Gene 558 (2015) 215–219

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Vitamin D receptor gene polymorphisms and steroid receptor status among Saudi women with breast cancer

Dalal M. Nemenqani [a,b], Rehab A. Karam [c,d,*], Mona G. Amer [e,f], Tamer M. Abd El Rahman [g,h]

[a] Department of Pathology and Cytopathology, College of Medicine, Taif University, Saudi Arabia
[b] Laboratory and Blood Bank, King Abdul Aziz Hospital, Taif, Saudi Arabia
[c] Department of Biochemistry, college of Medicine, Taif University, Al Taif, Saudi Arabia
[d] Departments of Biochemistry, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[e] Department of Anatomy and Histology, College of Medicine, Taif University, Al Taif, Saudi Arabia
[f] Departments of Histology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[g] Department of surgery, College of Medicine, Taif University, Al Taif, Saudi Arabia
[h] Department of surgery, Benha Teaching Hospital, Benha, Egypt

ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form 13 December 2014
Accepted 27 December 2014
Available online 2 January 2015

Keywords:
Vitamin D receptor
Single nucleotide polymorphisms
Breast cancer

ABSTRACT

The vitamin D receptor (VDR) is a mediator for the cellular effects of vitamin D and interacts with other cell signaling pathways that influence cancer development. We evaluated the associations of the FOK1 and Taq1 VDR polymorphisms and breast cancer risk and possible effect modification by steroid receptor status of the tumor. This case–control study includes 95 breast cancer patients and 100 age-matched controls. Genotyping for VDR FOK1 and Taq1 polymorphisms was performed using polymerase chain reaction-based restriction fragment length polymorphism. Level of 25(OH)D in serum was determined using ELISA. Immunohistochemical studies were performed for estrogen receptors (ER) and progesterone receptors (PR). The frequencies of ff genotype were significantly increased in the breast cancer group compared to the control group. Carriers of the f allele were significantly more likely to develop BC. We observed a statistically significant interaction for the Fok1 polymorphism and ER status. Our results demonstrated that FOK1 f. genotype and f allele have an important role in breast cancer risk in Saudi patients.

1. Introduction

Breast cancer is a leading malignancy among Saudi women comprising about 26% of all malignancies and is the foremost cause of cancer related death (Saudi National Cancer Registry, 2011). Moreover, carcinoma of the breast that developed before the age of 40 was significantly more common in Saudi women compared with patients in the United States (Elkum et al., 2007). This difference in the early onset of the disease in Saudi females could be due to considerable molecular differences both genetic and epigenetic between the ethnic groups. Also behavioral and geographical factors might play a role.

Several investigators hypothesized that vitamin D, which is synthesized in sunlight-exposed skin, may play a role in breast cancer prevention (Garland et al., 1990). A large body of experimental evidence supports the hypothesis that vitamin D plays an important role as a regulator of cell proliferation and differentiation in the breast and that vitamin D insufficiency can contribute to the pathogenesis of breast cancer (Welsh, 2007a).

Geographic and behavioral factors play important roles in determining exposure to ambient ultraviolet (UV) radiation and can therefore influence the ability to synthesize the vitamin D precursor 7-dehydrocholesterol cutaneously, for instance in high-latitude areas where UVB intensity is low and where more time may be spent indoors (particularly in winter), vitamin D deficiency may result among individuals with higher skin pigmentation (Webb et al., 1988; Kimlin, 2008).

The biologically most active form of vitamin D is 1,25(OH)2D, which mainly exerts its antiproliferative effects by binding to the vitamin D receptor (VDR) and acting in complex as a transcriptional factor for a variety of genes, including those involved in cell differentiation and cell growth (Hansen et al., 2001). The VDR are found in normal breast tissue and in breast tumors (Welsh, 2007b).

The VDR gene is located on chromosome 12 (12q13.11). Several common allelic variants have been identified in the VDR gene (Pedeutour et al., 1994). They include Bsm1, Apa1, Taq1 restriction sites, variable PolyA length and Fok1 restriction site (Köstner et al., 2009). Candidate gene approaches have reported single-nucleotide polymorphisms (SNPs) in this region to be associated with several

Abbreviations: VDR, vitamin D receptor; CA15.3, cancer antigen 15.3; CEA, carcinoembryonic antigen; SNPs, single-nucleotide polymorphisms; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism analysis

* Corresponding author at: Department of Biochemistry, college of Medicine, Taif University, Al Taif, Saudi Arabia.
E-mail address: rehab_atta@yahoo.com (R.A. Karam).

http://dx.doi.org/10.1016/j.gene.2014.12.065
0378-1119/

important diseases including susceptibility to lung (Fu et al., 2013), ovarian cancers (Song and Lee, 2013), breast cancer, prostate, skin, colorectal, ovary, and bladder cancers, and in renal cell carcinoma (Kostner et al., 2009).

Analysis of steroid receptor status has become the standard of care for patients with breast cancer. Estrogen receptor content has been correlated with prolonged disease-free survival and increased likelihood of response to endocrine therapy. Assessment of ER status by immunohistochemical analysis has been shown to have higher discriminating power than biochemical assays for predicting overall and disease-free survival (Lapidus et al., 1998).

Single-nucleotide polymorphisms (SNPs) in vitamin D related genes could represent risk factors for breast cancer, and thus potentially identify susceptible subgroups which might benefit from elevated and/or sustained levels of vitamin D. We therefore assessed the association between vit D status and VDR FokI, TaqI, SNPs with breast cancer risk and possible effect modification by steroid receptor status in Saudi women.

2. Subjects and methods

2.1. Subjects

The study protocol was approved by the ethical committee of Faculty of Medicine, Taif University, and informed consent for the experimental use of specimens was obtained from all participants.

Ninety five Saudi postmenopausal women (mean age 50.5 ± 9.8 years) with a clinical and histological diagnosis of BC were enrolled in this study from various surgical clinics at Taif, KSA, and 100 age-matched control women (mean age 51.2 ± 11.4) with no signs or symptoms of malignancy were randomly selected. Patients were eligible if they had histologically confirmed primary invasive or ductal carcinoma. Patients who have received chemotherapy or radiotherapy in the preoperative period have been excluded. The clinicopathological features of the patients and controls were summarized in Table 1.

As regards tissue samples, 4 μm thick sections from formalin-fixed, paraffin-embedded tissue blocks were stained with hematoxylin–eosin for morphological assessment. BC cases were graded according to the modified criteria as described by Bloom and Richardson (1957).

Table 1
The clinicopathological features of breast cancer patients (n = 95).

Patient characteristic	Number
Age (years)	50.5 ± 9.8
≤50	65
51–60	30
Tumor size	
T1 (≤2 cm)	53
T2 (>2 cm–5 cm)	23
T3 (>5 cm)	14
T4 (tumor of any size that has broken through (ulcerated) the skin, or is attached to the chest wall)	5
Lymph node status	
N0	50
N1	45
Histological grade (modified Bloom–Richardson score)	
I (well differentiated)	18
II (moderately differentiated)	50
III (poorly differentiated)	27
Hormone receptor status	
ER-positive	71
ER-negative	24
PR-positive	61
PR-negative	34

3. Methods

3.1. Genotype analysis

Half-milliliter of blood was collected in EDTA-treated tubes for DNA extraction. Genomic DNA was extracted from blood samples using Flexi-Gene DNA Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer's instruction.

Vitamin D receptor genotyping for studied single-nucleotide polymorphic sites (SNPs) was done by the PCR-RFLP method (polymerase chain reaction-restriction fragment length polymorphism analysis). We used the following flanking primers (Curran et al., 1999; Trabert et al., 2007). The VDR TaqI polymorphisms was detected using one primer in intron 8 (Forward: 5′-AGAGCATGGACAGGGAGCAAG-3′) and the other in exon 9 (Reverse: 5′-GCAACTCCTCATGGCTGAGGTCTCA-3′), yielding a 745 bp fragment. VDR-FokI polymorphic site was amplified using following primers Forward: 5′-GATGCCAGCTGGCCCTGGCA CTG-3′ and Reverse: 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′. PCR conditions were as follows: denaturation at 94 °C for 5 min, followed by 40 cycles of PCR at 94 °C (30 s), 60 °C–63.5 °C (30 s), and 72 °C (30 s). Annealing temperatures for TaqI and FokI PCR are 61 °C and 59.5 °C, respectively. Following PCR, aliquots of the amplified PCR products were digested with TaqI and FokI in accordance with the manufacturer's specifications (New England Biolabs, Beverly, MA, USA). The presence (lowercase) or absence (uppercase) of the enzyme recognition site was identified by ethidium bromide staining of fragments separated in a 2% agarose gel. Genotypes were assigned as TT, Tt and tt for the TaqI polymorphisms and FF, Ff and ff for the VDR-FokI polymorphisms. The PCR products digested with TaqI enzyme resulted in fragments of 496 bp and 249 bp in the presence of the polymorphic cutting site. The PCR products of VDR-FokI polymorphism (273 bp) digested with FokI enzyme result in 198 bp and 75 bp products in the presence of restriction site.

3.2. Measurement of CEA and CA15.3

Serum levels of carcinoembryonic antigen (CEA) and cancer antigen 15.3 (CA15.3) (DRG Diagnostics, GmbH, Germany) were measured by an enzyme-linked immunosorbent assay (ELISA) kit in duplicate according to the manufacturer's instructions.

3.3. Measurement of 25-hydroxyvitamin D

For quantification of 25(OH)D in serum we used the 25-hydroxyvitamin D ELISA kit supplied by (EUROIMMUN Medizinische Labordiagnostika AG, UK) in duplicate samples.

3.4. Immunohistochemical analysis

Histopathological examination and subsequent immunohistochemical (IHC) studies were performed for the biomarkers estrogen receptors (ER) and progesterone receptors (PR). Immunohistochemical reaction was carried out using avidin–biotin-complex immunoperoxidase system (Kiernan, 1999). Serial sections of paraffin embedded sections were deparaffinized on charged slides. The sections were incubated in hydrogen peroxide 0.1% (Merck; Darmstadt, Germany) for 30 min to block the endogenous peroxidase. To prevent non-specific binding of the primary antibody, the sections were incubated with diluted normal serum (10 ml PBS containing 150 μl horse serum; Vector Laboratories) for 20 min at room temperature. Sections were then incubated with the primary antibody. The primary antibody used was anti-ER (mouse monoclonal IgG, code number sc-56833, Santa Cruz Biotechnology, CA), anti-PR (rabbit polyclonal IgG, code number sc-539, Santa Cruz Biotechnology, CA). The chromagen 3,3′-diaminobenzidine (DAB; Dako, Glostrup, Denmark) was used for 8 to 10 min for visualization. Sections were counterstained with Mayer's acidic hematoxylin, dehydrated, and