

Genetic Factors of Hysteromyoma

Irina V. Krivoshei, Oksana B. Altuchova, Oleg V. Golovchenko,
Valentina S. Orlova, Alexey V. Polonikov and Mikhail I. Churnosov
Belgorod State University, Pobeda Street, 85, 308015 Belgorod, Russia

Abstract: Study concerns data of the bioinformative analysis of polymorphic options of genes at patients with hysteromyoma and women in control group. There were determined combinations genetic variations CC F13A1 with A FTO (OR = 1.55), C F13A1 with CC CNVs (OR = 1.58), G FTO with A KDM3B and C CNVs (OR = 1.38) with hysteromyoma developing in the women of Russia Central Region.

Key words: Hysteromyoma, polymorphisms, analysis, genes, variations

INTRODUCTION

Hysteromyoma is a common disease and its incidence in females >45 years old is >60% (Churnosov *et al.*, 2014a; Pachomov *et al.*, 2014; Tan, 2014; Ye *et al.*, 2015). Hysteromyomas are monoclonal tumors that arise from the uterine myometrium smooth muscle cells (Altuchova *et al.*, 2014b). According to the location of the growth, they can be categorised as submucosal when they grow just underneath the uterine lining, intramural when they are in between the muscles of the uterus and subserosal when they are on the outside of the uterus. A single fibroid can be less than one inch in size or can grow to eight inches or more. A group of fibroids can also vary in size. Fibroids may grow as a single tumour or in clusters. Although, hysteromyomas are benign, the tumors are able to trigger a number of diseases and cause severe complications including abnormal uterine bleeding, abdominal pain and discomfort, pregnancy complications and even infertility.

The cause of uterine fibroids is unknown, however genetic, hormonal, immunological and environmental factors may play a role in starting the growth of fibroids or in continuing that growth (Altuchova *et al.*, 2014a; Demakova *et al.*, 2014). In recent times, there are more and more data testifying that polymorphisms of some genes has a great importance in formation of underlying risk for hysteromyoma developing. According to literature data, >100 genes can take part in hysteromyoma formation (Rotili and Mai, 2011; Churnosov *et al.*, 2014b; Khan *et al.*, 2014).

MATERIALS AND METHODS

Investigation of associations of polymorphic markers genes under study was pursued at sampling of 1214 cases, among them, 227 are with hysteromyoma and

987 persons of control group. The sampling were women of Russian nationality coming from Russia Central Region and not being relatives. Clinic laboratory investigation was pursued based on gynecology department of perinatal center St. Joasaph Belgorod Regional Clinic Hospital. Patients with hysteromyoma were made an ultrasound investigation of pelvic organs, hysteroscopy with the following target biopsy of the lining of the uterus and scrape histologic examination there were applied general and laboratory study methods.

All the patients with hysteromyoma and the control group samples had typing of five molecular and genetic markers: F13A1 c.572-5671C>T (rs7766109), KDM3B c.193-1048G>A (rs757647), CNVs n.463+94569C>A (rs1782507), FTO c.1364+51665G>A (rs12324955), CD40LG c.1426C>T (rs92921).

Molecular genetic estimation of all the locuses was performed by method of polymerase chain reaction of DNA synthesis using oligonucleotide primers and probes (Miller *et al.*, 1988, Landi *et al.*, 2003). Genotyping of DNA markers is performed by method of TaqMan probes detection according to data of level value relative to fluorescence of each probe at «IQ5» amplificator with detecting system in real-time mode.

To estimate the correspondence of genotype distribution under study to the expected one and based on Hardy-Weinberg equilibrium, one used χ^2 -test. Estimation of role of genetic variants combinations in contraction of hysteromyoma is performed using the software APSampler using Markov chains Monte Carlo technique and Bayesian distribution-free statistics.

RESULTS AND DISCUSSION

After examination of 227 women with hysteromyoma and 987 women from the control group, it was determined that the control group is completely commensurable with

sampling of cases with hysterosarcoma by gender, age, nationality and place of birth and by height and weight ($p>0.05$). Main characteristics of the studied groups are given in Table 1.

Examination of alleles concentration of genes polymorphic markers under study showed that for all the examined locuses in the group of patients with hysterosarcoma and in population sampling, empiric genotype distribution corresponded to the expected one at Hardy-Weinberg equilibrium ($p>0.05$) (Table 2).

With the help of bioinformative approaches, it was stated that individuals with hysterosarcoma differ from control group by distribution of three various combinations of four polymorphic locuses F13A1 c.572-5671C>T (rs7766109), KDM3B c.193-1048G>A (rs757647), CNVs n.463+94569C>A (rs1782507), FTO c.1364+51665G>A (rs12324955) (Table 3).

FTO (fat mass and obesity associated gene) was the first obesity-susceptibility gene identified through GWAS and continues to be the locus with the largest effect on Body Mass Index (BMI) and obesity risk, most widely replicated with variety of obesity traits throughout the life course and across diverse ancestries (Loos and Yeo, 2014; Tung *et al.*, 2015). Obesity is an important risk factor for cardiovascular and metabolic disease (Lu and Loos, 2013). Hence, it comes as no surprise that because of the robust association between FTO and BMI, FTO SNPs are

also associated with a range of cardiometabolic traits (Fall *et al.*, 2013; Bravard *et al.*, 2014). In a recent large-scale meta-analysis of 36 studies ($n =$ up to 198,502) that examined FTO's effects on 24 cardiometabolic traits, the BMI-increasing allele of the FTO SNP was associated with increased risk of type 2 diabetes, heart failure, coronary heart disease, ever all-cause and ischemic stroke, hypertension, dyslipidemia, metabolic syndrome and mortality and also with increased fasting glucose and insulin levels, 2h-OGTT glucose levels, HbA1c, blood pressure, lipid levels, liver enzymes and inflammation markers (Freatly *et al.*, 2008).

Kdm3b is a Jumonji C domain-containing protein that demethylates mono- and di-methylated lysine 9 of histone H3 (H3K9me1 and H3K9me2). Although, the enzyme activity of Kdm3b is well characterized *in vitro*, its genetic and physiological function remains unknown. Disruption of Kdm3b function decreases IGFBP-3 expression which results in fast degradation of IGF-1 and small body size (Kasioulis *et al.*, 2014). The loss of Kdm3b function also prolongs female estrous cycle and decreases ovulation capacity, oocyte fertilization rate, embryo implantation, decidual response and embryo growth (Delahanty *et al.*, 2013). Together, these defects in reproductive function result in a female infertile phenotype. These defects are associated with extensive alterations of H3K9me1, H3K9me2 and/or H3K9me3 levels in the ovarian and uterine cells where Kdm3 is highly expressed (Kim *et al.*, 2012). These findings suggest that KDM3B mutation or disrupted pathways regulated by KDM3B might be present in human patients with growth retardation and/or female reproductive problems.

Accumulating evidence suggests a role of the blood coagulation factor gene *F13A1* in obesity (Zhang *et al.*,

Table 1: Characteristics of the subjects from the case and control groups

Characteristics	Cases	Controls
Total	227	987
Age (years)	40.05±10.08	42.2±8.5
Weight (kg)	60.8±1.9	63.4±2.8
Height (cm)	164.8±4.1	167.5±3.7

Table 2: Summary information about the studied polymorphisms

Polymorphism	Studied groups	Minor allele	MAF (%)	HWE	
				χ^2 -values	p-values
F13A1 c.572-5671C>T (rs7766109)	Case	T	51.13	0.360	>0.05
F13A1 c.572-5671C>T (rs7766109)	Control	T	53.50	1.110	>0.05
KDM3B c.193-1048G>A (rs757647)	Case	A	25.47	0.460	>0.05
KDM3B c.193-1048G>A (rs757647)	Control	A	23.98	1.660	>0.05
CNVs n.463+94569C>A (rs1782507)	Case	A	63.01	0.001	>0.05
CNVs n.463+94569C>A (rs1782507)	Control	A	65.13	1.870	>0.05
FTO c.1364+51665G>A (rs12324955)	Case	A	27.93	0.190	>0.05
FTO c.1364+51665G>A (rs12324955)	Control	A	28.06	0.740	>0.05
CD40LG c.1426C>T (rs92921)	Case	T	7.49	0.070	>0.05
CD40LG c.1426C>T (rs92921)	Control	T	7.85	0.840	>0.05

MAF: Minor Allele Frequency; Hardy: Weinberg equilibrium; p-values were calculated using the χ^2 -test

Table 3: Concentration combinations of alleles/genotypes of genes in patients with hysterosarcoma and in the control group

SNP 1	SNP 2	SNP 3	Carriage		Fisher's p-values	Odds ratio (95% CI)
			Case	Control		
CC F13A1	A FTO	-	13.57	9.20	0.03	1.55 (1.03-2.41)
C F13A1	CC CNVs	-	11.47	7.57	0.04	1.58 (0.98-2.55)
G FTO	A KDM3B	C CNVs	28.97	22.85	0.04	1.38 (0.99-1.92)

2015). Genotype frequencies of the F13A1 SNP rs7766109 were equivalent in PCOS and control women. In PCOS women, *F13A1* gene variants were significantly associated with Body Mass Index (BMI) ($p = 0.013$), systolic blood pressure ($p = 0.042$), insulin response (AUCins) ($p = 0.015$), Triglycerides (TG) ($p = 0.001$) and High Density Lipoprotein cholesterol (HDL) ($p = 0.012$) (Schweighofer *et al.*, 2012). In the subgroup of obese PCOS women Free Androgen Index (FAI), free testosterone and Sex Hormone Binding Globulin (SHBG) as well as glucose measurements showed a significantly different pattern across *F13A1* gene variants ($p = 0.043$; $p = 0.039$ and $p = 0.013$, respectively) (Schweighofer *et al.*, 2012).

CONCLUSION

Therefore, the results of work allow making a conclusion that combinations of genetic variations CC F13A1 with A FTO (OR = 1.55), C F13A1 with CC CNVs (OR = 1.58), G FTO with A KDM3B and C CNVs (OR = 1.38) are risk factors for hystero myoma in the women of Russia Central Region.

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