ACCELERATED PAPER

Germ line polymorphisms in cytochrome-P450 1A1 (C4887 CYP1A1) and methylenetetrahydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility

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We describe here a case-control study to identify associations between polymorphisms at the methylenetetrahydrofolate reductase (MTHFR) and cytochrome P-450 1A1 (CYP1A1) genes and susceptibility to endometrial cancer. Accordingly, genotype frequencies in 80 endometrial carcinoma patients were compared with frequencies in 60 controls. DNA analysis suggest a significantly increased endometrial cancer risk with an alanine to valine substitution at nucleotide 677 of MTHFR gene with an odds ratio of 2.8 (95% confidence interval: 1.36–6.14, P = 0.002). Moreover, the tumors from patients with the valine allele were more undifferentiated (P = 0.03). On the other hand, a recently described mutation in exon 7 of CYP1A1 gene (threonine exchanged to asparagine in codon 461) showed a strong association with endometrial cancer risk with an odds ratio of 6.36 (95% confidence interval: 1.99–26.5, P = 0.0004). Thus, this study suggests that polymorphisms at MTHFR and a novel CYP1A1 variant could influence susceptibility to endometrial cancer, although larger sample sizes would be required to corroborate these findings.

Introduction

Classical genetic approaches for identifying susceptibility genes, although successful for cancer with strong familial links, have not yet identified corresponding genes for sporadic cancers. For example, the BRCA1 and BRCA2 genes in breast cancer and several DNA mismatch repair genes in hereditary nonpolyposis colon cancer, although strongly associated with familial cancer, accounts for less than 10% of nonfamilial malignancies (1,2). In addition, phenotype variation between individuals carrying the same mutation in a high-penetrance gene has been described, i.e. the severity of duodenal polyposis in carriers of identical APC gene germ line mutations may be influenced for a locus on chromosome 1p35-p36 (3).

Such observations have led to a number of studies attempting to identify these low-penetrance genes that modify an individual’s risk of cancer. Because sporadic cancers result from mutations in transforming genes, and carcinogen detoxification influences the mutational events in these key genes, several polymorphic carcinogen-metabolism genes are potentially useful candidates. In this sense, three superfamilies have attracted interest: phase I cytochromes P450 (CYPs*) and phase II glutathione-S-transferases (GSTs) and N-acetyltransferases. In particular, certain variants at CYP1A1, CYP2D6, CYP2E1, GSTM1, GSTT1, NAT1 and NAT2 genes have been related with altered risk of various cancers (4).

Three polymorphisms had been described in the human CYP1A1 gene: a Msp I RFLP in the 3’-noncoding region (5), an adenine to guanine transition in the heme-binding domain of exon 7 (Ile >Val exchange in residue 462) (6), and an African-American-specific RFLP in intron 7 (7). The first two have been found overrepresented among lung cancer patients in Japan (8), but reports in Caucasians are inconclusive (4). Moreover, both have been related with a slightly elevated risk of colon and postmenopausal breast cancer (9–11). In addition, the Msp I polymorphism has been found associated with breast cancer in African-American women (12). Recently, we have found an enhanced endometrial cancer risk associated with the 3’-end and exon 7 CYP1A1 germ line polymorphisms (13). Finally, a new polymorphism in exon 7 of human CYP1A1 (C4887) has been recently described resulting in a threonine (Thr) to asparagine (Asn) exchange in codon 461 in the heme binding region of the protein (14). The significance of this variant is still unknown, although the exchange of the adjacent amino acid residues, isoleucine to valine, results in a change in enzyme activity (15).

On the other hand, germ line polymorphisms in the same oncogenes and tumor suppressor genes may also account for the different cancer susceptibility. In this sense, rare alleles of HRAS1 and p53 may confer an increased risk of certain types of cancer, including breast, ovarian and endometrial cancer (13,16–18). A disproportionally high rate of CpG > TpG transitions is frequently observed in tumor suppressor genes in various types of carcinomas (19) potentially due to deamination of 5-methylcytosine. In addition, methyldeficient diets may cause imbalances in the pools of nucleotide precursors leading to DNA strand breaks and mutations (20,21). Moreover, due to methylenetetrahydrofolate reductase (MTHFR) gene being a critical enzyme in the regulation of folate and methionine metabolism, both of which are important factors in DNA methylation and synthesis, germ line polymorphisms in the MTHFR gene could influence susceptibility to cancer. In this sense, an alanine (Ala) > valine (Val) (nucleotide 677: C >T) germ line polymorphism of the MTHFR gene been found to be related to the risk of colorectal cancer (22).

Although endometrial carcinoma is a common female malignancy, relatively little attention has been given to genetic susceptibility factors. The present study was undertaken to examine the recently described CYP1A1 and MTHFR germ line polymorphisms as potential molecular markers of endometrial carcinoma susceptibility.

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Material and methods

Study subjects

We analyzed DNA extracted from 80 unrelated Caucasian patients with histologically proven diagnosis of endometrial carcinoma recruited in the Department of Obstetrics and Gynecology at the Hospital Universitari Materno-Infantil Vall d’Hebron of Barcelona. The protocol was approved by the Institutional Review Board and informed consent was obtained from all the patients involved in the study. None of the patients had received radiation therapy or hormonal treatment prior to surgery. Their ages ranged from 45–82 years. Only five patients were premenopausal and the remaining 75 were postmenopausal. The stage distribution of the 80 patients according to the International Federation of Gynecology and Obstetrics (FIGO) staging system was Stage Ia (11 cases), Stage Ib (17 cases), Stage Ic (7 cases), Stage IIa (14 cases), Stage IIb (11 cases), Stage IIc (6 cases), Stage IIIa (9 cases), Stage IIIb (3 cases) and Stage IIIc (2 cases). Histologically, 74 of the 80 patients had endometrioid-type carcinomas whereas the remainder were four clear cell carcinomas and two papillary serous carcinomas. Among all surgically collected endometrial carcinomas, 40 were well-differentiated (G1), 82 years. Only five patients were premenopausal and the remaining 75 were postmenopausal. The stage distribution of the 80 patients according to the International Federation of Gynecology and Obstetrics (FIGO) staging system was Stage Ia (11 cases), Stage Ib (17 cases), Stage Ic (7 cases), Stage IIa (14 cases), Stage IIb (11 cases), Stage IIc (6 cases), Stage IIIa (9 cases), Stage IIIb (3 cases) and Stage IIIc (2 cases). Histologically, 74 of the 80 patients had endometrioid-type carcinomas whereas the remainder were four clear cell carcinomas and two papillary serous carcinomas. Among all surgically collected endometrial carcinomas, 40 were well-differentiated (G1), 23 were moderately differentiated (G2) and 17 were poorly differentiated (G3). The prevalence of the MTHFR and CYP1A1 polymorphisms studied were compared to those observed in a control group comprising 60 unrelated women from the same region and with the same ethnic background, attending to the Hospital Universitari Materno-Infantil Vall d’Hebron of Barcelona in the annual gynecological cancer screening program. Controls were randomly selected from those women who were free of clinical or histological malignancy. In addition, none had any personal history of cancer. Their ages ranged from 44–76 years. No differences were observed between cases and controls according to age distribution (P > 0.05). All endometrial cancer patients and control subjects were selected at Vall d’Hebron Hospital of Barcelona from January 1993 to December 1995. DNA was extracted from fresh endometrial tissue by proteinase K digestion and phenol/chloroform extraction (23).

Genotyping

The analysis of C>T transversion at position 677 of MTHFR, which results in the replacement of Ala by Val, was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) as described by Frosett and co-workers (24). The Hinf I RFLP analysis for MTHFR polymorphism is illustrated in Figure 1. A single undigested band at 198 base pair represents a homozygous wild-type allele, two bands at 198- and 175-base pairs represents the heterozygous genotype and a single band at 175 base pair represents a homozygous rare mutant allele.

The analysis of C>T transversion at position 4887 in exon 7 of CYP1A1, which results in the replacement of Thr by Asn at residue 461 in the heme binding region of the enzyme, was performed using PCR-RFLP as described by Cascorbi and co-workers (14). The Bsa I RFLP analysis for CYP1A1 polymorphism is illustrated in Figure 2. A single undigested band at 204 base pair represents a homozygous rare mutant allele, two bands at 204- and 139-base pairs represent the heterozygous genotype and a single band at 139 base pair represents a homozygous wild-type allele.

Statistical analysis

The odds ratio (OR) and 95% confidence intervals (CI) were calculated as a measure of the association between genotypes and endometrial cancer. The StatXact-Turbo statistical package was used to obtain exact P-values. Expected genotype frequencies were calculated by the Hardy-Weinberg equation (1 = p^2 + 2pq + q^2) from the allele frequencies.

Homozygous and heterozygous patients for the MTHFR Val variant where
No significant differences in the distribution of CYP1A1 rare mutant allele in endometrial cancer patients according to age at onset, F.I.G.O. stage, cellular differentiation grade, and histological type of the tumors were found (Table II). Composite genotype analysis of the codon 461-Val variant with the codon 462 and Msp I CYP1A1 gene variants previously studied (13), reveals that the simultaneous carriers of codon 461-codon 462 variants possess the higher endometrial cancer risk (P = 0.001).

In addition, the simultaneous carriers of MTHFR-Val and CYP1A1-codon 461 Val risk variants show a higher endometrial cancer risk (OR = 16, CI 95%; 2.61–163.29, P = 0.0003) compared with those that possess neither of them.

**Discussion**

Although endometrial carcinoma is the most frequently diagnosed neoplasm of the female genital tract, little is known about genetic factors in the etiology of the disease. Several studies have shown endometrial carcinoma to be a significant component in a dominantly inherited cancer syndrome known as hereditary non-polyposis colorectal cancer (HNPPC). The genetic background of HNPPC involves mutations in several genes including MSH2, MLH1, PMS1 and PMS2 (1). In addition, epidemiologic studies have shown that a woman who is the first-degree relative of a patient affected with endometrial cancer has a significantly increased cancer risk. For example, analysis of the Cancer and Steroid Hormone Study data indicated that mothers and sisters of endometrial cancer patients had 2.7 times the risk for endometrial cancer compared with those that possess neither of them.

Germ line variants of the MTHFR gene could be involved in endometrial cancer risk by altering DNA methylation or by influencing the rate of DNA mutation. The MTHFR mutation studied (677C>T Ala-to-Val) causes reduced enzyme activity (24). A decrease in the product of the MTHFR gene, 5-methylene tetrahydrofolate as (5-methylTHF), could contribute to carcinogenesis 5-methylTHF provides the methyl group for de novo methionine synthesis and DNA methylation (26). Abnormalities of DNA methylation are one of the most consistent molecular changes in human cancers. The alterations consist, simultaneously, of increased potential capacity for DNA methylation, widespread genomic hypomethylation, and more regional areas of hypermethylation; in this sense, selective growth and transformation of cells can result from DNA hypomethylation of protooncogenes or hypermethylation of tumor suppressor genes (27). Particularly for endometrial cancer, atypical distribution and increased levels of protein carboxyl methyltransferase (involved in methylation of ras and other GTP-binding proteins) (28,29) and abnormal methylation of estrogen receptor gene (30) have been described.

In addition, an imbalance of the MTHFR substrate, 5,10-methylenetetrahydrofolate (5,10-methyleneTHF), interferes with thymidylate biosynthesis, leading to accumulation of deoxyuridylate in DNA (31). Removal of this abnormal base might labilize DNA to strand breaks (32) and chromosome breaks appear to be important in nearly all human cancers (33).

Finally, our finding that a recently described CYP1A1 genetic polymorphism is associated with endometrial cancer risk, could be related with the known involvement of estrogen synthesis and metabolism in both initiation and promotion of endometrial cancer. In premenopausal women, persistent anovulation due to the polycystic ovary syndrome (34) and ovarian neoplasia (granulosa cell tumor, thecal cell tumor or adrenocortical hyperplasia) often causes an estrogen-predominant milieu associated with the occurrence of endometrial cancers; in addition, adipose tissues contribute to the formation of extra-glandular estrogen (mainly estrone), which is relevant for tumor risk factor, especially in perimenopause (35).
the fact that estrogen metabolism is partially determined by cytochrome P450 activity and is under the genetic control of both the CYP1A1 and CYP1A2 genes, the new CYP1A1 genetic polymorphism studied may influence the production of estrogen 2-hydroxylated metabolites (36) and therefore individual susceptibility to endometrial cancer.

In a parallel way, the individuals who inherit the rare CYP1A1 genotypes could suffer alterations in the metabolism of polycyclic aromatic hydrocarbons (PAHs) (known human carcinogens found in tobacco, smoke, food and combustion fumes) to reactive intermediates with mutagenic activity (37), contributing perhaps also to the higher endometrial cancer risk detected. In this sense, PAHs have a high capacity for adduct formation in human cancer cells (38) and benzop[a]pyrene-DNA adducts have been identified in human endometrium (39). In addition, PAHs metabolites have been shown to activate the H-ras protooncogene in vitro (40), a gene closely related to K-ras, which is found to be mutated in about one quarter of all sporadic endometrial carcinomas (41) and which is thought to be altered early in the progression from endometrial hyperplasia to endometrial carcinoma (42,43). Finally, we should point out that PAHs are lipophilic and stored in adipose tissue, and obesity is an epidemiological classic risk factor for endometrial carcinoma (44).

In conclusion, our preliminary data are in agreement with a genetic susceptibility to endometrial cancer associated with a MTHFR germline polymorphism and a recently described rare CYP1A1 variant. In addition to suggest the contribution of polymorphisms in DNA methylation-related genes and carcinogen-metabolism genes to an enhanced cancer risk, this study could provide a link with the epidemiological association between estrogen exposure, obesity and endometrial cancer. Therefore, studies in larger populations of sporadic endometrial cancer cases, in families with endometrial carcinoma (44).

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