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The role of *MMP-1* and *FGFR4-R388* gene polymorphisms in pituitary adenoma

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Background. The pathogenesis of pituitary adenoma (PA) is complex and poorly understood. It is thought that PA has a multifactorial aetiology; genetic factors also have an impact on PA development. Since *MMP1* and *FGFR4* genes play an important role in tumour growth, differentiation and progression, we decided to determine if the frequency of the genotypes of *MMP-1* and *FGFR4-R388* polymorphisms influence the development of PA.

Materials and methods. The study enrolled $n = 100$ patients with PA and $n = 200$ healthy controls (reference group). The genotyping tests of *MMP-1* and *FGFR4-R388* were carried out using the real-time polymerase chain reaction (PCR) method.

Results. The polymorphism in the *MMP-1* gene 1G/1G genotype was more frequent in the group of invasive PA than in the control group: 28.6% vs. 16.5%, $p = 0.044$. The 1G/2G genotype was more frequent in females of the control group compared to PA group females: 50.3% vs. 30.8%, $p = 0.011$. The polymorphism in the *MMP-1* gene 1G/1G genotype was more frequent in the active PA group than in the control group: 28.4% vs. 16.5%, $p = 0.044$. *FGFR4-R388* did not play any predominant role in PA development.

Conclusion. The *MMP-1* gene 1G/1G may play a role in invasive and active PA development.

Keywords: pituitary adenoma, matrix metalloproteinase-1, *FGFR4-R388*, gene polymorphism

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INTRODUCTION

Pituitary adenomas (PAs) are usually non-malignant monoclonal tumours with an overall prevalence of 16.7% (14.4% in autopsy studies and 22.5% in imaging studies) in the general population (1). The majority of PAs, however, are small and non-functional tumours, and only 0.16–0.2% of them are macroadenomas ≥10 mm in diameter (1, 2). The diagnosis of PAs has improved when MRI scans and hormone analysis in blood serum became more accessible (aggressive biomarkers). The accession of PAs is explained by better detection of microadenomas and the occurring symptoms of macroadenomas (3). Clinically, PAs are assorted to nonfunctional pituitary adenomas (NFPA) and functional pituitary adenomas (FPA) (4). Compared to FPA, NFPA are more aggressive and complicated to detect because of lack of symptoms that appear only when adenomas enlarge and start to compress surrounding structures (5). One of the most important behaviours of PA is invasiveness, which manifests itself by destroying surrounding structures thus triggering a lot of complications (6). Also, it has been demonstrated that invasiveness could be a sign of poor prognosis (6).

The pathogenesis of PA is complex and poorly understood. It is thought that PA has a multifactorial aetiology; also, genetic factors have an impact on PA development. MMPs have an important role in tumour progression due to the breakdown of their collagen, which is a fundamental structure within the extracellular matrix (7–9). Degradation of the extracellular matrix gradually increases depending on the level of *MMP-1* (10). The matrix metalloproteinase-1 enzyme is important in replacement of collagen fibres in the intercellular matrix (11). Rutter et al. (12) have announced that the insertion of a G nucleotide at –1607 bp in the nucleotide sequence of the MMP-1 gene promoter generates a new 5′-GGA-3′ sequence that matches the core recognition sequence of the binding site for members of the Ets family of transcription factors (11, 13). *MMP-1* levels increased approximately twice compared to normal concentration when this insertion was present, this allowed *MMP-1* to relieve tumour invasion and metastasis (14). *MMP-1* is also thought to be significant to tumour development (11). Altaş et al. researched the influence of 2G polymorphism on pituitary adenomas and found that 90% of invasive pituitary adenomas manifest in patients who are homozygous for this *MMP-1* SNP (11).

The *FGFR4-R388*G SNP is associated with *MMP* expression (13). Fibroblast growth factors (FGFs) and their receptors (FGFRs) are a family of ligands and receptors that regulate development, growth, differentiation, migration, and angiogenesis (1). It is thought that basic FGF (bFGF; FGF2) is found in bovine pituitary folliculostellate cells responsible for secretion of pituitary hormones (15). Deletion of FGF10 or its receptor can be accountable for the disruption of initial pituitary development (16). Ezzat et al. (17) demonstrated that the amount of FGF mRNA in blood serum correlates with aggressiveness of pituitary tumours.

The aim of our study was to determine if the frequency of the genotypes of *MMP-1* and *FGFR4-R388* had an influence on the development of non-invasive and invasive hypophyseal adenoma.

MATERIALS AND METHODS

Permission (No. P2-9/2003) to undertake the study was obtained from Kaunas Regional Biomedical Research Ethics Committee. The study was conducted in the departments of ophthalmology and neurosurgery of the Hospital of the Lithuanian University of Health Sciences.

Study participants comprised of 100 subjects with the diagnosis of pituitary adenoma, and the control group involved 200 subjects.

The control group was formed by taking into consideration the distribution of age and gender in the pituitary adenoma group. Therefore, the medians of the patients' age of the control group and the pituitary group did not differ statistically significantly ($p < 0.05$).

The demographic data of the study subjects are presented in Table 1.

The inclusion criteria of the PA group were as follows: 1) established and confirmed PA via MRI; 2) patient's general good condition; 3) patient's consent to take part in the study; 4) age ≥18 years, 5) no other brain or other localized tumours.

Invasiveness evaluation

All pituitary adenomas were analysed based on MRI findings. The suprasellar extension and sphenoid sinus invasion by PAs were classified according to the Wilson Hardy classification (the Hardy classifi-

Table 1. Demographic characteristics of patients with pituitary adenoma (PA) and the control group subjects

Characteristic	Group		*p* value
	PA *n* = 100	control *n* = 200	
Men, *n* (%)	35 (35)	49 (24.5)	NS
Women, *n* (%)	65 (65)	151 (75.5)	NS
Age, median	51.38	49.57	NS

NS – non-significant

cation, modified by Wilson) (18). The degree of suprasellar and parasellar extension was graded as stages A–E. The degree of sellar floor erosion was graded as grades I–IV. Grade III, localized sellar destruction, and grade IV, diffused destruction, were considered invasive PA. The Knosp classification system was used to quantify the invasion of the cavernous sinus, in which only grades III and IV define true invasion of the tumour into the cavernous sinus. Grade 0, no cavernous sinus involvement; grades I and II, the tumour pushes into the medial wall of the cavernous sinus, but does not go beyond the hypothetical line extending between the centres of the two segments of the internal carotid artery (grade I) or it goes beyond such a line, but without passing a line tangent to the lateral margins of the artery itself (grade II); grade III, the tumour extends laterally to the internal carotid artery within the cavernous sinus; grade 4, total encasement of the intracavernous carotid artery (19). So, grade III and IV tumours were considered to be invasive.

Activeness and recurrence evaluation

The analysis of all pituitary adenomas was based on histopathological findings of PA and hormone levels in blood serum before surgery. All 100 subjects were categorized into two groups – active or inactive PA. The active PA group was not broken down into smaller groups by the increase of specific hormone because dominant tumours were prolactinomas and others would not fill the optimal space in our study. Since some of the 100 subjects had already had surgery in recent years, we categorized them by the recurrence of pituitary adenoma into two groups – with PA and without recurrence.

DNA extraction and genotyping

The DNA extraction and analysis of the gene polymorphism of *MMP-1* Rs1799750 and *FGFR4-R388* Rs351855 were carried out at the Laboratory of Ophthalmology at the Institute of Neuroscience of the Lithuanian University of Health Sciences. 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 Scientific) or the silica-based membrane technology utilizing a genomic DNA extraction kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific), according to the manufacturer's recommendations.

The genotyping of MMP-1Rs1799750 and *FGFR4-R388* Rs351855 was carried out using the real-time PCR method. Both single-nucleotide polymorphisms were determined using TaqMan®Drug Metabolism assays (Thermo Scientific).

The genotyping was performed by a Rotor – Gene Q real-time PCR quantification system (Qiagen, USA), using 2X TaqMan® Universal Master Mix, TaqMan® Drug Metabolism assay, and nuclease-free water. Appropriate real-time PCR mixtures of MMP-1Rs1799750 and *FGFR4-R388* Rs351855 were prepared for determining single-nucleotide polymorphisms.

A PCR reaction mixture (9 μL) was poured into each of the 72 wells of the Rotor-Disc, and then 1 μL of matrix DNA of the samples (~10 ng) and 1 μL of negative control (–K) were added.

The Allelic Discrimination program was used during the real-time PCR. Then, the assay was continued following the manual provided by the manufacturer (www.qiagen.com, Allelic Discrimination). After that, the Allelic Discrimination program was completed, and the genotyping results were received. The program determined the individual genotypes according to the fluorescence intensity rate of different detectors (VIC and FAM).

Statistical analysis

Statistical analysis was performed using the SPSS/W 20.0 software (Statistical Package for the Social Sciences for Windows). The data are presented as absolute numbers with percentages

in brackets and average values. The frequencies of genotypes (in percentage) are presented in Table 2.

Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of Rs1799750 and Rs351855 using the χ^2 test in all groups. The distributions of the Rs1799750 and Rs351855 SNPs in the PA and control groups were compared using the χ^2 test or the Fisher exact test. Binomial logistic regression analysis was performed to estimate the impact of genotypes on PA development. Odds ratios and 95% confidence intervals are presented. The selection of the best genetic model was based on the Akaike Information Criterion (AIC); therefore, the best genetic models were those with the lowest AIC values. Differences were considered statistically significant when $p < 0.05$.

RESULTS

The frequency of polymorphisms in the *MMP-1* gene (c.-1607 2G) Rs1799750 and *FGFR4-R388* gene (G>A) Rs351855 was evaluated in both the PA and reference groups (Table 2). The distribution of the analyzed *MMP-1* genotypes and allele frequencies in patients with PA did not match and in the control group matched the Hardy-Weinberg equilibrium ($p > 0.05$). *MMP-1* gene polymorphism analysis in the overall group did not reveal any differences in the genotypes distribution between patients with PA and control group subjects (Table 2). The distribution of the analyzed *FGFR4-R388* genotypes and allele frequencies in the control group and in the PA group matched the Hardy-Weinberg equilibrium ($p > 0.05$). *FGFR4-R388* gene polymorphism analysis in the overall group did not reveal any differences in the genotype distribution between the patients with PA and the control group subjects (Table 2).

Binomial logistic regression analysis in the patients with PA and in the control group was performed (Table 3). This analysis revealed that there were no statistically significant variables in the models of the patients with PA and in the control group.

The frequency of the *MMP-1* and *FGFR4* genotypes in the patients with PA and in the control group

Table 2. The frequency of polymorphisms in the *MMP-1* gene (c.-1607 2G) Rs1799750 and *FGFR4-R388* gene (G>A) Rs351855 in the PA and reference groups

Gene	Genotype/ allele	Frequency (%)				
		Control group *n* (%) (*n* = 200)	***p* value-HWE**	**PA group *n* (%) (*n* = 100)**	***p* value HWE**	***p* value**
MMP-1	Genotype					
(c.-1607 2G)	1G/1G	33 (16.5)	0.768	25 (25)	0.022	0.155
Rs1799750	1G/2G	94 (47)		38 (38)		
	2G/2G	73 (36.5)		37 (37)		
	All	200 (100)		100 (100)		
	Allele					
	1G	160 (40.00)		88 (44.00)		
	2G	240 (60.00)		112 (56.00)		
FGFR4 (G>A)	Genotype					
Rs351855	G/G	95 (47.5)	0.134	45 (45.00)	0.119	0.885
	G/A	92 (46)		49 (49.00)		
	A/A	13 (6.5)		6 (6.00)		
	All	200 (100)		100 (100)		
	Allele					
	G	282 (70.5)		139 (69.5)		
	A	118 (29.5)		61 (30.5)		

PA – pituitary adenoma, *p* value – significance level, *p* value-HWE – significance level by Hardy-Weinberg equilibrium.

was evaluated by gender as well. The polymorphism in the *MMP-1* gene was statistically significant in the women's group ($p < 0.05$). The 1G/2G genotype was significantly less frequent in women in the PA group than in the control group: 30.8% vs. 50.3%, $p = 0.011$. The polymorphism of *FGFR4* was not statistically significant by gender ($p > 0.05$). The data are presented in Table 4.

Binomial logistic regression analysis was performed by gender. Analysis of the *MMP-1* gene

Table 3. Binomial logistic regression analysis in the patients with pituitary adenoma (PA) and in the control group

Model	Genotype	OR (CI, 95%)	*p* value	AIC
MMP-1				
Codominant	1G2G	0.798 (0.462; 1.377)	0.417	384.253
	1G1G	1.495 (0.778; 2.872)	0.228	
Dominant	1G/2G + 2G/2G	0.979 (0.595; 1.610)	0.932	385.901
Recessive	1G/1G	0.593 (0.330; 1.066)	0.081	382.911
Over-dominant	1G2G	0.691 (0.423; 1.128)	0.140	383.702
Additive	–	1.162 (0.836; 1.615)	0.370	385.105
FGFR4				
Codominant	GA	1.124 (0.685; 1.846)	0.643	387.665
	AA	0.974 (0.348; 2.730)	0.961	
Dominant	G/A+A/A	1.106 (0.683; 1/790)	0.682	385.741
Recessive	A/A	1.089 (0.401; 2.956)	0.867	385.880
Over-dominant	GA	1.128 (0.697; 1.824)	0.624	385.668
Additive	–	1.056 (0.711; 1.567)	0.788	385.836

Table 4. The frequency of the *MMP-1* and *FGFR4* genotypes in the patients with pituitary adenoma (PA) and in the control group, by gender

MMP-1 (c.-1607 2G) Rs1799750						
Genotype	Males		*p* value	Females		*p* value
	PA group *n* (%) (*n* = 35)	Control group *n* (%) (*n* = 49)		PA group *n* (%) (*n* = 65)	Control group *n* (%) (*n* = 151)	
1G1G (%)	8 (22.9)	10 (20.4)	0.794	17 (26.2)	23 (15.2)	0.053
1G2G (%)	18 (51.4)	18 (36.7)	0.190	20 (30.8)	76 (50.3)	0.011
2G2G (%)	9 (25.7)	21 (42.9)	0.165	28 (43.1)	52 (34.4)	0.282
Allele						
1G	34 (48.57)	38 (38.78)		54 (41.54)	122 (40.40)	
2G	36 (51.43)	60 (61.22)		76 (58.46)	180 (59.60)	
FGFR4 (G>A) Rs351855						
	PA group *n* (%) (*n* = 35)	Control group *n* (%) (*n* = 47)	*p* value	PA group *n* (%) (*n* = 65)	Control group *n* (%) (*n* = 153)	*p* value
GG (%)	21 (60.0)	20 (42.6)	0.258	24 (36.9)	75 (49.0)	0.105
GA (%)	12 (34.3)	23 (48.9)	0.259	37 (56.9)	69 (45.1)	0.138
AA (%)	2 (5.7)	4 (8.5)	0.697	4 (6.2)	9 (5.9)	1
Allele						
G	54 (77.14)	63 (67.02)		85 (65.38)	219 (71.57)	
A	16 (22.86)	27 (32.98)		45 (34.62)	87 (28.43)	

PA – pituitary adenoma, *p* value – significance level

in females revealed codominant (p = 0.037) and over-dominant (p = 0.009) variables were statistically significant, however, in males none of the models showed statistical significance ($p > 0.05$). Also, there was no statistical significance in the *FGFR4* polymorphism in either of the groups by gender ($p > 0.05$). The data are presented in Table 5.

The frequency of the *MMP-1* and *FGFR4* genotypes in the patients with PA and in the control group by invasiveness of PA was analyzed. Patients with invasive PA had the 1G/2G genotype more frequently than the subjects in the control group: 28.6% vs. 16.5%, p = 0.044. However, the 1G/2G genotype was less frequent in the non-invasive PA group than in the control group: 47% vs. 27%, p = 0.03. The non-invasive PA group did not match the Hardy-Weinberg equilibrium (HWE) for the *MMP-1* gene polymorphism (p = 0.02), although the invasive PA and the control group matched HWE for both polymorphisms ($P > 0.05$). The data are presented in Table 6.

Table 5. Binomial logistic regression analysis in pituitary adenoma (PA) and the control group by gender

Model	Genotype	OR (CI, 95%)	*p value*	AIC
MMP-1				
Females				
Codominant	1G2G	0.489 (0.249; 0.958)	0.037	262.394
	1G1G	1.373 (0.631; 2.986)	0.424	
Dominant	1G/2A + 1G/1G	0.694 (0.383; 1.258)	0.229	266.792
Recessive	1G/1G	0.507 (0.250; 1.031)	0.061	264.804
Over-dominant	1G2G	0.439 (0.237; 0.812)	0.009	261.029
Additive	–	1.045 (0.699; 1.562)	0.831	268.186
Males				
Codominant	1G2G	2.333 (0.843; 6.459)	0.103	117.289
	1G1G	1.867 (0.554; 6.286)	0.314	
Dominant	1G/2G + 1G/1G	2.167 (0.841; 5.579)	0.109	115.438
Recessive	1G1G	0.865 (0.302; 2.476)	0.787	118.032
Over-dominant	1G2G	0.312 (0.076; 1.284)	0.107	51.33
Additive	–	0.312 (0.792; 2.582)	0.236	116.679
FGFR4				
Females				
Codominant	GA	1.676 (0.911; 3.081)	0.097	268.847
	AA	1.389 (0.392; 4.918)	0.611	
Dominant	G/A+A/A	1.643 (0.906; 2.979)	0.102	268.936
Recessive	A/A	0.953 (0.283; 3.213)	0.938	269.650
Over-dominant	GA	1.609 (0.896; 2.888)	0.111	267.098
Additive	–	1.408 (0.869; 2.281)	0.165	267.722
Males				
Codominant	GA	0.497 (0.196; 1.258)	0.140	115.456
	AA	0.476 (0.078; 2.894)	0.420	
Dominant	G/A+A/A	0.494 (0.203; 1.202)	0.120	113.458
Recessive	A/A	1.535 (0.265; 8.894)	0.633	115.677
Over-dominant	GA	0.544 (0.221; 1.342)	0.187	114.138
Additive	–	0.585 (0.281; 1.217)	0.151	113.767

Table 6. The frequency of the *MMP-1* and *FGFR4* genotypes in the patients with pituitary adenoma (PA) and in the control group by the invasiveness of PA

Gene	Genotype/ allele	Frequency (%)					
		Control group *n* (%) (*n* = 200)	*p* value-HWE	Non-invasive PA group *n* (%) (*n* = 37)	*p* value-HWE	Invasive PA group *n* (%) (*n* = 63)	*p* value-HWE
MMP-1 (c.-1607 2G) Rs1799750	Genotype						
	1G/1G	33* (16.5)	0.768	7 (18.9)	0.020	18* (28.6)	0.379
	1G/2G	94** (47)		10** (27.0)		28 (44.4)	
	2G/2G	73 (36.5)		20 (54.1)		17 (27)	
	All	200 (100)		37 (100)		63 (100)	
	Allele						
	1G	160 (40.00)		24 (32.43)		64 (50.79)	
	2G	240 (60.00)		50 (67.57)		62 (49.21)	
FGFR4 (G>A) Rs351855	Genotype						
	G/G	95 (47.5)	0.134	17 (45.9)	0.659	28 (44.4)	0.102
	G/A	92 (46)		17 (45.9)		32 (50.8)	
	A/A	13 (6.5)		3 (8.1)		3 (4.8)	
	All	200 (100)		37 (100)		63 (100)	
	Allele						
	G	282 (70.5)		51 (68.92)		88 (69.84)	
	A	118 (29.5)		23 (31.08)		38 (30.16)	

PA – pituitary adenoma, *p* value – significance level

* $p = 0.044$

** $p = 0.030$

Binomial logistic regression analysis in the invasive PA group for the *MMP-1* gene polymorphism revealed that the dominant variable ($p = 0.037$) was statistically significant. There was no statistical significance in the *FGFR4* polymorphism in both groups by the invasiveness of PA ($p > 0.05$). The data are presented in Table 7.

We analyzed the frequency of the *MMP-1* and *FGFR4* genotypes in the patients with PA and in the control group by the activity of PA. The 1G/1G genotype was more frequent in the active PA group than in the control group: 28.4% vs. 16.5%, $p = 0.049$. The active PA group did not match the Hardy-Weinberg equilibrium for polymorphism in the *MMP-1* gene ($p = 0.04$), although all other groups matched the HWE by genotype distributions ($p > 0.05$). The data are presented in Table 8.

Table 7. Binomial logistic regression analysis in the PA group and the control group by the invasiveness of PA

Model	Genotype	OR (95% CI)	*p* value	AIC
MMP-1				
Non-invasive PA				
Codominant	1G2G	0.502 (0.177; 1.425)	0.195	207.767
	1G1G	1.292 (0.498; 3.353)	0.599	
Dominant	1G/2G + 1G/1G	0.489 (0.241; 2.992)	0.047	205.381
Recessive	1G/1G	1.181 (0.478; 2.914)	0.718	209.199
Over-dominant	1G2G	0.418 (0.192; 2.908)	0.028	204.051
Additive	–	1.069 (0.998; 1.145)	0.057	205.705
Invasive PA				
Codominant	1G2G	0.546 (0.268; 1.114)	0.096	290.891
	2G2G	0.427 (0.196; 0.931)	0.032	

Table 7. (continued)

Model	Genotype	OR (95% CI)	*p* value	AIC
Dominant	1G/2G + 2G/2G	0.494 (0.255; 0.958)	0.037	289.406
Recessive	2G/2G	0.634 (0.344; 1.203)	0.167	291.610
Over-dominant	1G2G	0.902 (0.511; 1.594)	0.723	293.465
Additive	–	0.658 (0.442; 0.978)	0.039	289.267
FGFR4				
Non-invasive PA				
Codominant	GA	0.801 (0.206; 3.113)	0.748	211.196
	AA	0.775 (0.200; 3.013)	0.713	
Dominant	G/A+A/A	0.788 (0.213; 2.913)	0.721	209.203
Recessive	A/A	0.939 (0.465; 1.899)	0.862	209.295
Over-dominant	GA	0.998 (0.494; 2.017)	0.995	209.326
Additive	–	0.920 (0.522; 1.622)	0.773	209.243
Invasive PA				
Codominant	GA	1.507 (0.403; 5.632)	0.542	295.013
	AA	1.277 (0.340; 4.801)	0.717	
Dominant	G/A+A/A	1.390 (0.383; 5.044)	0.616	293.324
Recessive	A/A	0.884 (0.500; 1.562)	0.672	293.411
Over-dominant	GA	1.212 (0.687; 2.136)	0.507	293.150
Additive	**–**	**0.964 (0.604; 1.541)**	**0.880**	**293.568**

Table 8. The frequency of the *MMP-1* and *FGFR4* genotypes in the patients with pituitary adenoma (PA) and in the control group by the activity of PA

Gene	Genotype/ allele	Frequency (%)					
		Control group *n* (%) (*n* = 200)	*p* value-HWE	Inactive PA group *n* (%) (*n* = 33)	*p* value-HWE	Active PA group *n* (%) (*n* = 67)	*p* value-HWE
MMP-1 Rs1799750	Genotype						
	1G/1G	33* (16.5)	0.768	6 (18.2)	0.349	19* (28.4)	0.040
	1G/2G	94 (47)		13 (39.4)		25 (37.3)	
	2G/2G	73 (36.5)		14 (42.4)		23 (34.3)	
	All	200 (100)		33 (100)		67 (100)	
	Allele						
	1G	160 (40.00)		25 (37.88)		63 (47.01)	
	2G	240 (60.00)		41 (62.12)		71 (52.99)	
FGFR4 Rs351855	Genotype						
	G/G	95 (47.5)	0.134	14 (42.4)	0.094	31 (46.3)	0.464
	G/A	92 (46)		18 (54.5)		31 (46.3)	
	A/A	13 (6.5)		1 (3.0)		5 (7.5)	
	All	200 (100)		33 (100)		67 (100)	
	Allele						
	G	282 (70.5)		46 (69.7)		93 (69.4)	
	A	118 (29.5)		20 (30.3)		41 (30.6)	

PA – pituitary adenoma, *p* value – significance level

* $p = 0.049$

Binomial logistic regression analysis in the active PA group for *MMP-1* revealed that the codominant (p = 0.035) and dominant (p = 0.036) variables were statistically significant, but in the inactive PA group no statistical significance ($p > 0.05$) was found. There was no statistical significance in the *FGFR4* polymorphism in both groups by the activity of PA ($p > 0.05$) as well. The data are presented in Table 9.

The frequency of the *MMP-1* and *FGFR4* genotypes in the patients with PA and in the control group by the recurrence of PA were investigated. The 1G/1G genotype was more frequent in the PA without recurrence group than in the control group: 27.7% vs. 16.5%, p = 0.034. The PA without recurrence group did not match the HWE (p = 0.013), although the PA group with recurrence and control groups matched ($p > 0.05$). All groups matched the HWE for genotype distributions of *FGFR4* ($p > 0.05$) (Table 10).

Binomial logistic regression analysis in PA without recurrence group for *MMP-1* revealed that the recessive (p = 0.033) variable was statistically

Table 9. Binomial logistic regression analysis in the PA group and the control group by the activity of PA

Model	Genotype	OR (95% CI)	*p* value	AIC
MMP-1				
Inactive PA				
Codominant	1G2G	0.721 (0.319; 1.628)	0.431	195.412
	2G2G	0.948 (0.335; 2.685)	0.920	
Dominant	1G/2G + 1G/1G	0.780 (0.369; 1.648)	0.515	193.668
Recessive	1G/1G	1.125 (0.431; 2.938)	0.811	194.031
Over-dominant	1G2G	0.733 (0.346; 1.554)	0.418	193.422
Additive	–	0.918 (0.543; 1.553)	0.749	193.985
Active PA				
Codominant	1G2G	0.462 (0.226; 0.946)	0.035	301.334
	2G2G	0.547 (0.263; 1.140)	0.107	
Dominant	1G/2G + 1G/1G	0.499 (0.261; 0.956)	0.036	300.600
Recessive	1G/1G	0.909 (0.509; 1.625)	0.749	304.732
Over-dominant	1G2G	0.671 (0.380; 1.184)	0.169	302.910
Additive	–	0.768 (0.525; 1.122)	0.172	302.971
FGFR4				
Inactive PA				
Codominant	GA	2.543 (0.313; 20.682)	0.383	194.832
	AA	1.916 (0.232; 15.801)	0.546	
Dominant	G/A + A/A	2.225 (0.281; 17.598)	0.449	193.377
Recessive	A/A	0.814 (0.387; 1.714)	0.589	193.793
Over-dominant	GA	1.409 (0.672; 2.951)	0.364	193.259
Additive	–	0.957 (0.520; 1.760)	0.887	194.067
Active PA				
Codominant	GA	0.876 (0.289; 2.655)	0.815	306.751
	AA	0.848 (0.280; 2.570)	0.771	
Dominant	G/A + A/A	0.862 (0.295; 2.515)	0.786	304.763
Recessive	A/A	0.952 (0.547; 1.657)	0.861	304.804
Over-dominant	GA	1.011 (0.580; 1.761)	0.970	304.834
Additive	–	0.944 (0.602; 1.479)	0.800	304.771

Table 10. The frequency of the *MMP-1* and *FGFR4* genotypes in the patients with the PA group and in the control group by the recurrence of PA

Gene	Genotype/ allele	Frequency (%)					
		Control group *n* (%) (*n* = 200)	*p* value-HWE	PA without recurrence group *n* (%) (*n* = 83)	*p* value-HWE	PA with recurrence group *n* (%) (*n* = 17)	*p* value-HWE
MMP-1 Rs1799750	Genotype						
	1G/1G	33* (16.5)	0.768	23* (27.7)	0.013	2 (11.8)	0.900
	1G/2G	94 (47)		30 (36.1)		8 (47.1)	
	2G/2G	73 (36.5)		30 (36.1)		7 (41.2)	
	All	200 (100)		83 (100)		17 (100)	
	Allele						
	1G	160 (40.00)		76 (45.78)		12 (35.29)	
	2G	240 (60.00)		90 (54.22)		22 (64.71)	
FGFR4 Rs351855	Genotype						
	G/G	95 (47.5)	0.134	39 (47.0)	0.239	6 (35.3)	0.235
	G/A	92 (46)		39 (47.0)		10 (58.8)	
	A/A	13 (6.5)		5 (6.0)		1 (5.9)	
	All	200 (100)		83 (100)		17 (100)	
	Allele						
	G	282 (70.50)		117 (70.48)		22 (64.71)	
	A	118 (29.50)		49 (29.52)		12 (35.29)	

PA – pituitary adenoma, *p* value – significance level

* $p = 0.034$

significant. There was no statistical significance of variables in analysis of *FGFR4* in either of the groups by the recurrence of PA ($p > 0.05$). The data are presented in Table 11.

DISCUSSION

Our results revealed that the *MMP-1* gene 1G/1G genotype was more frequent in the invasive PA

Table 11. Binomial logistic regression analysis in the PA group and the control group by the recurrence of PA

Model	Genotype	OR (95% CI)	*p* value	AIC
MMP-1				
		PA without recurrence		
Codominant	1G2G	0.777 (0.430; 1.403)	0.02	343.331
	2G2G	1.696 (0.858; 3.352)	0.129	
Dominant	1G/2G + 1G/1G	1.015 (0.596; 1.729)	0.955	346.465
Recessive	1G/1G	1.940 (1.055; 3.565)	0.033	342.033
Over-dominant	1G2G	0.638 (0.377; 1.081)	0.095	343.630
Additive	–	1.240 (0.875; 1.759)	0.226	345.005
		PA with recurrence		
Codominant	1G2G	0.888 (0.308; 2.560)	0.825	124.891
	2G2G	0.632 (0.125; 3.208)	0.580	
Dominant	1G/2G + 1G1G	0.821 (0.300; 2.250)	0.702	123.074
Recessive	1G/1G	0.675 (0.147; 3.091)	0.612	124.939

Table 11. (continued)

Model	Genotype	OR (95% CI)	*p value*	AIC
Over-dominant	1G2G	1.002 (0.372; 2.703)	0.996	123.219
Additive	–	0.821 (0.397; 1.696)	0.594	122.930
FGFR4				
		PA without recurrence		
Codominant	GA	1.102 (0.368; 3.302)	0.862	348.432
	AA	1.067 (0.356; 3.196)	0.907	
Dominant	G/A+A/A	1.084 (0.374; 3.145)	0.881	346.446
Recessive	A/A	0.980 (0.587; 1.636)	0.937	346.462
Over-dominant	GA	1.041 (0.623; 1.738)	0.879	346.445
Additive	–	0.999 (0.656; 1.522)	0.996	346.468
		PA with recurrence		
Codominant	GA	1.413 (0.167; 11.963)	0.751	124.155
	AA	0.821 (0.091; 7.372)	0.860	
Dominant	G/A+A/A	1.112 (0.137; 9.056)	0.921	123.209
Recessive	A/A	0.603 (0.215; 1.693)	0.337	122.264
Over-dominant	GA	1.677 (0.614; 4.582)	0.313	122.185
Additive	–	0.738 (0.334; 1.629)	0.452	122.662

group than in the control group: 28.6% vs. 16.5%, $p = 0.044$, and the 1G/2G genotype was less frequent in non-invasive PA compared to the control group: 27% vs. 47%, $p = 0.030$. To our knowledge, there has been only one study by Altaş M. et al. (11) analyzing *MMP-1* gene polymorphism on the development of pituitary adenoma. Scientists in this research revealed that the prevalence of the *MMP-1* gene was: 36.6% had the 2G/2G genotype, 46.6% had the 1G/2G genotype, and 16.6% had the 1G/1G genotype. The 2G allele frequency was found to be 83.4%. In 90% of cases of invasive adenoma, a homozygous 2G/2G genotype was detected (11). So we are in disagreement with the study done by Altaş et al. who did not find the same association as we did (11). To our knowledge, the study of Altaş et al., did not evaluate either the association between PA hormonal activity or the association with gender. Our results revealed that the 1G/2G genotype was more frequent in women in the control group than in the PA group: 50.3% vs. 30.8%, $p = 0.011$, and the polymorphism in the *MMP-1* gene 1G/1G genotype was more frequent in the active PA group than in the control group: 28.4% vs. 16.5%, $p = 0.044$. Monsalves et al. found that *MMP-1* immunoreactivity was observed in 93% of PAs (20).

Other researchers state that *MMP-1* expression is associated with a poor outcome in such cancers as oral carcinoma (21, 22), nasopharyngeal carcinoma (23), colorectal cancer (CRC) (24), gastric cancer (25), and pancreatic cancer (26); the relevance of *MMP-1* in both CRC and esophageal cancer has been reported by Murray et al. (27, 28). Also, a few studies have shown that an elevated expression of *MMP-1* can promote local growth and formation of brain metastases by breast cancer cells (29, 30). Other studies have revealed that SLFN5 (human schlafen 5) increases *MMP-1* and *MMP-13* which promote malignant cell motility in renal cell carcinoma (31), although in meningiomas *MMP-1* did not have an effect on initiation, growth, or progression (32). Monsalves et al. state that the high *FGFR4* levels of PAs seem to be responsible for the induction of *MMP-1* expression in PAs. A similar correlation has been observed with the *FGFR4* expression levels and *MMP-1* score of pituitary tumour groups (20).

It was discovered that the other gene investigated in our study, *FGFR4-R388*, did not play any predominant role in PA development. It is thought that ptd-*FGFR4* expression can provoke invasive growth of pituitary tumour cells *in vivo* because of deprivation of membranous

N-cadherin (1, 33, 34). The FGF family has been described as having an impact on pituitary tumour activeness, aggressiveness, and invasiveness (11, 13, 15). A total of 23 FGF ligands have been identified. FGF signals are transduced through FGF receptors with specific affinity for selected receptor isoforms. Although the *FGFR4* was found to be an aggressive pituitary tumour marker (35–37), we did not discover any linkage between FGFR SNP and hypophyseal tumour development, invasiveness, and activeness, and we are in disagreement with these studies. Other researchers state that *FGFR4-R388* single nucleotide polymorphism is found in up to 50% of the population and has an impact on advanced or treatment-resistant prostate carcinoma, head and neck carcinomas, breast carcinoma, sarcomas, and colorectal carcinoma (38–41).

At this moment there are an insufficient number of studies in the literature that have investigated the polymorphism of the *MMP-1* and *FGFR4*-G388 genes promoter region in brain tumours, especially in PA. Therefore additional studies should address the progression and invasiveness of PA. The effect of the *MMP-1* and *FGFR4*-G388 promoter polymorphisms will become clearer through these types of studies and such studies will help us understand the relationship between polymorphisms of these genes and progression and invasiveness of the PA tumour.

CONCLUSIONS

The Rs1799750 polymorphism 1G/1G in the *MMP-1* gene may play a role in invasive and active PA development. This discovery contradicts with the study by Altaş M. et al. who did not find the same association as we did. It is important to research the effect of *MMP-1* in brain tumours to find out the link between this polymorphism and the progression, recurrence, activeness, and invasiveness of PA.

CONFLICT OF INTEREST

The authors of the paper declare no conflict of interest.

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MMP-1 IR *FGFR4-R388* GENŲ POLIMORFIZMŲ ĮTAKA HIPOFIZĖS ADENOMOS VYSTYMUISI

Santrauka

Įvadas. Hipofizės adenomos (toliau – HA) patogenezė yra kompleksinė ir iki šiol mažai išaiškinta. Manoma, kad HA yra daugiaveiksnės etiologijos, todėl ir genetiniai veiksniai gali turėti įtakos HA vystymuisi. *MMP-1* ir *FGFR4* genai yra susiję su auglio augimu, diferenciacija bei progresavimu. Dėl šios priežasties nusprendėme nustatyti *MMP-1* ir *FGFR4-R388* polimorfizmų genotipų įtaką HA vystymuisi.

Metodai. Tyrime dalyvavo 100 HA sergančių ir 200 sveikų kontrolinės grupės asmenų. *MMP-1* ir *FGFR4-R388* genotipavimas atliktas tikro laiko polimerazės grandinės reakcijos metodu.

Rezultatai. *MMP-1* geno polimorfizmo 1G/1G genotipas buvo statistiškai reikšmingai dažnesnis invazyvia HA sergančiųjų grupėje nei kontrolinėje grupėje: 28,6 % vs. 16,5 %, $p = 0{,}044$. 1G / 2G genotipas buvo statistiškai reikšmingai dažnesnis sveikoms moterims nei sergančioms HA: 50,3 % vs. 30,8 %, $p = 0{,}011$. Taip pat *MMP-1* geno polimorfizmo 1G/1G genotipas buvo statistiškai reikšmingai dažnesnis sergantiems aktyvia HA nei kontrolinės grupės asmenims: 28,4 % vs. 16,5 %, $p = 0{,}044$. *FGFR4-R388* polimorfizmo asociacijų su HA vystymusi nustatyta nebuvo.

Išvados. *MMP-1* geno polimorfizmo 1G/1G genotipas gali būti susijęs su invazyvios ir aktyvios HA vystymusi.

Raktažodžiai: hipofizės adenoma, matrikso metaloproteinazė-1, *FGFR4-R388*, geno polimorfizmas