3 rs744166 | Co-dominant | A/A      | 1.000           |         | 801.223 |
|            |           | G/G      | 0.879 (0.603-1.282) | 0.504   |         |
|            |           | G/G      | 0.396 (0.211-0.743) | 0.004   |         |
|            | Recessive | A/A+G    | 1.000           |         | 799.670 |
|            |           | G/G      | 0.425 (0.234-0.771) | 0.005   |         |
|            | Additive  | -        | 0.702 (0.541-0.911) | 0.008   | 801.881 |

OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion; SIRT1, sirtuin 1; STAT3, signal transducer and activator of transcription 3.

**Table V. Binomial logistic regression analysis in patients with pituitary adenoma and control subjects according to gender.**

| Gene       | Gender  | Model     | Genotype | OR (95% CI)     | P-value | AIC     |
|------------|---------|-----------|----------|-----------------|---------|---------|
| SIRT1 rs12778366 | Male    | Co-dominant | T/T      | 1.000           |         | 275.783 |
|            |         |           | T/C      | 0.000 (0.000)   | 0.997   |         |
|            |         |           | C/C      | 8.889 (3.076-25.683) | <0.001 |         |
|            | Female  | Recessive | T/T+C    | 1.000           |         | 290.082 |
|            |         |           | C/C      | 10.815 (3.748-31.205) | <0.001 |         |
|            | Male    | Co-dominant | T/T      | 1.000           |         | 450.358 |
|            |         |           | T/C      | 0.000 (0.000)   | 0.996   |         |
|            |         |           | C/C      | 6.961 (3.285-14.750) | <0.001 |         |
|            | Female  | Recessive | T/T+C    | 1.000           |         | 474.428 |
|            |         |           | C/C      | 8.483 (4.008-17.955) | <0.001 |         |
|            | Male    | Additive  | A/A      | 1.580 (1.100-2.271) | 0.013   | 497.979 |
|            |         |           | G/A      | 0.875 (0.542-1.411) | 0.583   |         |
|            |         |           | G/G      | 0.314 (0.135-0.727) | 0.007   |         |
|            | Female  | Co-dominant | A/A      | 1.000           |         | 496.249 |
|            |         |           | G/A      | 0.875 (0.542-1.411) | 0.583   |         |
|            |         |           | G/G      | 0.314 (0.135-0.727) | 0.007   |         |
|            | Recessive | A/A+G    | 1.000           |         | 494.549 |
|            |         |           | G/G      | 0.337 (0.151-0.752) | 0.008   |         |
|            | Female  | Additive  | A/A      | 0.654 (0.469-0.912) | 0.012   | 497.077 |
|            |         |           | G/G      | 0.337 (0.151-0.752) | 0.008   |         |

OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion; SIRT1, sirtuin 1; STAT3, signal transducer and activator of transcription 3.

FGFR2 rs2981582 polymorphism analysis by gender was performed, but it did not reveal any genotype distribution differences between females and males.

**STAT3 rs744166 polymorphism analysis did not reveal any significant differences between females and males with PA in the genotype (G/G, G/A and A/A) distribution (8.0, 48.9 and 43.2% vs. 10.9, 45.5 and 43.6%, respectively; Table III) either.** When comparing STAT3 genotype distribution between healthy
Table VI. Frequency of SNPs in patients with PA and in control group according to PA invasiveness.

| Gene marker       | Control group, n (%) | P-value HWE | Non invasive PA group, n (%) | P-value HWE | Invasive PA group, n (%) | P-value HWE |
|-------------------|----------------------|-------------|------------------------------|-------------|--------------------------|-------------|
| **SIRT1 rs12778366** |                      |             |                              |             |                          |             |
| Genotype          |                      |             |                              |             |                          |             |
| T/T               | 647 (80.1)           | <0.001      | 47 (81.0)                    | <0.001      | 69 (81.2)                | <0.001      |
| T/C               | 141 (17.5)           |             | 0 (0.0)                      |             | 0 (0.0)                  |             |
| C/C               | 20 (2.5)             |             | 11 (19.0)                    |             | 16 (18.8)                |             |
| Total             | 808 (100.0)          |             | 58 (100.0)                   |             | 85 (100.0)               |             |
| Allele            |                      |             |                              |             |                          |             |
| T                 | 1,435 (88.8)         |             | 94 (81.0)                    |             | 138 (81.2)               |             |
| C                 | 181 (11.2)           |             | 22 (19.0)                    |             | 32 (18.8)                |             |
| **FGFR2 rs2981582** |                      |             |                              |             |                          |             |
| Genotype          |                      |             |                              |             |                          |             |
| G/G               | 336 (41.6)           | <0.001      | 16 (27.6)                    | <0.001      | 40 (47.1)                | 0.043       |
| G/A               | 429 (53.1)           |             | 42 (72.4)                    |             | 42 (49.4)                |             |
| A/A               | 43 (5.3)             |             | 0 (0.0)                      |             | 3 (3.5)                  |             |
| Total             | 808 (100.0)          |             | 58 (100.0)                   |             | 85 (100.0)               |             |
| Allele            |                      |             |                              |             |                          |             |
| G                 | 1,101 (68.1)         |             | 74 (63.8)                    |             | 122 (71.8)               |             |
| A                 | 515 (31.9)           |             | 42 (36.2)                    |             | 48 (28.2)                |             |
| **STAT3 rs744166** |                      |             |                              |             |                          |             |
| Genotype          |                      |             |                              |             |                          |             |
| G/G               | 154 (19.1)           | <0.001      | 9 (15.5)                     | 0.313       | 4 (4.7)                  | 0.031       |
| G/A               | 363 (44.9)           |             | 23 (39.7)                    |             | 45 (52.9)                |             |
| A/A               | 291 (36.0)           |             | 26 (44.8)                    |             | 36 (42.4)                |             |
| Total             | 808 (100.0)          |             | 58 (100.0)                   |             | 85 (100.0)               |             |
| Allele            |                      |             |                              |             |                          |             |
| G                 | 671 (41.5)           |             | 41 (35.3)                    |             | 53 (31.2)                |             |
| A                 | 945 (58.5)           |             | 75 (64.7)                    |             | 117 (68.8)               |             |

In all analyses, the T/C genotype is significantly less frequent (P=0.021) in non-invasive PA compared with the control group. The T/C genotype is significantly less frequent (P=0.001) in invasive PA compared with the control group. The T/C genotype is significantly less frequent (P=0.041) in non-invasive PA compared with the control group. The T/C genotype is significantly less frequent (P=0.001) in invasive PA compared with the control group. The G/A genotype is significantly less frequent (P=0.038) in non-invasive PA compared with the control group. The G/A genotype is significantly less frequent (P=0.024) in invasive PA compared with the non-invasive PA group. The G/A genotype is significantly more frequent (P=0.004) in non-invasive PA compared with the control group. The G/A genotype is significantly less frequent (P=0.009) in invasive PA compared with the non-invasive PA group. The G/A genotype is significantly less frequent (P=0.001) in invasive PA compared with the control group. The G/A genotype is significantly less frequent (P=0.038) in invasive PA compared with the non-invasive PA group. PA, pituitary adenoma; SIRT1, sirtuin 1; FGFR2, fibroblast growth factor receptor 2; STAT3, signal transducer and activator of transcription 3; HWE, Hardy-Weinberg equilibrium.

Binomial logistic regression analysis of the patients with PA and the control group. Binomial logistic regression analysis of the patients with PA and the control group was performed (Table IV). SIRT1 rs12778366 analysis revealed that there were significant variables in the co-dominant (OR=7.530; 95% CI: 4.087-13.873; P<0.001), recessive (OR=9.171; 95% CI: 4.982-16.881; P<0.001) and additive (OR=1.584; 95% CI: 1.187-2.115; P=0.002) models of the patients with PA and the control group (Table IV). FGFR2 rs2981582 analysis did not reveal any significant variables.

STAT3 rs744166 analysis revealed that there were significant variables in the co-dominant (OR=0.396; 95% CI: 0.211-0.743; P=0.004), recessive (OR=0.425; 95% CI: 0.250-0.717; P=0.002) and additive (OR=1.584; 95% CI: 1.187-2.115; P=0.002) models of the patients with PA and the control group (Table IV).
0.234-0.771; P=0.005) and additive (OR=0.702; 95% CI: 0.541-0.911; P=0.008) models of the patients with PA and the control group (Table IV).

Binomial logistic regression analysis in the patients with PA and the control group according to gender was performed (Table V). In the SIRT1 rs12778366 analysis there were statistically significant variables in the co-dominant (P<0.001) and recessive (P<0.001) models of males. The co-dominant (P<0.001), recessive (P<0.001) and additive (P=0.013) variables were also significant in females.

Binomial logistic regression analysis of FGFR2 rs2981582 in the patients with PA and in the control group according to gender showed statistically significant variables only in the co-dominant (P=0.007), recessive (P=0.008) and additive (P=0.012) models of females (Table V).

Genotype distribution in the control group and the PA patients by different PA subgroups. Analysis of SIRT1 rs1277836, FGFR2 rs2981582 and STAT3 rs744166 polymorphisms was performed by different PA subgroups (Tables VI-VIII).

The SIRT1 rs12778366 T/C genotype was less frequently observed in non-invasive, non-recurrent and inactive PA subgroups compared with healthy controls (0 vs. 17.5%, P=0.021; 0 vs. 17.5%, P<0.001; 0 vs. 17.5%, P<0.001, respectively). However, no differences were observed between non-invasive and invasive, non-recurrent and recurrent, and inactive and active PA subgroups (Tables VI-VIII).

**Table VII. Frequency of single nucleotide polymorphisms in patients with PA and in the control group according to PA recurrences.**

| Gene marker | Control group, n (%) | P-value | Non-recurrent PA group, n (%) | P-value | Recurrent PA group, n (%) | P-value |
|-------------|----------------------|---------|-----------------------------|---------|--------------------------|---------|
| **SIRT1 rs12778366** | | | | | | |
| Genotype | | | | | | |
| T/T | 647 (80.1) | <0.001 | 91 (81.3) | <0.001 | 25 (80.6) | <0.001 |
| T/C | 141 (17.5)b | 0 (0.0)a | 0 (0.0)b | | | |
| C/C | 20 (2.5)d | 21 (18.8)c | 6 (19.4)d | | | |
| Total | 808 (100) | 112 (100.0) | 31 (100.0) | | | |
| Allele | | | | | | |
| T | 1,435 (88.8) | 182 (81.3) | 50 (80.6) | | | |
| C | 181 (11.2) | 42 (18.8) | 12 (19.4) | | | |

**FGFR2 rs2981582**

| Genotype | | | | | | |
| G/G | 336 (41.6) | <0.001 | 44 (39.3) | <0.001 | 12 (38.7) | 0.067 |
| G/A | 429 (53.1) | 66 (58.9) | 18 (58.1) | | | |
| A/A | 43 (5.3) | 2 (1.8) | 1 (3.2) | | | |
| Total | 808 (100.0) | 112 (100.0) | 31 (100.0) | | | |

**STAT3 rs744166**

| Genotype | | | | | | |
| G/G | 154 (19.1)e | <0.001 | 7 (6.3)e | 0.083 | 6 (19.4)f | 0.305 |
| G/A | 363 (44.9) | 56 (50.0) | 12 (38.7) | | | |
| A/A | 291 (36.0) | 49 (43.8) | 13 (41.9) | | | |
| Total | 808 (100.0) | 112 (100.0) | 31 (100.0) | | | |

Note: The T/C genotype is significantly less frequent (P<0.001) in non-recurrent PA compared with the control group. The T/C genotype is significantly less frequent (P=0.005) in recurrent PA compared with the control group. The C/C genotype is significantly more frequent (P<0.001) in non-recurrent PA compared with the control group. The C/C genotype is significantly more frequent (P=0.047) in recurrent PA compared with the control group. The G/G genotype is significantly less frequent (P<0.001) in non-recurrent PA compared with the control group. The FGFR2 rs2981582 G/G genotype is statistically more frequent (P=0.036) in recurrent PA compared with non-recurrent PA. PA, pituitary adenoma; SIRT1, sirtuin 1; FGFR2, fibroblast growth factor receptor 2; STAT3, signal transducer and activator of transcription 3; HWE, Hardy-Weinberg equilibrium.
Additional analysis revealed that the C/C genotype was more frequent in invasive, recurrent and active PA subgroups compared with the healthy controls (18.8 vs. 2.5%, P=0.041; 19.4 vs. 2.5%, P=0.047; 15.0 vs. 2.5%, P<0.001, respectively; Tables VI, VII and VIII).

The FGFR2 rs2981582 G/G genotype was less frequently observed in the non-invasive PA subgroup compared with the healthy controls (27.6 vs. 41.6%, respectively; P=0.038), but the G/A genotype was more frequently observed in the non-invasive PA subgroup compared with the control group (72.4 vs. 53.1%, respectively; P=0.004) and the invasive PA subgroup (72.4 vs. 49.4% respectively; P=0.009) (Table VI).

Statistical analysis was performed to evaluate the FGFR2 rs2981582 association with PA activity and recurrence (Tables VII and VIII). This analysis did not reveal any association between SNP and active or non-active PA, and PA without recurrence or with recurrence.

The STAT3 rs744166 A/A genotype was more frequent in the active PA subgroup compared with the control group (48.8 vs. 36.0%, respectively; P=0.029). There were
differences between non-invasive and invasive, non-recurrent and recurrent PA subgroups as well, with the exception of comparing inactive and active PA. The \textit{STAT3 rs744166 G/G} genotype was more frequent in non-invasive PA compared with invasive PA (15.5 vs. 4.7%, respectively; \(P=0.038\)) and in recurrent PA group comparing to non-recurrent PA (19.4 vs. 6.2%, respectively; \(P=0.036\)) (Tables VI-VIII).

\textbf{Binomial logistic regression analysis of the control group and the PA patients by different PA subgroups.} Binomial logistic regression analysis in the non-invasive PA, invasive PA and control groups was performed (Table IX). Analysing the \textit{SIRT1} polymorphism in non-invasive PA group and control group this analysis showed that the co-dominant (\(P<0.001\)), recessive (\(P<0.001\)) and additive (\(P=0.025\)) variables were significant. Binomial logistic regression analysis in the patients with invasive PA and the control group revealed significance of the same co-dominant (\(P<0.001\)), recessive (\(P<0.001\)) and additive (\(P=0.010\)) variables (Table IX).

Binomial logistic regression analysis of \textit{FGFR2 rs2981582} in the non-invasive PA and control groups showed that the co-dominant (\(P=0.017\)), dominant (\(P=0.039\)) and over-dominant (\(P=0.005\)) variables were significant, but this analysis in the patients with invasive PA and the control group did not reveal any significance of these models.

Binomial logistic regression analysis of \textit{STAT3 rs744166} was also performed (Table IX). The analysis showed that the co-dominant (\(P=0.004\)), recessive (\(P=0.003\)) and additive (\(P=0.011\)) variables were statistically significant only in the invasive PA and control groups (Table IX).

Binomial logistic regression analysis in the inactive PA and control groups, and in the active PA and control groups, was performed for all 3 SNPs (Table X).

Inactive PA group analysis of \textit{SIRT1 rs12778366} showed that the co-dominant (\(P=0.001\)), recessive (\(P=0.001\)) and additive (\(P<0.001\)) variables were significant. The analysis of the active PA group revealed significance in the co-dominant (\(P<0.001\)) and recessive (\(P<0.001\)) models (Table X).

Binomial logistic regression analysis of \textit{FGFR2 rs2981582} was performed in the inactive PA, active PA and control groups, but this analysis did not reveal significance in these models.

\textit{STAT3 rs744166} analysis in the inactive PA group showed that there were no significant variables. Analysing the active PA group, the present study revealed significance
Table X. Binomial logistic regression analysis in inactive and active PA, and control groups.

| Gene         | PA subgroup | Model   | Genotype | OR (95% CI) | P-value | AIC     |
|--------------|-------------|---------|----------|-------------|---------|---------|
| **SIRT1 rs12778366** | Inactive    | Co-dominant | T/T       | 1.000       |         | 402.990 |
|              |             |         | T/C       | 0.000 (0.000) |         | <0.001  |
|              |              |         | C/C       | 10.109 (4.868-20.996) |         |         |
|              | Recessive   | T/T+T/C | 1.000     |             |         | 419.306 |
|              |             |         | C/C       | 12.312 (5.933-25.552) |         | <0.001  |
|              | Additive    | -       | T/C       | 2.045 (1.388-3.015) |         | <0.001  |
|              | Active      | Co-dominant | T/T       | 1.000       |         | 497.635 |
|              |              |         | T/C       | 0.000 (0.000) |         | 0.996   |
|              |              |         | C/C       | 5.709 (2.675-12.183) |         | <0.001  |
|              | Recessive   | T/T+T/C | 1.000     |             |         | 512.245 |
|              |             |         | C/C       | 6.953 (3.260-14.828) |         | <0.001  |
| **STAT3 rs744166** | Active      | Co-dominant | A/A       | 1.000       |         | 535.474 |
|              |              |         | G/A       | 0.669 (0.430-1.135) |         | 0.148   |
|              |              |         | G/G       | 0.339 (0.148-0.776) |         | 0.010   |
|              | Dominant    | A/A     | 1.000     |             |         | 536.765 |
|              |              |         | G/A+G/G   | 0.592 (0.373-0.939) |         | 0.026   |
|              | Recessive   | A/A+G/A | 1.000     |             |         | 535.576 |
|              |              |         | G/G       | 0.407 (0.184-0.902) |         | 0.027   |
|              | Additive    | -       |           | 0.622 (0.442-0.887) |         | 0.007   |

PA, pituitary adenoma; SIRT1, sirtuin 1; STAT3, signal transducer and activator of transcription 3; OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion.

Table XI. Binomial logistic regression analysis in non-recurrent and recurrent PA and control groups.

| Gene         | PA subgroup | Model   | Genotype | OR (95% CI) | P-value | AIC     |
|--------------|-------------|---------|----------|-------------|---------|---------|
| **SIRT1 rs12778366** | Non-recurrent | Co-dominant | T/T       | 1.000       |         | 614.042 |
|              |             |         | T/C       | 0.000 (0.000) |         | <0.001  |
|              |              |         | C/C       | 7.465 (3.896-14.307) |         |         |
|              | Recessive   | T/T+T/C | 1.000     |             |         | 645.812 |
|              |             |         | C/C       | 9.092 (4.748-17.411) |         | <0.001  |
|              | Additive    | -       | T/T       | 1.592 (1.153-2.199) |         | 0.005   |
|              | Recurrent   | Co-dominant | T/T       | 1.000       |         | 247.718 |
|              |              |         | T/C       | 0.000 (0.000) |         | 0.996   |
|              |              |         | C/C       | 7.764 (2.868-21.019) |         | <0.001  |
|              | Recessive   | T/T+T/C | 1.000     |             |         | 255.407 |
|              |             |         | C/C       | 9.456 (3.495-25.586) |         | <0.001  |
| **STAT3 rs744166** | Non-recurrent | Co-dominant | A/A       | 1.000       |         | 673.559 |
|              |              |         | G/A       | 0.916 (0.606-1.385) |         | 0.678   |
|              |              |         | G/G       | 0.270 (0.119-0.610) |         | 0.002   |
|              | Recessive   | A/A+G/A | 1.000     |             |         | 671.731 |
|              |             |         | G/G       | 0.283 (0.129-0.621) |         | 0.002   |
|              | Additive    | -       |           | 0.653 (0.487-0.876) |         | 0.005   |

PA, pituitary adenoma; SIRT1, sirtuin 1; STAT3, signal transducer and activator of transcription 3; OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion.

in the co-dominant (P=0.010), dominant (P=0.026), recessive (P=0.027) and additive (P=0.007) models (Table X).

Additional binomial logistic regression analysis of SNPs was performed in non-recurrence, recurrence and control
groups. The analysis in the non-recurrent PA and control groups showed that the co-dominant (P<0.001), recessive (P<0.001) and additive (P=0.005) variables were statistically significant (Table XI). In the analysis of PA with recurrence, the co-dominant (P<0.001) and recessive (P<0.001) variables were also significant.

**FGFR2 rs2981582** polymorphism analysis did not show any statistical significance.

Binomial logistic regression analysis of **STAT3 rs744166** in the non-recurrent and recurrent PA groups, and the control group was performed. This revealed that in the non-recurrent PA and control groups, the co-dominant (P=0.002), recessive (P=0.002) and additive (P=0.005) variables were significant (Table XI). In the analysis of PA with recurrence there were no statistically significant variables.

**Discussion**

The impact of **SIRT1, FGFR2** and **STAT3** gene polymorphisms on the development of various tumours has been analysed in numerous studies (38,42-52,60,63-65), but no studies have investigated the associations with PA development, invasiveness, activity and recurrence.

A study conducted by Rizk et al (37) investigated **SIRT1** gene single nucleotide polymorphism rs12778366 in patients with breast cancer, revealing that the **SIRT1 rs12778366** T/T genotypes were more frequent, exhibited higher SIRT1 levels than the C/C and C/T genotypes, and were associated with histological grade and lymph node status. The T allele frequency was higher in patients with breast cancer compared with that in normal subjects.

The present study was the first to assess the association between **SIRT1 rs12778366** and PA. It was found that the T/C genotype was less frequent in the PA group compared with the healthy controls (0 vs. 17.5%, respectively; P<0.001) and that the C/C genotype was more frequent in the PA group compared with the healthy control group (18.9 vs. 2.5%, respectively; P<0.001).

Numerous studies have investigated the **FGFR2 rs2981582** polymorphism in breast cancer patients, and have provided controversial data on the impact of this polymorphism on tumour development. Chen et al (43) revealed that the G/A and A/A genotypes of **FGFR2 rs2981582** were associated with lower mammographic density and a reduced risk of breast cancer, and Butt et al (42) revealed a statistically significant association between the **FGFR2 rs2981582** A/A genotype and breast cancer risk. Shan et al (66) also revealed that patients with the A/A genotype of **FGFR2 rs2981582** exhibited an increased risk of breast cancer, while Ledwoń et al (47) revealed that the **rs2981582 SNP** showed significant association with the familial and sporadic types of breast cancer. On the basis of these findings, the present study aimed to examine whether the polymorphism in the **FGFR2** promoter may affect the risk of PA development, activity, recurrence or invasiveness. No differences in genotype (G/G, G/A and A/A) distribution were observed between the control and PA groups (41.6 vs. 39.2%, 53.1 vs. 58.7%, and 3.3 vs. 2.1%, respectively; P=0.174). No significant differences were observed between genotype distribution according to gender, PA activity, invasiveness or recurrence.

Several studies have analysed the **STAT3 rs744166** polymorphism in association with various types of tumour, but none have investigated the association between **STAT3 rs744166** and PA. Rocha et al (61) reported that the **rs744166** polymorphic G allele was associated with gastric cancer, and a significantly decreased risk of non-small cell lung cancer was observed for carriers of **STAT3 rs744166** in a study by Jiang et al (64). The present study demonstrated the differences in the distribution of the **STAT3 rs744166** polymorphism between patients with PA and control groups (P=0.012). The G/G genotype was less frequent in the PA group compared with the healthy controls (9.1 vs. 19.1%, respectively; P=0.003). Analysis in different PA subgroups showed that the **STAT3 rs744166** G/G genotype was more frequent in non-invasive PA compared with invasive PA (15.5 vs. 4.7%; P=0.038) and in recurrent PA compared with the non-recurrent PA (19.4 vs. 6.2%, respectively; P=0.036).

Overall, the present study demonstrated that the SNPs **SIRT1 rs12778366** and **STAT3** require replication in future larger studies, particularly with increased sample sizes to confirm the association of **SIRT1** and **STAT3** in patients with PA.

**Acknowledgements**

The present study received funding from the Research Council of Lithuania (grant no. MIP-008/2014).

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