

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 *FGFR2* 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*, *FGFR2* and *STAT3* as tumour promoters. However, an association between *SIRT1*, *FGFR2* 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 (Omniscan; 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,

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 ^a	0.882

^aP-value for comparison of the median age between the PA and control groups. PA, pituitary adenoma; min, minimum; max, maximum.

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, *FGFR2* 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 μ l 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, *FGFR2* 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 II. Frequency of single nucleotide polymorphisms in patients with PA and the control group.

Gene marker	Control group, n (%)	P-value HWE	PA group, n (%)	P-value HWE	χ^2	P-value
<i>SIRT1 rs12778366</i>						
Genotype						
T/T	647 (80.1)	<0.01	116 (81.1)	<0.001	91.139	<0.001
T/C	141 (17.5) ^a		0 (0.0) ^a			
C/C	20 (2.5) ^b		27 (18.9) ^b			
Total	808 (100.0)		143 (100)			
Allele						
T	1,435 (88.8)		232 (81.1)			
C	181 (11.2)		54 (18.9)			
<i>FGFR2 rs2981582</i>						
Genotype						
G/G	336 (41.6)	<0.001	56 (39.2)	<0.001	3.502	0.174
G/A	429 (53.1)		84 (58.7)			
A/A	43 (5.3)		3 (2.1)			
Total	808 (100.0)		143 (100.0)			
Allele						
G	1,101 (68.1)		196 (68.53)			
A	515 (31.9)		90 (31.47)			
<i>STAT3 rs744166</i>						
Genotype						
G/G	154 (19.1) ^c	<0.001	13 (9.1) ^c	0.354	8.825	0.012
G/A	363 (44.9)		68 (47.6)			
A/A	291 (36.0)		62 (43.4)			
Total	808 (100.0)		143 (100.0)			
Allele						
G	671 (41.5)		94 (32.9)			
A	945 (58.5)		192 (67.1)			

^a*SIRT1 rs12778366* T/C genotype was significantly less frequent ($P<0.001$) in the PA group compared with the control group. ^b*SIRT1 rs12778366* C/C genotype was significantly more frequent ($P<0.001$) in the PA group compared with the control group. ^c*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.

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 rs12778366, rs2981582 and rs744166 using the χ^2 test in all groups. The distribution of rs12778366, rs2981582 and rs744166 single-nucleotide polymorphisms (SNPs) in the PA and control groups was compared using the χ^2 test or Fisher's exact test. Binomial logistic regression analysis was performed to estimate the impact of genotypes on PA development. Odds ratios (ORs) and 95% confidence intervals (CIs) are presented. Only statistically significant variables are presented in the tables. The selection of the most suitable genetic model was based on the Akaike Information Criterion (AIC), whereby the best genetic models were those with the lowest AIC values (65). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Genotype distribution in the PA patients and the control group. The genotyping of *SIRT1 rs12778366*, *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 rs12778366* 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 (0 vs. 17.5%; $P<0.001$) 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

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 HWE	P-value	Females	Males	P-value HWE	P-value
<i>SIRT1 rs12778366</i>								
Genotype								
T/T	407 (79.8)	240 (80.5)	0.811	0.801	71 (80.7)	45 (81.8)	0.866	0.866
T/C	89 (17.5) ^a	52 (17.4) ^b		1.000	0 (0.0) ^a	0 (0.0) ^b		1.00
C/C	14 (2.7) ^c	6 (2.0) ^d		0.518	17 (19.3) ^c	10 (18.2) ^d		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)		
<i>FGFR2 rs2981582</i>								
Genotype								
G/G	218 (42.7)	118 (39.6)	0.675	0.381	39 (44.3)	17 (30.9)	0.197	0.117
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)		
<i>STAT3 rs744166</i>								
Genotype								
G/G	104 (20.4) ^e	50 (16.8)	0.378	0.207	7 (8.0) ^e	6 (10.9)	0.815	0.563
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 (42.84)	234 (39.3)			57 (32.4)	37 (33.6)		
A	583 (57.2)	362 (60.7)			119 (67.6)	73 (66.4)		

^a*SIRT1 rs12778366* T/C genotype is significantly less frequent ($P<0.001$) in PA females compared with control females. ^b*SIRT1 rs12778366* T/C genotype is significantly less frequent ($P<0.001$) in PA males compared with control males. ^c*SIRT1 rs12778366* C/C genotype is significantly more frequent ($P<0.001$) in PA females compared with control females. ^d*SIRT1 rs12778366* C/C genotype is significantly more frequent ($P<0.001$) in PA males compared with control males. ^e*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.

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).

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 revealed 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%,

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

Gene	Model	Genotype	OR (95% CI)	P-value	AIC
<i>SIRT1 rs12778366</i>	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+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
<i>STAT3 rs744166</i>	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/A	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
<i>SIRT1 rs12778366</i>	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	
		Recessive	T/T+T/C	1.000		290.082
			C/C	10.815 (3.748-31.205)	<0.001	
	Female	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	
		Recessive	T/T+T/C	1.000		474.428
			C/C	8.483 (4.008-17.955)	<0.001	
<i>STAT3 rs744166</i>	Female	Additive	-	1.580 (1.100-2.271)	0.013	497.979
		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/A	1.000		494.549
			G/G	0.337 (0.151-0.752)	0.008	
		Additive	-	0.654 (0.469-0.912)	0.012	497.077

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

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).

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
<i>SIRT1 rs12778366</i>						
Genotype						
T/T	647 (80.1)	<0.001	47 (81.0)	<0.001	69 (81.2)	<0.001
T/C	141 (17.5) ^{a,b}		0 (0.0) ^a		0 (0.0) ^b	
C/C	20 (2.5) ^{c,d}		11 (19.0) ^c		16 (18.8) ^d	
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)	
<i>FGFR2 rs2981582</i>						
Genotype						
G/G	336 (41.6) ^e	<0.001	16 (27.6) ^{e,f}	<0.001	40 (47.1) ^f	0.043
G/A	429 (53.1) ^g		42 (72.4) ^{g,h}		42 (49.4) ^h	
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)	
<i>STAT3 rs744166</i>						
Genotype						
G/G	154 (19.1) ⁱ	<0.001	9 (15.5) ^j	0.313	4 (4.7) ^{i,j}	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)	

^a*SIRT1 rs12778366* T/C genotype is significantly less frequent (P=0.021) in non-invasive PA compared with the control group. ^b*SIRT1 rs12778366* T/C genotype is significantly less frequent (P<0.001) in invasive PA compared with the control group. ^c*SIRT1 rs12778366* C/C genotype is significantly more frequent (P=0.041) in non-invasive PA compared with the control group. ^d*SIRT1 rs12778366* C/C genotype is significantly less frequent (P<0.001) in invasive PA compared with the control group. ^e*FGFR2 rs2981582* G/G genotype is significantly less frequent (P=0.038) in non-invasive PA compared with the control group. ^f*FGFR2 rs2981582* G/G genotype is significantly more frequent (P=0.024) in invasive PA compared with the non-invasive PA group. ^g*FGFR2 rs2981582* G/A genotype is significantly more frequent (P=0.004) in non-invasive PA compared with the control group. ^h*FGFR2 rs2981582* G/A genotype is significantly less frequent (P=0.009) in invasive PA compared with the non-invasive PA group. ⁱ*STAT3 rs744166* G/G genotype is significantly less frequent (P<0.001) in invasive PA compared with the control group. ^j*STAT3 rs744166* G/G 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.

females and females with PA, there were significant differences. The *STAT3 rs744166* G/G genotype was less frequently present in PA females compared with healthy control females (8.0 vs. 20.4%, respectively; P=0.004). The *STAT3 rs744166* G/A and A/A genotype distribution did not exhibit any significant differences when healthy females and females with PA were compared. *STAT3 rs744166* analysis between male groups did not reveal any statistically significant differences (Table III).

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:

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 HWE	Non-recurrent PA group, n (%)	P-value HWE	Recurrent PA group, n (%)	P-value HWE
<i>SIRT1 rs12778366</i>						
Genotype						
T/T	647 (80.1)	<0.001	91 (81.3)	<0.001	25 (80.6)	<0.001
T/C	141 (17.5) ^{a,b}		0 (0.0) ^a		0 (0.0) ^b	
C/C	20 (2.5) ^{c,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)	
<i>FGFR2 rs2981582</i>						
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)	
Allele						
G	1,101 (68.1)		154 (68.8)		42 (67.7)	
A	515 (31.9)		70 (31.3)		20 (32.3)	
<i>STAT3 rs744166</i>						
Genotype						
G/G	154 (19.1) ^e	<0.001	7 (6.3) ^{e,f}	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)	
Allele						
G	671 (41.5)		70 (31.3)		24 (38.7)	
A	945 (58.5)		154 (68.8)		38 (61.3)	

^a*SIRT1 rs12778366* T/C genotype is significantly less frequent ($P<0.001$) in non-recurrent PA compared with the control group. ^b*SIRT1 rs12778366* T/C genotype is significantly less frequent ($^bP=0.005$) in recurrent PA compared with the control group. ^c*SIRT1 rs12778366* C/C genotype is significantly more frequent ($P<0.001$) in non-recurrent PA compared with the control group. ^d*SIRT1 rs12778366* C/C genotype is significantly more frequent ($P=0.047$) in recurrent PA compared with the control group. ^e*STAT3 rs744166* G/G genotype is significantly less frequent ($P<0.001$) in non-recurrent PA compared with the control group. ^f*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.

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 was performed, but no significant variables were observed.

However, binomial logistic regression analysis of *STAT3 rs744166* 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 rs12778366*, *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 VIII. Frequency of single nucleotide polymorphisms in patients with PA and in the control group according to PA activity.

Gene marker	Control group, n (%)	P-value HWE	Inactive PA group, n (%)	P-value HWE	Active PA group, n (%)	P-value HWE
<i>SIRT1</i> rs12778366						
Genotype						
T/T	647 (80.1)	<0.001	48 (76.2)	0.007	68 (85.0)	0.044
T/C	141 (17.5) ^{a,b}		0 (0.0) ^a		0 (0.0) ^b	
C/C	20 (2.5) ^{c,d}		15 (23.8) ^d		12 (15.0) ^c	
Total	808 (100.0)		63 (100.0)		80 (100.0)	
Allele						
T	1,435 (88.8)		96 (76.2)		136 (85.0)	
C	181 (11.2)		30 (23.8)		24 (15.0)	
<i>FGFR2</i> rs2981582						
Genotype						
G/G	336 (41.6)	<0.001	25 (39.7)	<0.001	31 (38.8)	0.005
G/A	429 (53.1)		38 (60.3)		46 (57.5)	
A/A	43 (5.3)		0 (0.0)		3 (3.8)	
Total	808 (100.0)		63 (100.0)		80 (100.0)	
Allele						
G	1,101 (68.1)		88 (69.8)		108 (67.5)	
A	515 (31.9)		38 (30.2)		52 (32.5)	
<i>STAT3</i> rs744166						
Genotype						
G/G	154 (19.1) ^e	<0.001	6 (9.5)	0.193	7 (8.8) ^e	0.915
G/A	363 (44.9)		34 (54.0)		34 (42.5)	
A/A	291 (36.0) ^f		23 (36.5)		39 (48.8) ^f	
Total	808 (100.0)		63 (100.0)		80 (100.0)	
Allele						
G	671 (41.5)		46 (36.5)		48 (30.0)	
A	945 (58.5)		80 (63.5)		112 (70.0)	

^a*SIRT1* rs12778366 T/C genotype is significantly less frequent ($P<0.001$) in inactive PA compared with the control group.

^b*SIRT1* rs12778366 T/C genotype is significantly less frequent ($P<0.001$) in active PA compared with the control group. ^c*SIRT1* rs12778366 C/C genotype is significantly less frequent ($P<0.001$) in active PA compared with the control group. ^d*SIRT1* rs12778366 C/C genotype is significantly more frequent ($P<0.001$) in inactive PA compared with the control group. ^e*FGFR2* rs2981582 G/G genotype is significantly less frequent ($P=0.022$) in active PA compared with the control group. ^f*FGFR2* rs2981582 A/A genotype is significantly more frequent ($P=0.029$) in active PA 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.

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 G/G genotype was less frequently observed in invasive, non-recurrent and active PA subgroups compared with healthy controls (4.7 vs. 19.1%, $P<0.001$; 6.2 vs. 19.1%, $P<0.001$; 8.8 vs. 19.1%, $P=0.022$, respectively).

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

Table IX. Binomial logistic regression analysis in non-invasive and invasive PA, and the control group.

Gene	PA subgroup	Model	Genotype	OR (95% CI)	P-value	AIC
SIRT1 rs12778366	Non-invasive	Co-dominant	T/T	1.000		390.145
			T/C	0.000 (0.000)	0.996	
			C/C	7.571 (3.426-16.734)	<0.001	
		Recessive	T/T+T/C	1.000		406.092
			C/C	9.221 (4.175-20.367)	<0.001	
		Additive	-	1.649 (1.065-2.554)	0.025	425.148
	Invasive	Co-dominant	T/T	1.000		509.448
			T/C	0.000 (0.000)	0.996	
			C/C	7.501 (3.715-15.147)	<0.001	
		Recessive	T/T+T/C	1.000		533.418
			C/C	9.136 (4.528-18.434)	<0.001	
		Additive	-	1.616 (1.120-2.330)	0.010	459.515
FGFR2 rs2981582	Non-invasive	Co-dominant	G/G	1.000		419.170
			G/A	2.056 (1.136-3.721)	0.017	
			A/A	0.000 (0.000)	0.998	
		Dominant	G/G	1.000		425.028
			G/A+A/A	1.869 (1.033-3.380)	0.039	
		Over-dominant	G/G+A/A	1.000		420.961
			G/A	2.319 (1.283-4.193)	0.005	
STAT3 rs744166	Invasive	Co-dominant	A/A	1.000		553.313
			G/A	1.002 (0.630-1.595)	0.993	
			G/G	0.210 (0.073-0.601)	0.004	
		Recessive	A/A+G/A	1.000		551.313
			G/G	0.210 (0.076-0.581)	0.003	
		Additive	-	0.651 (0.467-0.908)	0.011	558.771

PA, pituitary adenoma; *SIRT1*, sirtuin 1; *FGFR2*, fibroblast growth factor receptor 2; *STAT3*, signal transducer and activator of transcription 3; OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion.

differences between non-invasive and invasive, non-recurrent and recurrent PA subgroups as well, with the exception of comparing inactive and active PA. The *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).

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 *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 *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 *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 *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 *FGFR2 rs2981582* was performed in the inactive PA, active PA and control groups, but this analysis did not reveal significance in these models.

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
<i>SIRT1 rs12778366</i>	Inactive	Co-dominant	T/T	1.000		402.990
			T/C	0.000 (0.000)	0.996	
			C/C	10.109 (4.868-20.996)	<0.001	
	Active	Recessive	T/T+T/C	1.000		419.306
			C/C	12.312 (5.933-25.552)	<0.001	
		Additive	-	2.045 (1.388-3.015)	<0.001	444.842
		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		521.245
			C/C	6.953 (3.260-14.828)	<0.001	
<i>STAT3 rs744166</i>	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-.0776)	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	533.914

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
<i>SIRT1 rs12778366</i>	Non-recurrent	Co-dominant	T/T	1.000		614.042
			T/C	0.000 (0.000)	0.995	
			C/C	7.465 (3.896-14.307)	<0.001	
		Recessive	T/T+T/C	1.000		645.812
			C/C	9.092 (4.748-17.411)	<0.001	
		Additive	-	1.592 (1.153-2.199)	0.005	678.223
	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	
<i>STAT3 rs744166</i>	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	677.047

PA, pituitary adenoma; *SIRT1*, sirtuin 1; *STAT3*, signal transducer and activator of transcription 3; OR, odds ratio; CI, confidence interval; 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%, $P<0.001$).

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 5.3 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 group subjects ($P=0.012$). 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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