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Validation of PCR-RFLP techniques for the evaluation of codon 72 of p53 and CYP1A1 gene's polymorphisms in relation with ovarian cancer in a Romanian population

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Abstract

Epidemiological studies suggest that the onset and progression of ovarian cancer are associated with the presence of estrogens. CYP1A1 gene has two polymorphisms, which may affect the estrogens' metabolites and contribute to increased susceptibility to neoplastic transformation of ovarian cells. P53 is a tumor suppressor gene, which acts to preserve stability of human genome. Codon 72 polymorphism of p53 gene was correlated with susceptibility for ovarian cancer. The aim of our study was to validate the use of PCR-RFLP techniques for the evaluation of p53 codon 72 and CYP1A1 gene polymorphisms in relation with ovarian cancer in a Romanian population and to evaluate gene–environment interaction in this context. The case-control study included 42 subjects. The assessment of risk and protective factors was performed using a questionnaire. Polymorphisms of CYP1A1 and p53 genes were assessed using the validated PCR-RFLP techniques. The statistical analysis was performed using Epi Info 3.5.1 software. Frequency of Arg/Arg genotype of p53 gene was higher among cases (43%) compared with controls (33.3%), but the difference was not statistically significant (p=0.75). The presence of Ile/Val polymorphism of CYP1A1 gene was identified in 9.5% of the cases and the MspI polymorphism of CYP1A1 gene was not identified in our subjects. Validation of techniques consisted in the optimization of RFLP methods for p53 and CYP1A1 genes polymorphism analyzing that allowed highlighting the existence of codon 72 polymorphism of p53 gene and Ile/Val polymorphism of CYP1A1 gene in the population from this region.

Keywords: ovarian cancer, risk factors, CYP1A1 polymorphism, p53 polymorphism.

Introduction

One of the modern directions of epidemiology is *molecular epidemiology*, which focuses on the potential contribution of genetic and other risk factors, identified at the molecular level, to the etiology, distribution and prevention of disease in different families or populations. It can be defined briefly as the application of molecular biology techniques to the study of populations, with a particular focus on investigating diseases [1, 2].

In molecular epidemiology studies, the main research methodology issues include: the information provided to subjects and type of consent obtained from participants in the study (written, clear and informed consent for participation in the study, from both cases and controls); how to collect, transport and store the samples, in relation to the stability of different components; the amount of blood to be collected; reproducibility and repeatability of laboratory tests; heterogeneity of results (measurement errors, selection errors, inter-population and intra-population variability in response, unpredictable interactions between environmental exposures and genetic susceptibility);confounding factors; publication bias; laboratory methods used; biological interpretation of the tests; the power of association; statistical considerations (need of stratification to remove bias); ethical considerations (follow respectful protocols to obtain information; establish confidentiality and security safeguards; manage anonymisation of database in case of cooperation; ownership and intellectual property policies; respectful written, clear and informed consent of subjects participating in the study; be clear about communication protocol (whether and how subjects will be informed) of findings that might be medically helpful for them [3], and Ethics Committee approval).

Epidemiological research findings suggest that the onset and progression of ovarian cancer are associated with the presence of estrogens. CYP1A1 is a gene that encodes a member of the cytochrome P450 enzyme superfamilies, which acts in the oxidation of several chemicals, including estrogens. They are metabolized by cytochrome P450 and converted into 4-OH catechol-estrogens, which are carcinogens. CYP1A1 gene has several polymorphisms, the most common being T6235C transition in the non-coding 3'-flanking region (MspI

polymorphism) and another one being translation in exon 7 A4889G (Ile/Val polymorphism), resulting in the replacement of isoleucine (Ile) by valine (Val) [4–6]. These polymorphisms may affect the estrogens' metabolites and contribute to increased susceptibility to neoplastic transformation of ovarian cells. Estrogens play two roles in carcinogenesis, as a hormone stimulating cell proliferation and as procarcinogen inducing genetic alterations by the action of free radicals [7].

P53 is a tumor suppressor gene, located on the short arm of chromosome 17, involved in multiple pathways including apoptosis, cellular transcriptional control and cell cycle regulation [8]. The role of this gene is to preserve stability of human genome. It has a polymorphism at codon 72, with a single base change causing an amino acid substitution in the transactivation domain of the protein – Arg (CGC) by Pro (CCC) [9]. There was recently suggested that this polymorphism influence the risk for ovarian cancer [10], but the correlation to the susceptibility for such malignancies has not been very much studied, and the results of previous studies are contradictory.

A large number of studies have been conducted to investigate the direct modulator effect of genetic polymorphisms on the risk, prognosis and survival in ovarian cancer, but most of them have reported negative results, and, relating to gene-environment interaction, a small number of studies have been developed [5, 7–13].

The aim of our study was to validate the use of PCR-RFLP techniques for the evaluation of p53 codon 72 and CYP1A1 gene polymorphisms in relation with ovarian cancer in a Romanian population and to evaluate gene-environment interaction in this context.

Materials and Methods

We conducted a case-control study in which 42 subjects were included. Ovarian cancer cases were selected from the Oncology Clinic of "St. Spiridon" University Hospital Iassy, in 2009–2010. The control subjects were selected from persons who were hospitalized in the Infectious Diseases Clinics for different acute diseases or carers of children with acute illnesses. The assessment of risk and protective factors was performed using a questionnaire, validated in a previous study [14]. MspI and Ile/Val polymorphisms of CYP1A1 and p53 codon 72 polymorphism were assessed using the validated PCR-RFLP techniques. Collected epidemiological data were included in an Excel database which was then processed descriptive (means, medians, frequencies) and analytical (odds ratio) using Epi Info 3.5.1 software. For the evaluation of statistical significance, *t*-Student and χ^2 tests were used. In interpreting the results, a level of $p<0.05$ for a 95% confidence interval were considered significant.

After obtaining informed consent, 10 mL of peripheral blood from each patient were collected, distributed in two tubes of 5 mL each filled with anticoagulant (heparin, EDTA or sodium citrate). DNA extraction was performed on whole blood collected on the same day or within 24 hours before and preserved in a refrigerator at 4^0C. Samples were processed separately, identification data recorded for each sample (date of collection, date of extraction, blood volume, anti-coagulant used, the patient, code number). Aliquots for each DNA extraction were marked with 1 and 2. After rehydration phase, DNA solutions were quantified spectrophotometrically by measuring optical density at 260 and 280 nm wavelengths.

PCR (Polymerase Chain Reaction) is a selective enzymatic amplification of a DNA fragment of known sequence. Amplification is done in several cycles, each cycle consisting of three steps: DNA denaturation (separation of double-stranded DNA in two single-stranded DNA); annealing of the primers to this single-stranded DNA template; elongation of the strands by DNA-polymerase (Taq) in the direction 5'→3'. Thus, it produces copies of DNA region to which primers were bind. Number of DNA copies increases exponentially reaching one million copies of the original DNA sequence in a couple of hours.

PCR is one of the most used methods, both in cloning techniques, as well as molecular diagnostic method.

Parameters which has been taken into account in the optimization of PCR were as follow: the quantity and quality of DNA matrix (in our case, genomic DNA); the quantity and quality of primers; respective primer/matrix ratio and primer/primer ratio for each pair of chosen primers; the temperature of hybridization (annealing), depending on Tm values of each primer; amplification program – during each phase, the number of cycles (the possibility of using gradient, increment, hot-start or touch-down functions); the final concentration of magnesium, added to the $MgCl_2$ or $MgSO_4$ solution; free nucleotide concentration and quality; enzyme quality and quantity (Taq polymerase).

Primers were designed using Primer3 Input program or taken from the literature [7, 8], checked in NIH (National Institutes of Health) database, using BLAST program against false hybridizations. Also, the NetPrimer program was used to verify prospective primer dimers or cross-dimer.

PCR-RFLP (Restriction Fragment Length Polymorphism) is a method used in molecular epidemiology studies for genetic polymorphisms genotyping. It is a reliable method, based on property of nucleic acid fragments, obtained after digestion with specific restriction enzyme, to migrate different in a electrophoresis gel depending on their size (smaller fragments migrate faster on), resulting in a gene restriction profile. The structure of restriction profile is dependent on the origin sequence and number of restriction sites contained therein.

DNA was amplified by PCR and then was cut into restriction fragments using appropriate endonucleases. Restriction fragments were separated, according to length, by 2% agarose gel electrophoresis and were visualized before and after treatment in agarose gel, using visualization with ethidium bromide. The presence or absence of restriction sites vary in the different alleles due to gene polymorphisms. This makes the length of

the fragments to be not identical and the position of amplicons to differ between individuals (polymorphism).

Results

In our study, DNA extraction was optimized with the adoption of the variant using Wizard® Genomic DNA purification kit and 10 mL of whole blood protocol. DNA collection was realized from all DNA samples extracted started from samples of studied subjects, resulting in eight aliquots for each sample (two stored at -80^{0}C for collection; four stored at -22^{0}C for other molecular epidemiology determination; two in the refrigerator for current handling – PCR-RFLP).

For all DNA samples was obtained DNA quantitatively adequate (more than 50 µg) and qualitatively (report DO260/DO280 as close to 2) for PCR amplification.

For our applications, PCR-RFLP was based on the existence of polymorphic sites that can be recognized by restriction enzymes (MspI, HincII, BstUI). A mutation or a polymorphism at the DNA level can remove or create a site restriction, which will be recognized as such by a specific restriction enzyme. Following enzymatic digestion of DNA molecule (amplicon), depending on the presence or not of characteristic site, is obtained a specific pattern of DNA fragments, which can be electrophoretic identified. Restriction profile of the normal sequence (wild-type) will be different from that of a polymorphic sequence, heterozygotes individuals with a restriction profile generated by both alleles, wild-type and polymorphic.

For MspI polymorphism of CYP1A1 gene (T6235C), T (thymine) nucleotide substitution with a C (cytosine) generates a CCGG sequence, which represents a restriction site for MspI enzyme. Thus, the enzyme will split C polymorphic allele (generating a fragment of 140 bases pairs (bp) and one of 200 bp), while T wild-type allele will not be split, remaining at 340 bp. After restriction digestion, wild-type individuals will have an electrophoretic profile of 340 bp (without digestion), heterozygotes individuals a profile of 340 + 200 + 140 bp (two alleles, one split and one not), and polymorphic homozygotes individuals will have a 200 + 140 bp profile (both alleles split) (Figure 1).

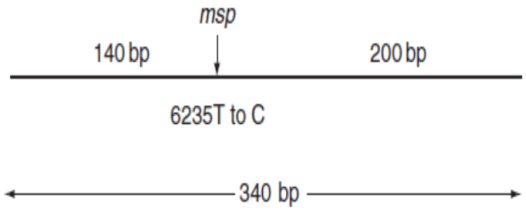

Figure 1 – ***Fragments resulting from amplicons digestion of CAP1A1 gene with MspI enzyme (MspI polymorphism).***

MspI enzyme functions effectively at 37^{0}C, in the NEB4 buffer and can be heat inactivated at 80^{0}C. We used 20 enzyme units for each reaction. Digestion was initially done for one hour, after which we tried long incubation time, without changing the restriction profiles.

For Ile/Val polymorphism of CYP1A1 gene (A4889G), A (arginine) nucleotide substitution with a G (guanine) generates a GTTGAC sequence which is a restriction site for HincII enzyme. Another HincII restriction site exists in the same amplicon, as a GTCAAC sequence upstream. Thus, the enzyme will split the polymorphic G allele twice (resulting in a fragment of 120 bp, one of 48 bp and a small unobservable fragment of 19 bp), while wild-type T allele will be cut only once (generating fragments of 48 and 139 bases pairs). After restriction digestion, wild-type individuals will have an electrophoretic profile of 139 + 48 bp, heterozygotes individuals a profile of 139 + 120 + 48 + 19 bp, and polymorphic homozygotes individuals will have a profile of 120 + 48 + 19 bp. Given the difficulty of observation of 19 bp fragment, the difference between genotype scan be made by 139 and 120 bp fragments. The existence of an additional HincII site in the amplicons automatically provides digestion positive control to verify the functionality of the enzyme (Figure 2).

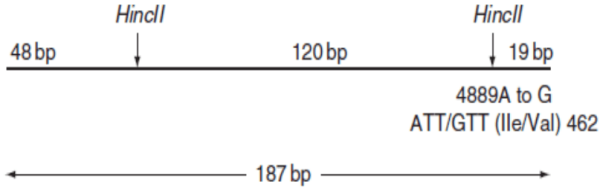

Figure 2 – ***Fragments resulting from amplicons digestion of CAP1A1 gene with HincII enzyme (Ile/Val polymorphism).***

HincII enzyme functions effectively at 37^{0}C, in the NEB3 buffer and can be heat inactivated to 80^{0}C. We used 10 enzyme units for each reaction. Digestion was initially done for one hour, after which we noted that the incubation period extended to two hours allows a more efficient and complete amplicons digestion.

For Arg72Pro polymorphism in codon 72 of p53 gene, G (guanine) nucleotide substitution with a C (cytosine) destroys original CGCG sequence, which represents a restriction site for BstUI enzyme. Thus, the enzyme will cleave wild-type allele G (generating a fragment of 113 bp and one of 86 bp), while the polymorphic C allele will not be split. After restriction digestion, wild-type individuals will have an electrophoretic profile of 113 + 86 bp (both alleles split), individuals heterozygotes a profile of 199 + 113 + 86 bp (one allele split and one not), and polymorphic homozygotes individuals profile will have a 199 bp (no digestion) (Figure 3).

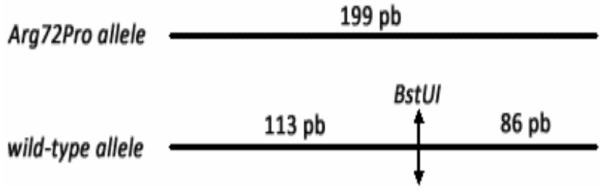

Figure 3 – ***Fragments resulting from amplicons digestion of p53 gene with BstUI enzyme (Arg72Pro polymorphism).***

BstUI enzyme functions effectively at a temperature of 60^0C, in the NEB4 buffer and cannot be heat inactivated. We used 10 enzyme units for each reaction. Digestion was carried out for an hour, enough time for complete amplicons digestion.

MspI and Ile/Val polymorphisms of CYP1A1 gene and pArg72Pro polymorphism of p53 gene were investigated by optimized PCR-RFLP techniques, in the group of 21 patients with ovarian cancer and control group of 21 women without cancer. Given the absence of the MspI polymorphic patients, positive control was represented by a p53 amplicon split by the same enzyme MspI. The Figures 4–6 show specific aspects from MspI, Ile/Val, and Arg72Pro polymorphisms analysis by PCR-RFLP.

Through amplification, programs and using reagents concentrations previously optimized, were realized the amplification of region of interest for each patient and each controls. Thus, was successfully achieved amplification of the 3'-UTR region of CYP1A1 gene (for MspI polymorphism analysis, 200 ng DNA was used), amplification of the region corresponding to exon 7 of CYP1A1 gene (for Ile/Val polymorphism analysis, 200 ng DNA was used) and amplification of the codon 72 corresponding region of p53 gene (Arg72Pro polymorphism analysis, 50 ng DNA was used). All these amplifications were performed for the group of 21 patients with ovarian cancer and for the control group of 21 women. For each amplification, were systematically performed, negative controls without DNA matrix, in order to verify the absence of contamination.

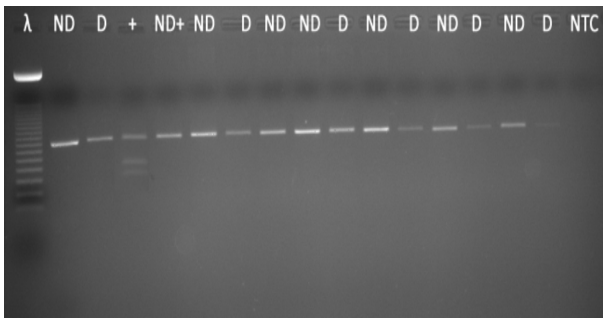

Figure 4 – ***MspI polymorphism analysis in the 3'-UTR region of CYP1A1 by PCR-RFLP (λ – 50 bp marker size; ND – Undigested amplicon; D – MspI digestion; + – MspI positive control; NTC – No template control).***

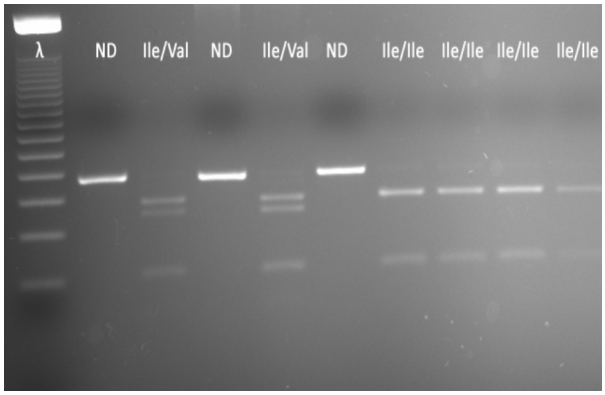

Figure 5 – ***Ile/Val polymorphism analysis at the exon 7 of CYP1A1 by PCR-RFLP (λ – 50 bp marker size; ND – Undigested amplicon; Ile/Val – Heterozygotes individual; Ile/Ile – Wild-type homozygotes individual).***

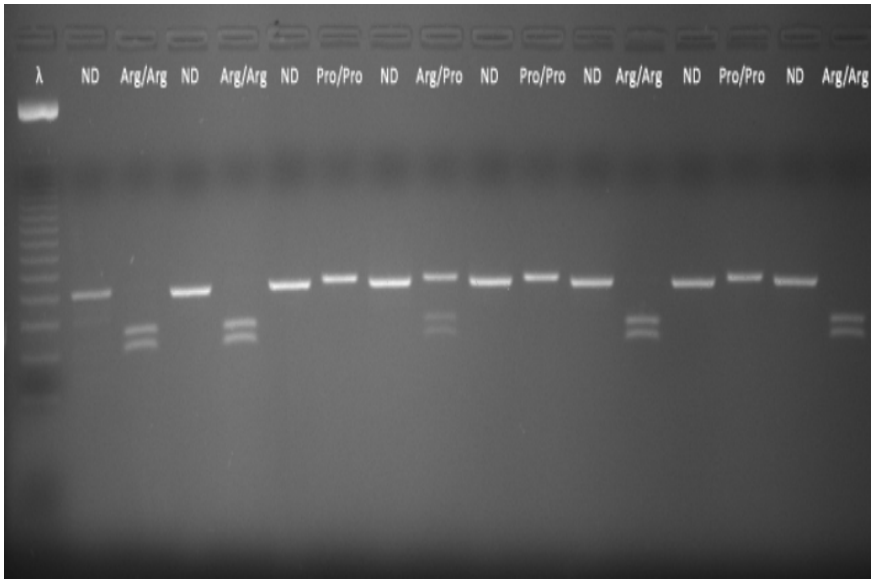

Figure 6 – ***Arg72Pro polymorphism analysis of p53 gene by PCR-RFLP (λ – 50 bp marker size; ND – Undigested amplicon; Arg/Arg – Wild-type homozygotes individual; Arg/Pro – Heterozygotes individual; Pro/Pro – Polymorphic homozygotes individual).***

MspI polymorphism (T6235C) in 3'-UTR region of CYP1A1 gene was not identified, nor in the group of cases, neither in the controls, nor homozygote state nor heterozygote state.

Ile/Val polymorphism (A4889G) in exon 7 of CYP1A1 gene was identified in heterozygote state (Ile/Val) in two subjects from the patients group. The other 19 patients and all women in the control group were wild-type genotyped (Ile/Ile).

Arg72Pro polymorphism of p53 gene was identified in homozygote form (Pro/Pro) in six patients with ovarian cancer and in seven women in the control group. In the heterozygote state (Arg/Pro), the same polymorphism was identified in six patients with ovarian cancer and seven women in the control group. Nine patients with ovarian cancer and seven women in the control group had wild-type homozygotes status (Arg/Arg). Table 1 shows systematized data regarding the frequency of investigated polymorphisms, in ovarian cancer cases and subjects of control group.

Table 1 – ***Distribution of investigated polymorphisms in the studied population***

Population	Ile/Val polymorphism of CYP1A1				
	Ile/Ile (wild-type) No. (%)	***Ile/Val No. (%)***	***Val/Val Nor. (%)***	***Ile allele No. (%)***	***Val allele No. (%)***
Cases	19 (90.5)	2 (9.5)	0	40 (95)	2 (5)
Controls	21 (100)	0	0	42 (100)	0
	MspI polymorphism of CYP1A1				
	TT (wild-type) No. (%)	***TC No. (%)***	***CC No. (%)***	***T allele No. (%)***	***C allele No. (%)***
Cases	21 (100)	0	0	42 (100)	0
Controls	21 (100)	0	0	42 (100)	0
	P53 codon 72 polymorphism				
	Arg/Arg (wild-type) No. (%)	***Arg/Pro No. (%)***	***Pro/Pro No. (%)***	***Arg allele No. (%)***	***Pro allele No. (%)***
Cases	9 (43)	6 (28.5)	6 (28.5)	24 (57)	21 (50)
Controls	7 (33.33)	7 (33.33)	7 (33.33)	18 (43)	21 (50)
	OR=0.67	IC=0.16–2.78	*p*=0.75		
	OR_{allele}=0.75	IC=0.29–1.33	*p*=0.51		

In the group of subjects with ovarian cancer, for codon 72 polymorphism of p53 gene were revealed nine Arg/Arg homozygotes carriers (43%), six Pro/Pro polymorphic homozygotes (28.5%) and six Arg/Pro heterozygotes (28.5%). Also, were identified 12 subjects who have at least one Pro allele (57%). For Ile/Val polymorphism of CYP1A1gene were identified 19 Ile/Ile homozygotes carriers (90.5%) and two Ile/Val heterozygotes (9.5%) (associated with increased cancer risk); for MspI polymorphism of CYP1A1 gene we did not identified homozygotes or heterozygotes carriers.

In terms of Arg allele frequency, was higher among cases compared with controls (57% *vs.* 43%) and the Pro allele was equal in both populations (50%).

In women from the control group, for the codon 72 polymorphism of p53 gene, seven Arg/Arg homozygotes carriers were identified (33.33%), seven Pro/Pro homozygotes (33.33%), seven Arg/Pro heterozygotes (33.33%) and 14 (66.66%) subjects who have at least one Pro allele; for Ile/Val polymorphism of CYP1A1 gene all recruited subjects were Ile/Ile homozygotes carriers (21 control subjects); as in subjects with ovarian cancer, MspI polymorphism of CYP1A1 gene was not identified.

In our research, the three polymorphisms were not highlighted as significantly associated with risk of ovarian cancer. Also, in Tables 2 and 3 are presented information regarding genotypes and allele frequency of codon 72 of p53 gene and of CYP1A1 gene, according to the presence of epidemiological variables.

Table 2 – ***Frequency of genotypes and alleles of codon 72 of p53 according to the presence of epidemiological variables***

No.	Variables	Genotypes frequency			Alleles frequency	
		Arg/Arg No. (%)	***Arg/Pro No. (%)***	***Pro/Pro No. (%)***	***Arg No. (%)***	***Pro No. (%)***
1.	*Menopausal status*					
	▪ Premenopausal	4 (33.33)	4 (33.33)	4 (33.33)	12 (50)	12 (50)
	▪ Postmenopausal	5 (55.55)	2 (22.22)	2 (22.22)	12 (66.66)	6 (33.33)
2.	*Obesity (BMI)*					
	▪ >25 kg/m^2	5 (41.66)	4 (33.33)	3 (25)	14 (58.33)	10 (41.66)
	▪ ≤25 kg/m^2	4 (44.44)	2 (22.22)	3 (33.33)	10 (55.55)	8 (8.88)
3.	*Histologic type*					
	▪ Serous	6 (40)	4 (26.66)	5 (33.33)	16 (53.33)	14 (46.66)
	▪ Mucinous	0	0	0	0	0
4.	*Number of births*					
	▪ <2	3 (42.85)	2 (28.57)	2 (28.57)	8 (57.14)	6 (42.85)
	▪ ≥2	6 (42.85)	4 (28.57)	4 (28.57)	16 (57.14)	12 (42.85)
5.	*Breastfeeding*					
	▪ Yes	7 (41.17)	6 (35.29)	4 (23.52)	20 (58.82)	14 (41.17)
	▪ No	2 (50)	0	2 (50)	4 (50)	4 (50)
6.	*Ovulatory cycles during the life*					
	▪ <22 years	4 (28.57)	5 (35.71)	5 (35.71)	13 (46.42)	15 (53.57)
	▪ 23–29 years	3 (75)	1 (25)	0	7 (87.5)	1 (12.5)
	▪ 30–34 years	2 (100)	0	0	4 (100)	0
	▪ ≥35 years	0	0	1 (100)	0	1 (100)

Table 3 – ***Frequency of genotypes and alleles of CYP1A1 according to the presence of epidemiological variables***

No.	Variables	Genotypes frequency			Alleles frequency	
		Ile/Ile No. (%)	*Ile/Val No. (%)*	*Val/Val No. (%)*	*Ile No. (%)*	*Val No. (%)*
1.	*Menopausal status*					
	▪ Premenopausal	11 (91.66)	1 (8.33)	0	23 (95.83)	1 (4.16)
	▪ Postmenopausal	8 (88.88)	1 (22.22)	0	17 (94.44)	1 (5.55)
2.	*Obesity (BMI)*					
	▪ >25 kg/m^2	12 (100)	0	0	24 (100)	0
	▪ ≤25 kg/m^2	7 (77.77)	2 (22.22)	0	16 (88.88)	2 (11.11)
3.	*Histologic type*					
	▪ Serous	14 (93.33)	1 (6.66)	0	29 (96.66)	1 (3.33)
	▪ Mucinous	0	0	0	0	0
4.	*Number of births*					
	▪ <2	6 (85.71)	1 (14.28)	0	13 (92.85)	1 (7.14)
	▪ ≥2	13 (92.85)	1 (7.14)	0	27 (96.42)	1 (3.57)
5.	*Breastfeeding*					
	▪ Yes	15 (88.23)	2 (11.76)	0	32 (94.11)	2 (5.88)
	▪ No	4 (100)	0	0	8 (100)	0
6.	*Ovulatory cycles during the life*					
	▪ <22 years	13 (92.85)	1 (7.14)	0	27 (96.42)	1 (3.57)
	▪ 23–29 years	3 (75)	1 (25)	0	7 (87.5)	1 (12.5)
	▪ 30–34 years	2 (100)	0	0	4 (100)	0
	▪ ≥35 years	1 (100)	0	0	1 (100)	0

Discussion

There are a large number of materials in the literature, which suggest that factors depending on the host characteristics, including genetic polymorphisms, may explain some individual differences in the occurrence of ovarian cancer [15].

In the current study, we found a higher frequency of homozygote genotype Arg/Arg (wild-type) or Arg allele in the cases group compared with controls. It is considered necessary to include a larger number of subjects, to allow the generalization of the results for the female population of Northeastern region of Romania, which can be done concurrently with the development of DNA collection. Another aspect that would help the best possible interpretation of molecular epidemiology results regarding the involvement of codon 72 polymorphism of p53 gene and MspI and Ile/Val polymorphisms of CYP1A1 gene in increasing the ovarian cancer risk is the knowledge of the frequency of these polymorphisms in the general population, another direction for study emerged as a result of the present study.

Our results were different from those of Ueda M *et al.* [8], who found a higher frequency of these genotypes in the controls group, but in agreement with those of Agorastos T *et al.* [10] and Pegoraro RJ *et al.* [16]. Other authors have identified a frequency of 30–40% for Arg/Arg homozygote genotype among controls group in a Japanese population [5, 17].

As mentioned above, Agorastos T *et al.* [10] and Pegoraro RJ *et al.* [16] reported that women with ovarian cancer showed Arg/Arg genotype or Arg allele more frequently than controls. In contrast, Wang Y *et al.* [18] have found that patients with ovarian cancer showed a more increased frequency of homozygotes/heterozygotes for the Pro allele, which is closely linked with a reserved prognosis. Chemotherapy resistance is a complex problem in ovarian cancer and is one of the causes of this poor prognosis. Unlike the results of Wang's team [18], in our research the frequency of homozygotes Pro/Pro among cases (6 – 28.5%) and among those who have at least one Pro allele (12 – 57%) was lower compared with controls (7 – 33.33%, respective 14 – 66.66%). From this point of view, our data are consistent with those of Ueda's team [8].

Other studies have reported that wild-type Arg allele of p53 gene (allele more frequent in the population) may be more effective in the multi-drug resistance gene MDR1 suppression and induction of proapoptotic oncogene BAX, while wild-type Pro allele of p53 gene is involved in promoting cell cycle and DNA repair by inducing p21-waf and GADD45 [8].

Recently, ovarian cancers were classified into two categories called cancers type I and type II, which are consistent with the two main pathways of carcinogenesis [19]. These two types of cancers have different genetic mutations including p53 mutations. Although these models are related to tumorigenesis mechanisms and not to histological types, it would be interesting to assess the correlation between genotypes and allele frequency of codon 72 of p53 gene and histopathological types of ovarian cancer.

CYP1A1 polymorphisms were associated with some cancers such as lung cancer, colorectal cancer, the breast, endometrial, but also with ovarian neoplasms [7, 11, 12]. Mentioned polymorphisms could explain some differences in susceptibility for ovarian cancer. Also, it was shown that MspI polymorphism in the 3'

non-coding region control CYP1A1 gene expression and Ile/Val polymorphism in the heme-binding region modulates the activity of CYP1A1 enzymes [11]. They can increase the enzymatic activity of steroids hydroxylation.

Among cases, Sugawara T *et al.* [7] reveals a frequency of 64.4% (29 cases) for Ile/Ile homozygotes genotype of CYP1A1 gene, of 33.3% (15 subjects) for Ile/Val heterozygotes, of 2.2% (one case) for the Val/Val homozygotes and of 35.6% (16 women) for the Val allele. The risk for subjects who presented at least one Val allele compared with those showing Ile/Ile genotype was OR=1.16 (p=0.96). Regarding the population variant (wild-type – Wt) and the MspI polymorphism of CYP1A1 gene (variant-type – Vt), values were 41.3% Wt/Wt (19 subjects), 50% Wt/Vt (23 cases), 8.7% Vt/Vt (four cases) and 58.7% for Vt allele (27 cases). The risk for subjects with a Vt allele compared with those who had Wt/Wt genotype was OR=1.33 (p=0.70) [7]. The conclusion of the research team was that they found no statistically significant association between the two polymorphisms of CYP1A1 gene and ovarian cancer.

In a recent study, ovarian cancer was associated with Ile/Val polymorphisms in Turkish population [12]. Aktas D *et al.* reported that Ile/Val genotype of CYP1A1 gene significantly increases the risk for ovarian cancer (OR=5.7, 95%CI=3.34–9.76). Also, the researchers team found a statistically significant difference in Val/Val genotype distribution of CYP1A1 gene among subjects included in the study (OR=5.85, 95%CI=2.40–14.25), suggesting that the presence of Val allele significantly increases the risk for epithelial ovarian neoplasm. The team led by Aktas D identified an increase of 6.5 to 10 times for the risk of serous ovarian carcinoma in carriers of Ile/Val or Val/Val genotypes and 5.4 to 10 times for the mucinous type. They found no significant difference in the distribution of Val allele among patients with endometrioid type.

The presence of this polymorphism in our cases was identified in 9.5% (two subjects), but among controls was not identified any such individual. The second studied polymorphism of CYP1A1 gene (MspI) was not identified in our population, neither cases nor controls.

Conclusions

With the achievement of this research, the successful implementation of molecular biology techniques was succeeded (such as DNA extraction, DNA amplification and PCR-RFLP) in the Molecular Epidemiology Laboratory of the Department of Primary Health Care and Epidemiology, Faculty of Medicine, University of Medicine and Pharmacy "Grigore T. Popa" Iassy.

Validation of techniques consisted in the optimization of RFLP methods for p53 and CYP1A1 genes polymorphism analyzing that allowed highlighting the existence of codon 72 polymorphism of p53 gene and Ile/Val polymorphism of CYP1A1 gene in the population from this region.

Frequency of Arg/Arg homozygotes genotype of p53 gene was higher among cases (43%) compared with controls (33.3%), but the difference was not statistically significant (p=0.75). The presence of Ile/Val polymorphism of CYP1A1 gene, associated with an augmentation of ovarian cancer risk, was identified in 9.5% (two subjects) of the cases and the MspI polymorphism of CYP1A1 gene was not identified in the studied population.

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