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The assessment of GWAS — identified polymorphisms associated with infertility risk in Polish women with endometriosis

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ABSTRACT

Objectives: Genome-wide association studies in patients with endometriosis revealed ten significant single nucleotide polymorphisms (SNPs) in the Caucasian population, which include rs12700667 near *NFE2L3*, rs12037376 in *WNT4*, rs7521902 near *WNT4*, rs13394619 in *GREB1*, rs10859871 near *VEZT*, rs1537377 near *CDKN2B-AS1*, rs4141819 near *ETAA1*, rs7739264 near *ID4*, rs1519761 near *RND3* and rs6542095 near *IL1A*.

Material and methods: We replicated ten polymorphisms among infertile women with endometriosis (n = 315) and healthy fertile women (n = 406) in the Polish Caucasian population. Genotyping was conducted either by high-resolution melting curve analysis or by a pre-designed TaqMan probe.

Results: For all infertile women with endometriosis, the p values of the Cochran-Armitage trend test for the rs12700667 SNP was p_{trend} = 0.038 and the odds ratio (OR) for the risk allele frequency (RAF) of rs12700667 was 1.304 (95% CI = 1.009–1.685; p = 0.042). In patients with endometriosis with severity stages III/IV, p_{trend} for rs12700667 SNP was 0.036 and OR for the RAF was 1.394 (95% CI = 1.010–1.923; p = 0.043). In infertile women with endometriosis with severity stages III/IV for rs4141819 SNP, we observed p_{trend} = 0.026 and for RAF the OR = 1.350 (95% CI = 1.032–1.766; p = 0.029).

Conclusions: Our results demonstrate association of RAF of rs12700667 and rs4141819 SNPs with infertility in Polish women with advanced endometriosis.

Key words: GWAS, endometriosis, infertility

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INTRODUCTION

Endometriosis is a complex gynaecological health problem in women, which is characterized by the aberrant presence of endometrial cells outside the uterus [1]. This benign disease is usually limited to the pelvis and is characterized by peritoneal inflammation, neovascularization, fibrosis, and ovarian cysts [1]. The morbidity for endometriosis ranges from 5–10% and is accompanied by subfertility in women at reproductive age [2, 3]. Despite the extensive conducted studies, the aetiology of endometriosis is still unclear. The presence of endometrial tissue and cells in the peritoneal cavity has been explained by the standard theory of retrograde menstruation along the fallopian tubes [4]. Although retrograde menstruation is observed in 90% of women, merely 10–20% of women suffer from endometriosis, which suggests the involvement of different genetic and environmental components in *etiopathogenesis* [5, 6]. Recently, the genetic and epigenetic background of endometriosis has been demonstrated [5]. The aetiology of endometriosis may be related to genetic factors, which increases oestrogen activity, the production of prostaglandins, cytokines, and metalloproteinases, as well as enhances of oncogenic

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Table 1. Characteristics of SNPs genotyped in the data set

SNP	Locus	Location (bp)[a]	Alleles[b]	SNP location	Gene[c]
rs12700667	7p15.2	25901639	A / **G**	intergenic	*NPVF / NFE2L3*
rs12037376	1p36.12	22462111	**A** / G	intronic	WNT4
rs7521902	1p36.12	22490724	**A** / C	intergenic	*WNT4 / ZBTB40*
rs13394619	2p25.1	11727507	**A** / G	intronic (function: splice-3)	GREB1
rs10859871	12q22	95711876	A / **C**	intergenic	*VEZT / METAP2*
rs1537377	9p21.3	22169700	**C** / T	intergenic	*CDKN2B / DMRTA1*
rs4141819	2p14	67864675	**C** / T	intergenic	*ETAA1 / C1D*
rs7739264	6p22.3	19785588	**C** / T	intergenic	*RNF144B / ID4*
rs1519761	2q23.3	151633204	A / **G**	intergenic	*RND3 / RBM43*
rs6542095	2q13	113529183	**C** / T	intergenic	*CKAP2L / IL1A*

[a] — NCBI build 37 / hg19; [b] — underline denotes the minor allele; [c] — genes separated by forward slash indicate nearest protein coding genes upstream / downstream of the SNP

pathways [5]. To date, five genome-wide association studies (GWAS) have been conducted on four independent groups, encompassing women with endometriosis [7–13]. These GWAS analyses have revealed ten genome-wide significant loci in Caucasians, presented in Table 1 [7–13]. Despite finding several genome-wide significant loci linked to endometriosis, there is still a need for confirmation in different populations.

We aimed to assess the rs12700667, rs12037376, rs7521902, rs13394619, rs10859871, rs1537377, rs4141819, rs7739264, rs1519761 and rs6542095 SNPs genome-wide significant loci (Tab. 1) as possible infertility risk in Polish women with endometriosis.

MATERIAL AND METHODS

A case-control study

A case-control study design was used in 315 patients with endometriosis and 406 matched controls (Tab. 2). Peripheral blood samples were randomly obtained between November 2014 and December 2017 from infertile women with endometriosis and control women of similar ages from the Gynaecologic and Obstetrical University Hospital, Division of Reproduction at Poznan University of Medical Sciences, Poland (Tab. 1). After informed consent was obtained, approximately 7 mL venous blood was drawn and collected into the Vacutainer System with EDTA (Sarstedt, Germany) and stored at –20°C until DNA isolation was performed.

The patients with infertility and endometriosis underwent laparoscopy and had a histologically confirmed diagnosis at the Gynaecologic and Obstetrical University Hospital, Division of Reproduction at Poznan University of Medical Sciences, Poland. Patients with endometriosis were divided into two subgroups according to the revised American Society for Reproductive Medicine (rASRM) classification system [14]; n = 142 patients had minimal or mild endometriosis (stages I–II), n = 166 had moderate or severe endometriosis (stages III–IV), and n = 7 patients had an undefined stage of endometriosis (Tab. 2). The control group was comprised of healthy women (n = 406), without history of infertility, who had a caesarean section performed (Tab. 2).

The inclusion and exclusion criteria for the infertile women with endometriosis and the women without disease were previously described [15]. The inclusion criteria for infertile women diagnosed with endometriosis were as follows: regular menses, no anatomical changes in the reproductive tract, no hormonal treatments, and a minimum of one year of infertility with a current desire for conception. The exclusion criteria were as follows: mechanical distortion of the endometrial cavity by fibroids, bilateral tubal occlusion, male factor infertility, adenomyosis, polycystic ovary syndrome (PCOS) and benign or malignant gynaecological diseases. The inclusion criteria for fertile control women were as follows: performed caesarean section, regular menses, no anatomical changes in the reproductive tract, no hormonal treatments, and at least one child born no more than one year before the study (Tab. 2). The exclusion criteria were as follows: signs of past or present inflammation, pelvic abnormalities, endometriosis, adenomyosis, PCOS or any other benign or malignant gynaecological diseases, which was confirmed during surgical exploration. Both patients with endometriosis and healthy controls were all Caucasians of Polish ancestry (Tab. 2).

Ethical approval

All procedures performed in this study which involved human participants were in accordance with the ethical standards of the ethics committee of Poznan University of Medical Sciences and with the 1964 Helsinki declaration and its ethical standards. Informed consent was obtained from all individual participants included in the study.

Table 2. Characteristics of the populations of infertile women with endometriosis

Characteristics and fertile healthy women	Infertile women with endometriosis	Fertile healthy women
Numbers	315	406
Age (years)	31 (21–36)[a]	31 (19–38)[a]
Parity	NA	1 (1–4)[a]
Duration of infertility (years)	3 (1–7)[a]	NA
rASRM (stage)[b]	Stage I and II (n = 142 Stage III and IV (n = 166) Undefined (n = 7)	NA

[a] — median (range); [b] — revised American Society for Reproductive Medicine (rASRM) [14]; NA — not applicable

Genotyping of rs12700667, rs12037376, rs7521902, rs13394619, rs10859871, rs1537377, rs4141819, rs7739264, rs1519761 and rs6542095 SNPs

Genomic DNA was isolated from peripheral blood leukocytes by salt extraction. The SNPs evaluated in this study were selected based on GWAS and meta-analyses studies [8, 9, 11–13]. Genotyping of rs12700667, rs12037376, rs7521902, rs13394619, rs10859871, rs1537377, rs4141819, rs7739264, and rs1519761 variants was carried out by high resolution melting curve analysis (HRM) on the LightCycler 96 system (Roche Diagnostics, Mannheim, Germany) with the use of 5x HOT FIREPol EvaGreen HRM Mix (Solis BioDyne, Tartu, Estonia). The PCR programme consisted of an initial step at 95°C for 15 minutes to activate HOT FIREPol DNA polymerase, followed by 50 amplification cycles of denaturation at 95°C for 10 seconds, primer-dependent annealing (Tab. 3) for 10 seconds, and elongation at 72°C for 15 seconds. Amplified DNA fragments were then subjected to HRM with 0.1°C increments in temperatures ranging from 65 to 95°C (Tab. 3). The final concentrations of reagents in HRM reactions were as follows: 1x HOT FIREPol EvaGreen HRM Mix, 0.2 pmol/μL of each primer and 2 ng/μL DNA template. The HRM reactions were performed in a 10 μL volume. Genotyping of rs6542095 was carried out on the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany) using pre-designed TaqMan SNP, according to the manufacturer's instructions provided by Applied Biosystems (Applied Biosystems, Foster City, CA, USA) (Tab. 3).

Data analysis

Hardy-Weinberg equilibrium (HWE) was assessed by Pearson's goodness-of-fit Chi-square (χ^2) statistic. The SNPs were studied for associations with endometriosis using the

Table 3. Primers and HRM conditions for replication genotyping

rs no.	Alleles	Primers for PCR amplification (5′–3′)	PCR product length (bp)	Annealing temp. (°C)	Melt. temp. range (°C)
rs12700667	A / G	F: GAGAGTGAAAATGTGACAAAAGTGA	90	55	74–89
		R: AAGCGCCACACCATATACATC			
rs12037376	A / G	F: GAGACCACAGGCTTCCATA	78	55	78–93
		R: TTCAGGAGTAAGGGGTGCT			
rs7521902	A / C	F: GCTCTGTCTTCGAGGCACTT	104	53	80–95
		R: TCCCAATTACATGATCCTCTCC			
rs13394619	A / G	F: CCCCTTGTCACTTCTCTGTC	119	55	75–90
		R: TACCATTTGGGTAGCACCA			
rs10859871	A / C	F: CAAGTGGGCAATTTATTTCTCTG	144	58	74–89
		R: TGCAATAGGATTCTCACATTAACCT			
rs1537377	C / T	F: CAGCTCTACTCTTGGATTTGG	110	55	72–87
		R: ATGCATAACAGTCTATAAGTAGG			
rs4141819	C / T	F: CCTCAGGTGAAAGTTCATGC	85	55	75–90
		R: TGAGGAAAGTGGCTAGAGGA			
rs7739264	C / T	F: GAGGCCACTCACTACAATGC	137	53	75–90
		R: CCTCTTGGACAGATTTTCCTG			
rs1519761	A / G	F: CAAAAATATGTTGTATATGAG	91	53	65–80
		R: TAATCCATGTTTTCCTAC			

Genotyping of rs6542095 was carried out on the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany) using pre-designed TaqMan SNP Genotyping Assay according to the manufacturer's instructions provided by Applied Biosystems (Applied Biosystems, Foster City, CA)

Cochran-Armitage trend test. Differences in the risk allele frequencies (RAF) between the cases and controls were calculated using χ^2 analysis. The odds ratio (OR) and 95% confidence intervals (95% CI) were also calculated. The statistical analyses were conducted with Statistica version 10 (2011, Stat Soft, Inc., Tulsa, USA).

RESULTS

The comparison of rs12700667, rs12037376, rs7521902, rs13394619 rs10859871, rs1537377, rs4141819, rs7739264, rs1519761 and rs6542095 genotypes and allele frequencies between all infertile women with endometriosis and fertile healthy women

There was no divergence from the HWE in the frequency of genotypes between all infertile women with endometriosis and the fertile women groups (Tab. 4). The prevalence of the genotype and allele frequencies, OR and 95% CI calculated for the ten SNPs are presented in Table 4. The statistical significance of the p values of the Cochran-Armitage trend test (p_{trend} = 0.038) was found for the rs12700667 SNP and the OR for RAF was 1.304 (95% CI = 1.009–1.685; p = 0.042). However, none of the SNPs, rs12037376, rs7521902, rs13394619, rs10859871, rs1537377, rs4141819, rs7739264, rs1519761 or rs6542095 displayed a significant association with all infertile women with endometriosis in the additive inheritance model (Tab. 4).

The comparison of rs12700667, rs12037376, rs7521902, rs13394619, rs10859871, rs1537377, rs4141819, rs7739264, rs1519761 and rs6542095 genotypes and allele frequencies between infertile women with endometriosis in stages III and IV and fertile healthy women

We found divergence from the HWE in the frequency of genotypes in the subgroup with endometriosis with severity stage I/II for rs1519761 but no divergence was observed from the HWE for other subgroups of women with

Table 4. Association between GWAS-identified polymorphisms and the risk of endometriosis

SNP	Genotypes	RAF[a]	p_{trend}	$OR_{allelic}$ (95%CI)[b]; p value[c]	HW[d]
rs12700667	**AA / AG / GG**	**A — major**			
Controls	229 / 154 / 19	0.76			0.560
Cases	201 / 101 / 10	0.81	**0.038**	**1,304 (1,009–1,685); 0.042**	0.821
Cases stage I and II	91 / 42 / 7	0.80	0.178	1,255 (0.898–1,754); 0.183	0.761
Cases stage III and IV	108 / 55 / 3	0.82	**0.036**	**1,394 (1,010–1,923); 0.043**	0.403
rs12037376	**GG / AG / AA**	**A — minor**			
Controls	285 / 112 / 8	0.16			0.732
Cases	211 / 89 / 11	0.18	0.302	1,157 (0.876–1,530); 0.304	0.914
Cases stage I and II	97 / 39 / 5	0.17	0.533	1,121 (0.781–1,608); 0.537	0.909
Cases stage III and IV	109 / 48 / 6	0.18	0.281	1,202 (0.857–1,685); 0.286	0.969
rs7521902	**CC / AC / AA**	**A — minor**			
Controls	218 / 155 / 24	0.26			0.876
Cases	159 / 133 / 18	0.27	0.462	1,091 (0.860–1,384); 0.474	0.354
Cases stage I and II	71 / 63 / 5	0.26	0.814	1,037 (0.760–1,415); 0.820	0.133
Cases stage III and IV	85 / 68 / 12	0.28	0.417	1,125 (0.843–1,502); 0.422	0.950
rs13394619	**GG / AG / AA**	**A — minor**			
Controls	126 / 184 / 89	0.45			0.379
Cases	96 / 154 / 59	0.44	0.618	0.947 (0.767–1,170); 0.612	0.981
Cases stage I and II	44 / 75 / 22	0.42	0.365	0.879 (0.668–1,157); 0.358	0.562
Cases stage III and IV	52 / 74 / 37	0.45	0.992	1,001 (0.773–1,297); 0.991	0.561
rs10859871	**AA / AC / CC**	**C — minor**			
Controls	197 / 166 / 42	0.31			0.728
Cases	140 / 151 / 23	0.31	0.835	1,024 (0.818–1,282); 0.837	0.117
Cases stage I and II	69 / 65 / 8	0.29	0.459	0.894 (0.664–1,204); 0.460	0.344
Cases stage III and IV	67 / 85 / 13	0.34	0.357	1,135 (0.864–1,491); 0.362	0.142

Table 4 (cont.). Association between GWAS-identified polymorphisms and the risk of endometriosis

SNP	Genotypes	RAF[a]	p_{trend}	$OR_{allelic}$ (95%CI)[b]; p value[c]	HW[d]
rs1537377	**TT / CT / CC**	**C — minor**			
Controls	144 / 198 / 58	0.39			0.749
Cases	101 / 165 / 45	0.41	0.491	1,075 (0.869–1,332); 0.505	0.234
Cases stage I and II	47 / 78 / 16	0.39	0.941	0.990 (0.750–1,307); 0.943	0.155
Cases stage III and IV	50 / 84 / 29	0.44	0.173	1,194 (0.920–1,551); 0.182	0.828
rs4141819	**TT / CT / CC**	**C — minor**			
Controls	195 / 171 / 38	0.31			0.998
Cases	124 / 151 / 31	0.35	0.0856	1,212 (0.9693–1,517) 0.0913	0.311
Cases stage I and II	64 / 64 / 13	0.32	0.672	1,065 (0.795–1,425); 0.674	0.870
Cases stage III and IV	60 / 87 / 18	0.37	**0.026**	**1,350 (1,032–1,766); 0.029**	0.261
rs7739264	**TT / CT / CC**	**C — minor**			
Controls	124 / 191 / 89	0.46			0.636
Cases	92 / 149 / 70	0.46	0.770	1,033 (0.837–1,273); 0.765	0.808
Cases stage I and II	42 / 62 / 37	0.48	0.473	1,108 (0.845–1,454); 0.458	0.366
Cases stage III and IV	47 / 84 / 32	0.45	0.935	0.989 (0.764–1,281); 0.934	0.881
rs1519761	**AA / AG / GG**	**G — minor**			
Controls	125 / 209 / 65	0.42			0.357
Cases	104 / 162 / 44	0.40	0.394	0.915 (0.739–1,132); 0.413	0.319
Cases stage I and II	42 / 84 / 14	0.40	0.441	0.903 (0.684–1,191); 0.469	0.013
Cases stage III and IV	59 / 76 / 28	0.40	0.531	0.921 (0.709–1,197); 0.539	0.918
rs6542095	**TT / CT / CC**	**C – minor**			
Controls	201 / 178 / 25	0.28			0.212
Cases	168 / 126 / 21	0.27	0.501	0.925 (0.732–1,169); 0.514	0.922
Cases stage I and II	76 / 53 / 13	0.28	0.985	0.980 (0.725–1,325); 0.897	0.702
Cases stage III and IV	87 / 71 / 8	0.26	0.467	0.903 (0.677–1,206); 0.490	0.394

RAF — risk allele frequency

endometriosis with severity stages I/II and III/IV (Tab. 4). The prevalence of the genotype and allele frequencies, OR, and 95% CI calculated for the ten SNPs for both fertile healthy women and women with endometriosis stages I/II and III/IV are presented in Table 4. In patients with endometriosis with severity stages III/IV, for the rs12700667 SNP, we found statistically significant p values of the Cochran-Armitage trend test (p_{trend} = 0.036) and OR for the RAF of 1.394 (95% CI = = 1.010–1.923; p = 0.043). In infertile women with endometriosis stages III/IV, for rs4141819 SNP, we observed statistically significant p values of the Cochran-Armitage trend test (p_{trend} = 0.026) and OR for the RAF of 1.350 (95% CI = 1.032– –1.766; p = 0.029). However, we did not find any association between rs12700667 and rs4141819 SNPs in women with endometriosis severity stages I/II. Moreover, rs12037376, rs7521902, rs13394619, rs10859871, rs1537377, rs7739264, rs1519761 and rs6542095 failed to display a significant association with infertile women with endometriosis in stages I/II and III/IV in additive inheritance models (Tab. 4).

DISCUSSION

Large twin studies revealed approximately 50% heritability of endometriosis [16]. Numerous investigations on various candidate genes have been carried out to study the genetic background of endometriosis; however, some studies did not replicate these results [16, 17]. Previous efforts to study the genetic background of endometriosis suggest that it is a highly complex field [5, 16, 17].

Previous replication studies and meta-analysis studies conducted on GWAS in European ancestry cohorts found ten genome-wide significant single nucleotide polymorphisms (SNPs) associated with advanced endometriosis in Caucasians [7–13,18]. These include rs12700667 on 7p15.2 near NFE2L3 (erythroid-derived 2-like 3), rs12037376 in WNT4 (wingless-type MMTV integration site family, member 4), rs7521902 on 1p36.12 near WNT4, rs13394619 on 2p25.1 in GREB1 (growth regulation by oestrogen in breast cancer 1), rs10859871 on 12q22 near VEZT (vezatin, adherens junctions transmembrane protein),

rs1537377 on 9p21.3 near CDKN2B-AS1 (cyclin-dependent kinase inhibitor 2B antisense RNA), rs4141819 on 2p14 near ETAA1 (ewing tumour-associated antigen 1), rs7739264 on 6p22.3 near ID4 (inhibitor of DNA binding 4, dominant negative helix-loop-helix protein), rs1519761 on 2q23.3 near RND3 (Rho family GTPase 3 gene) and rs6542095 on 2q13 near IL1A (interleukin 1A) (Tab. 1) [7–13,18].

In our study we found association of the rs12700667 polymorphism with infertility in Polish women with advanced endometriosis. The GWAS study conducted by Painter_et al. 2011 demonstrated rs12700667 SNP association with advanced endometriosis in Australia and the UK cohort and was replicated in independent cohort from the United States [10]. The rs12700667 SNP is located ~290.2 kb upstream of the nuclear factor NFE2L3. Recently, in a Chinese population, it was reported that rs12700667 significantly increased the risk of ovarian endometriosis [19].

We also found contribution of the rs4141819 polymorphism to infertility in Polish women with advanced endometriosis. The GWAS study carried out by Nyholt et al. 2012 revealed in European and Japanese ancestry rs4141819 SNP as risk factor for advanced endometriosis [9]. The rs4141819 SNP is situated ~227.0 kb downstream of the ETAA1 gene that encodes a tumour-specific cell surface antigen in the Ewing family of tumours [20]. The rs4141819 SNP is also located in the intronic region of a long non-coding RNA (lncRNA), AC007422.1, which has an unknown biological function. The association of rs12700667 and rs4141819 SNPs should be repeated in other independent Polish cohorts to assess the possible diagnostic value of these polymorphism in the development of infertility in Polish women with endometriosis.

However, we did not observe an association between rs12037376, rs7521902, rs13394619, rs10859871, rs1537377, rs7739264, rs1519761 and rs6542095 SNPs with infertility in Polish women with advanced endometriosis.

The GWAS study conducted in endometriotic women with European ancestry found association with the intrononic rs12037376 SNP of *WNT4* and rs7521902 SNP situated ~21.3 kb downstream of *WNT4* [8, 9]. *WNT4* is considered to be associated with the development of the female genital tract and steroidogenesis [21].

Both rs13394619 and rs10859871 are endometriosis susceptible SNPs, that have been identified by GWAS meta-analysis in Japanese and European populations [9]. The rs13394619 SNP is situated in the intron region between exon 9 and exon 10 in the *GREB1* gene, which is involved in hormone-dependent breast cancer cell growth [22]. Elevated expression of *GREB1* has been suggested to be involved in oestrogen-dependent growth in peritoneal endometriosis [23]. The rs10859871 SNP is located ~15.3 kb downstream of the *VEZT* gene, which is a putative tumour suppressor gene encoding an adherens junction transmembrane protein [24]. The rs10859871 SNP was replicated in Italian Caucasian women with endometriosis [25]. Recently, Holdsworth-Carson et al. (2016) reported the association of the rs10859871 SNP with increased *VEZT* expression in the secretory phase of the menstrual cycle in endometrial glands of women with endometriosis [26].

In the Nyholt et al., (2012) study of European ancestry they found an association of rs1537377 and rs7739264 SNPs with endometriosis, excluding cases with minimal or unknown severity [9]. The rs1537377 SNP is located ~48 kb upstream of the *CDKN2B-AS1* gene. This gene, which is at the 9p21.3. locus, was first demonstrated in GWAS of endometriosis in a Japanese population [11]. CDKN2B-AS1 regulates *CDKN2A*, *CDKN2B* and *ARF* expression, which are known as tumour suppressor genes [27–29]. *CDKN2A* inactivation has been observed in endometriosis and endometrial cancer via loss of heterozygosity and promoter hypermethylation [30, 31]. The rs7739264 SNP is situated ~52.0 kb upstream of the *ID4* gene and in the intronic region of lncRNA, *RP1–167F1.2*, with unknown function. Changes in *ID4* expression is involved in ovarian and breast carcinogenesis [32, 33].

The GWAS study carried out by Albertsen et al. (2013) has identified rs1519761 SNP to be associated with endometriosis in a European cohort [8]. The rs1519761 is located in the intergenic region ~289 kb downstream of the *RND3* gene (OMIM *602924).

The Sapkota et al. (2015) study on a population of European ancestry revealed an association of rs6542095 SNP with a genome-wide significance in patients with moderate-to-severe endometriosis [13]. The rs6542095 SNP is situated ~2.3 kb downstream of the *IL1A* gene. This SNP may regulate the expression of other genes, which could support the evidence for a relationship between the pathogenesis of endometriosis and inflammatory responses [34].

CONCLUSIONS

In our studies we replicated the rs12700667 and rs4141819 association of RAF with infertility in Polish women with advanced endometriosis. However, we did not replicate rs12037376, rs7521902, rs13394619, rs10859871, rs1537377, rs7739264, rs1519761 and rs6542095 SNPs in infertile women, neither with all stages of endometriosis nor advanced endometriosis. The frequency of genetic polymorphisms varies according to ethnic groups, which may have effect on the sample size to get statistical power of study. Our study attributes the small population and lack of sufficient power for replication. Therefore, this study should be replicated in other independent cohorts.

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