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RESEARCH

Polymorphisms in the MUC16 Gene: Potential Implication in Epithelial Ovarian Cancer

Houda Bouanene · Hassen Hadj Kacem · Leila Ben Fatma · Halima Ben Limem · Slim Ben Ahmed · Salwa Yakoub · Abdelhédi Miled

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Abstract MUC16 plays an important role in epithelial ovarian cancer. In this paper, we studied the association between two tags SNPs of MUC16 and the risk of epithelial ovarian cancer. We aimed also to test the association between these tags SNPs and elevated level of the protein CA125. We analyzed a collection of 117 cases. Forty-one samples of patients with epithelial ovarian cancer and 76 samples from Tunisian volunteers were genotyped for two synonymous coding tags SNPs of the MUC16 gene (rs1596797, A/C and rs2547065, C/G) using polymerase chain reaction and sequencing. For the rs1596797 SNP, there was no significant difference in genotype distribution, a rare variation observed in only one patient. For the polymorphism rs2547065, mean CA125 levels were 24 and 78 UI/ml in patients with GG and GC genotypes versus 230 UI/ml in patients with CC genotype (P=0.36). Compared to the C/C genotype, the 'G' allele (C/G+G/G genotypes) did not significantly modified the risk of developping epithelial ovarian cancer (OR=0.43; 95% CI). As for the polymorphism rs1596797, compared to the C/C genotype, the 'A' allele (C/A+A/A genotypes) did not significantly modified the risk of developping epithelial ovarian cancer (OR=881.7; 95% CI). MUC16 gene polymorphisms selected in this study are neither involved in genetic predisposition to epithelial ovarian cancer nor associated with CA125 level.

Keywords MUC16 · Polymorphism · CA125 · Epithelial ovarian cancer

H. Bouanene (✉) · H. Ben Limem · A. Miled
Laboratory of Biochemistry—CHU Farhat Hached,
Sousse 4000, Tunisia
e-mail: bouanenehouda2@yahoo.fr

H. Hadj Kacem
Center of Biotechnology,
Sfax, Tunisia

L. Ben Fatma · S. Ben Ahmed
Service of Oncology—CHU Farhat Hached,
Sousse, Tunisia

S. Yakoub
Center of Blood Transfusion,
Sousse, Tunisia

Introduction

Approximately 90% of epithelial ovarian cancers (EOC) are considered to be sporadic events, without evidence of an autosomal dominant hereditary predisposition. The remaining 5% to 10% are primarily associated with germline mutations of Breast Cancer 1 (BRCA1; 17q12-21) and BRCA2 (13q12-13) genes and account for 95% of hereditary ovarian carcinomas [1, 2]. Studies to identify other chromosomal regions that might harbor major genes for ovarian cancer risk provide little evidence for the existence of additional high-risk genes for ovarian cancer susceptibility [3]. Thus, there is emerging consensus that most of the genetic component of ovarian cancer risk is due to genetic polymorphisms that confer low to moderate risk. A common approach to identify risk variants is to rely on known biology to identify plausible candidate genes [4]. A gene thought to have an important role in ovarian cancer is MUC16 (mucin 16) gene that encodes the tumor marker CA125 [5–7]. This gene is located on chromosome 19p13.2 [8] and is coded by sequences present within approximately 179 kb of genomic DNA. As per the present available information, there is a discrepancy regarding the total number of exons present in MUC16 genomic DNA. This discrepancy is due to the absence/presence of some of the

genomic sequences (particularly for the repeat regions) in the available genomic databases [9]. It is a member of the mucin family [10], a family of large glycoproteins showing common features: large size of their mRNAs, large nucleotide tandem repeat domains, complex expression both at tissular and cellular level [11]. Many single nucleotide polymorphisms (SNPs) in MUC16 were identified through the dbSNP database but none study has reported the potential implication on any of these. A selected set of sequence polymorphisms can serve as genetic markers to detect association between a particular region and the disease, whether or not the markers themselves have a functional effect [12]. It is therefore not necessary to test each polymorphism individually. Because most SNPs are correlated with nearby polymorphisms, risk-related SNPs will be correlated with one or more assayed SNPs [13].To clarify the role of MUC16 in the predisposition to epithelial ovarian cancer (EOC), we tested the association of two common polymorphisms (tags SNPs) with the disease in a case-control study of Tunisian women. We aimed also to test the association between these tags SNPs and elevated level of the protein CA125 encoded by MUC16 gene.

Materials and Methods

Patients and Controls

We analyzed a collection of 117 cases. Forty-one blood samples were drawn post-operatively for patients with epithelial ovarian cancer from Service of Oncology—CHU Farhat Hached Sousse-Tunisia were histopathologically diagnosed as having an epithelial ovarian cancer. Women taking part in the study were asked to provide a 10 ml blood sample for DNA analysis. 76 Samples from Tunisian volunteers were included in this analysis. The protocol of the study was approved by the local Ethic Committee.

SNP Identification and Selection

SNPs with validated frequency data were identified through the dbSNP database http://www.ncbi.nlm.nih.gov/SNP/. Selection of informative single nucleotide polymorphisms (SNPs) using data from a dense network of SNPs that have been genotyped for the gene MUC16 was a crucial step. One possible approach would be to select tagging SNPs (tSNPs) according the HapMap information from http://www.hapmap.org/. We selected randomly two synonymous coding tags SNPs: SNP 1 (rs1596797, A/C) in exon 1 and SNP 2(rs2547065, C/G) in exon 2 of the gene MUC16.

Isolation of Genomic DNA and Genotyping of MUC16 Polymorphisms by PCR/RFLP (Polymerase Chain Reaction/Restriction Fragment Length Polymorphism)

Genomic DNA from anticoagulated peripheral blood was then extracted with the Wizard Genomic DNA Purification Kit (Promega). DNA was quantified photometrically. The PCR reactions were performed in a 50 μL reaction volume containing 70 ng genomic DNA, 20 μM each primer, 10 mM/L dNTP, 10×PCR buffer (Tris–HCl, KCl, Triton X-100, pH 9.0), 25 mM MgCl2 and 5 units of Taq DNA polymerase (Promega). The PCR cycle conditions consisted of an initial denaturation step at 95° for 5 min, followed by 35 cycles of 30 s at 95°C, 20 s at Tm (58°C for rs1596797 and 62°C for rs2547065), 30 s at 72°C, and a final elongation at 72°C for 10 min. The PCR fragments were amplified using sets of primers listed in Table 1. Following PCR, 10 μl of PCR products digested with 20 units of two separate enzymes including BsrI (Fermentas) for rs2547065 and TasI (Fermentas) for rs1596797 in a total volume of 30 μl at 65°C overnight. The digested-amplification products were electrophoretically separated on 2% agarose gels and visualized by ethidium bromide staining under UV-light. Quantitative determination of products was carried out using scan analysis software with photographs of the gels.

DNA Sequencing

Fragments obtained from polymerase chain reaction (PCR) for different genotypes for both polymorphisms rs2547065 and rs1596797 were purified with Wizard® SV Gel and PCR Clean-up System kit (Promega) and sequenced on ABI prism 310 (Applied BioSystems, États-Unis) to confirm different genotypes for both polymorphisms.

CA125 Enzyme Immunoassay

Measurement of serum CA125 was done for each woman enrolled in this study. The serum CA125 values were

Table 1 PCR primers used in the detection of tags SNPs in MUC16

Tag SNPs	Primers (F, forward; R, reverse)	Product Size (bp)
rs1596797	(F) GCTTGGCATCTTGTCCTCAT (R) GGTTTTACTACTGAAGCCATGGTAAT	375
rs2547065	(F) ATTGCCCTTTTCTTTCAGCA (R) CTTGGGTGGCATTAGCAGAG	171

quantitatively measured with a commercially available automated microparticle enzyme immunoassay method (Abbott AxSym system), following the manufacturer's recommendations. The cut-off value for "normal" in this system was <35 U/ml.

Statistical Analysis

Genotypic and allelic frequencies were compared by χ^2 testing. The association between MUC16 tags polymorphisms and CA125 serum level was analyzed using analysis of variance. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model. A probability level of 5% was considered significant. Statistical analysis of the data was performed using SPSS 10.0 software.

Results

Subject Characteristics

The median age was 56 years for patients and 52 years for controls. FIGO (International Federation of Gynecology and Obstetrics) Stages at primary diagnosis in patients with epithelial ovarian cancer are as follow: 72.22% stage III, 11.11% stage I and IV each one and 5.55% stage II. For histological type, the serous type was the most frequent with 76.47%. Serum CA125 levels in epithelial ovarian cancer patients were ranged from 4.1 to 4328 UI/ml with a mean of 288.11 UI/ml. Serum CA125 levels in controls were ranged from 1.7 to 40 UI/ml with a mean of 15.78 UI/ml.

Detection of MUC16 Gene Polymorphism

For the rs2547065 polymorphism, after digestion with BsrI, the product was separated on a 2% agarose gel stained with ethidium bromide. As a result, CC alleles were represented by DNA band with size of 171 bp, GG alleles were represented by a DNA band with sizes of 111 bp, and 59 bp, whereas heterozygotes displayed a combination of both alleles (171 bp, 111 bp and 59 bp) (Fig. 1). For rs1596797 polymorphism, as a result for digestion with TasI, CC alleles were represented by a DNA band with a size of 375 bp and CA alleles were represented by a DNA band with sizes of 375 bp and 348 bp (Fig. 2).

Genotype Distribution

The distribution of the MUC16 genotypes in the control and patients group significantly deviates from that expected for Hardy–Weinberg equilibrium. There was no significant difference in genotype distribution between epithelial ovarian cancer patients and controls (all P value >0.05).

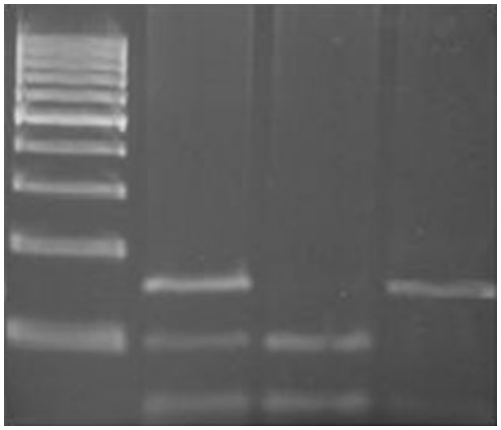

Fig. 1 MUC16 rs2547065 polymorphism genotyping by PCR–RFLP analysis followed by separation on 2% agarose gel. Lane 1 = 100 bp ladder; lane 2 = C/G; lane 3 = G/G; lane 4 = C/C

The frequencies of rs2547065 C/C, C/G and G/G genotypes were 33.82%, 48.53% and 17.65% in controls and 48.65%, 40.54% and 10.81% in patients, respectively (Table 2). The distribution of rs2547065 genotypes was not significantly different in controls and patients (χ^2=2.42, P=0.29). The frequency of rs1596797 C/C genotype was 100% in controls, the genotypes C/A and A/A were absent. In patients, the frequencies of rs1596797 C/C, C/A, A/A genotypes were 97.3%, 2.7% and 0%, respectively with no significant difference (χ^2=1.29, P=0.5).

For the polymorphism rs2547065, mean CA125 levels were 24 and 78 UI/ml in patients with GG and GC genotypes versus 230 UI/ml in patients with CC genotype (P=0.36). Compared to the C/C genotype, the 'G' allele (C/G+G/G genotypes) did not significantly modified the risk of developping epithelial ovarian cancer; odds ratio was 0.43 (95% CI). As for the polymorphism rs1596797, compared to the C/C genotype, the 'A' allele (C/A+A/A

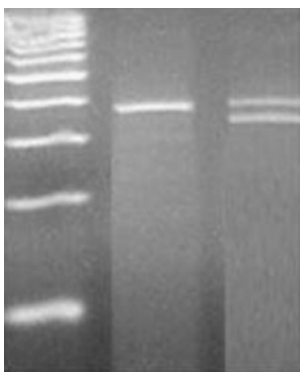

Fig. 2 MUC16 rs1596797 polymorphism genotyping by PCR–RFLP analysis followed by separation on 2% agarose gel. Lane 1 = 123 bp ladder; lane 2 = C/C; lane 3 = C/A

Table 2 Distribution of genotypes of two single nucleotide polymorphisms in MUC16

SNP	% of genotypes in controls (group 1)			% of genotypes in patients (group 2)		
rs1596797	C/C	C/A	A/A	C/C	C/A	A/A
	100	0	0	97, 3	2,7	0
rs2547065	C/C	C/G	G/G	C/C	C/G	G/G
	33,82	48,53	17,65	48,65	40,54	10,81

genotypes) did not significantly modified the risk of developping epithelial ovarian cancer; odds ratio was 881.7 (95% CI).

Discussion

The present study is the first to investigate the genetic contribution of MUC16, the gene that encodes the tumor marker CA125, to epithelial ovarian cancer. Given the nature of the current work as a preliminary investigation, it will provide guidance for future MUC16 genetic and ovarian cancer-associated studies that are currently needed to better understand the molecular biology of this lethal gynecological malignancy.

Numerous data support a pathogenic role for MUC16, such as altering the phenotype of natural killer cells in epithelial ovarian cancer patients. Such phenotypic changes in the natural killer cells of patients may have a profound impact on the success of immunological therapies [7]. Seelenmeyer et al. suggest that one role of MUC16 is to prevent efficient anti-tumor immune responses [14]. Another postulated function of MUC16 is to facilitate cell–cell interactions [5].

Enumeration of the basic structure of MUC16 has led recent studies to define the biological properties of this mucin. The over expression of MUC16 by many tumors of epithelial origin suggest an important role for this mucin in tumorigenesis [5]. Moreover, given that CA125 overexpression was demonstrated in patients with epithelial ovarian cancer [15], MUC16 could be a good candidate gene in the predisposition to this disease. We performed a study of two tags SNPs in MUC16 gene. No association study has previously reported any association between MUC16 polymorphism and ovarian cancer risk. The findings presented here demonstrate that MUC16 gene polymorphisms are neither associated with EOC risk nor with serum CA125 levels. Likewise, the absence of genetic involvement of interleukine-1(IL-1) in EOC was reported [16].

For the rs1596797 SNP, there was no significant difference in genotype distribution, a rare variation observed in only one patient. The rs1596797 polymorphism has been selected for this study because of the possible functional consequences of the amino acid change from Lys to Asn in the motif Asn-X-Ser of the protein which is a potential site for N-glycosylation. This amino acid change may contribute to the functional protein modification via its N-glycans which could be employed as functional groups to induce suppression of both innate and adaptive arms of the human immune response [17].

The rs1596797 polymorphism is located in exon 1 that is included in the glycosylated extracellular sequence of MUC16 [9]. This SNP may lead to different glycoprotein folding properties or variable levels of glycosylation. Clinically, these changes may alter mucin function. The same for MUC2 and MUC5AC mucin gene polymorphisms that occur in an exon corresponding to a highly glycosylated portion of the protein. These polymorphisms have the potential for clinically significant implications resulting from a different length glycoprotein [18].

It should be mentioned that there did exist differences in genotype distribution of the rs2547065 SNP between ovarian cancer patients and controls. The differences did not reach statistical significance, but this is likely related to relatively small patient numbers closely linked to the low frequency of the disease in Tunisia (3.2% of women's cancers) [19]. Besides, there is no obvious biochemical benefit in being homozygous CC/GG or heterozygous C/G for MUC16 gene polymorphism rs2547065. The absence of concordance between CA125 serum level and studied SNP may be related to the fact that assessments of CA125 level were carried on patients after receiving chemotherapy that makes the CA125 level modulated according to the response to treatment that may be different from patient to other. McLemore et al. suggested if variations within the tumor marker CA125 could be identified and related to genotypic data, it might be possible to change reference values of the tumor marker based on genotype to allow for population-based screening and more precise decision making [20].

Finally, in our population, the two selected tags SPNs are neither involved in genetic predisposition to epithelial ovarian cancer nor associated with CA125 level. However, we cannot exclude the possibility that these two SNPs might have a differential effect in another ethnic group via gene–gene or gene–environment interactions, or that a predisposing SNP might be present exclusively in another population. A larger study set would be needed to identify other MUC16 polymorphisms involved in epithelial ovarian cancer predisposition.

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