

Case control study

Estrogen receptor alpha dinucleotide repeat polymorphism in Japanese patients with autoimmune thyroid diseases

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Abstract

Background: The autoimmune thyroid diseases (AITDs), comprising Graves' disease (GD) and Hashimoto's thyroiditis (HT), appear to develop as a result of complex interactions between predisposing genes and environmental triggers. Susceptibility to AITDs is conferred by genes in the human leukocyte antigen (HLA) and genes unlinked to HLA, including the CTLA-4 gene. Recently, an association to some estrogen receptor (ER) α genotypes with breast cancer, hypertension, osteoporosis, generalized osteoarthritis, and some autoimmune diseases such as rheumatoid arthritis has been reported. We have analyzed a dinucleotide (TA) n repeat polymorphism lying upstream of the human ER α gene in patients with AITDs and in normal subjects.

Results: Seventeen different alleles were found in 130 patients with GD, 93 patients with HT, and 190 control subjects. There was no significant difference in the distributions of ER α alleles between patients and controls.

Conclusions: The present results do not support an association between the ER α gene and AITD in the Japanese population.

Background

Autoimmune thyroid diseases (AITDs), including Graves' disease (GD) and Hashimoto's thyroiditis (HT), are the most common human autoimmune diseases and are responsible for significant morbidity in premenopausal women. AITDs are caused by immune responses to the thyroid gland. In GD, the autoimmune process results in production of thyroid-stimulating antibodies and leads to hyperthyroidism, whereas in HT, the end result is thyroid cell death and hypothyroidism [1,2].

The pathogenesis of AITDs involves complex interactions between genetic and environmental factors [3,4]. The genetic relations between GD and HT and the famil-

ial and sporadic forms of these diseases are unclear. This problem, until now, has been addressed by studying a variety of candidate genes, primarily via association studies. Because it has been assumed that immune dysregulation and/or thyroid antigen presentation are involved in AITD, the candidate genes that have been tested comprised genes involved in immunoregulatory pathways and genes encoding for the major thyroid autoantigens. These genes included the human leukocyte antigen (HLA) [5], immunoglobulin H heavy chain [6], T cell receptor [7], interleukin-1 receptor antagonist [8], interferon- γ [9], thyroid stimulating hormone receptor [10,11], thyroid peroxidase [12], and cytotoxic T lymphocyte antigen-4 (CTLA-4) genes [13]. With the excep-

tion of the HLA and CTLA-4 loci, the candidate genes examined gave either negative or equivocal results. Recently, the existence of an estrogen receptor (ER) α gene polymorphism has made clear, and its association to some variant ER α genotypes with breast cancer [14,15], hypertension [16], osteoporosis [17,18], generalized osteoarthritis [19], and some autoimmune diseases such as rheumatoid arthritis [20] has been reported.

The ER α gene on chromosome 6q24-q27 is organized into eight exons and seven introns extending over approximately 140 kilobases [21]. ER α belongs to the nuclear hormone receptor superfamily and modulates the transcription of target genes in response to estrogen, a potent immunomodulatory hormone [22]. Estrogens appear to play a central role in the immune response and immune-mediated diseases [22]. In view of the possible

role of estrogens in the pathogenesis of AITDs, we have analyzed a dinucleotide (TA) n repeat polymorphism lying upstream of the human ER α gene in patients with AITDs and in normal subjects.

Results

Seventeen different alleles were found in 130 patients with GD, 93 patients with HT, and 190 controls subjects. The various alleles were designated as ER α *1 through ER α *17 according to their sizes, which ranged from 164 to 198 bp. The distribution of ER α alleles in the three groups is shown in Table 1. Allele frequencies in our GD patients and our control subjects did not differ significantly ($X^2 = 15.49$, 16 degrees of freedom, $p = 0.49$). Allele frequencies in our HT patients and our control subjects also did not differ significantly ($X^2 = 14.62$, 16 degrees of freedom, $p = 0.55$).

Table 1: Allele frequencies of the ER α gene polymorphism in patients with AITDs and in control subjects

ER α polymorphism	Graves' disease n=130, (260 alleles), (%)	Hashimoto's thyroiditis n=93, (186 alleles), (%)	Controls n=190, (380 alleles), (%)
allele *1	20 (7.7%)	13 (7.0%)	24 (6.3%)
allele *2	40 (15.3%)	34 (18.3%)	88 (23.2%)
allele *3	49 (18.7%)	25 (13.4%)	58 (15.3%)
allele *4	32 (12.3%)	26 (14.0%)	39 (10.3%)
allele *5	7 (2.7%)	2 (1.1%)	13 (3.4%)
allele *6	10 (3.8%)	8 (4.3%)	12 (3.2%)
allele *7	19 (7.3%)	12 (6.5%)	23 (6.1%)
allele *8	10 (3.8%)	12 (6.5%)	16 (4.2%)
allele *9	15 (5.8%)	10 (5.4%)	23 (6.1%)
allele *10	11 (4.2%)	10 (5.4%)	15 (3.9%)
allele *11	17 (6.5%)	12 (6.5%)	37 (9.7%)
allele *12	14 (5.4%)	12 (6.5%)	18 (4.7%)
allele *13	10 (3.8%)	7 (3.8%)	10 (2.6%)
allele *14	5 (1.9%)	2 (1.1%)	2 (0.5%)
allele *15	1 (0.4%)	0 (0%)	1 (0.3%)
allele *16	0 (0%)	0 (0%)	1 (0.3%)
allele *17	1 (0.4%)	1 (0.5%)	0 (0%)

The number of subjects is shown with the corresponding percentage in parentheses.

Discussion and Conclusions

ER α belongs to the nuclear hormone receptor superfamily and modulates the transcription of target genes in response to estrogen [22]. Recent studies have shown the presence of ER α s on the cells involved in the immune response, namely thymocyte, macrophages and endothelial cells [22]. Particular attention has been focused on the dose-dependent influence of estrogen on the immune response, which appears to be related to the clinical symptoms of autoimmunity (i.e. the effects of pregnancy or oral contraceptive pills) [22]. The influence of estrogens on cytokine production by target cells, through interference with their transcriptional activity, has also been the

focus of various studies [22]. The effect of estrogens on the expression of the protooncogenes and oncosuppressor genes involved in programmed cell death (apoptosis) might also be relevant to human autoimmunity, in particular the uncontrolled synovial lining cell hyperplasia associated with rheumatoid arthritis and the prolonged T-cell survival in systemic lupus erythematosus [22]. Thus, we investigated the relation between a dinucleotide (TA) n repeat polymorphism lying upstream of the human ER α gene and AITDs. Our data did not appear to indicate any association between the ER α gene and the AITDs analyzed. This result might indicate of a large diversity in the genetic background of AITDs, although this

observation deserves further analysis in a larger group of AITD patients.

Materials and Methods

Subjects

One hundred and thirty unrelated Japanese women with GD and 93 unrelated Japanese women with HT were included in this study. GD was diagnosed on the basis of clinical symptoms and biochemical confirmation of hyperthyroidism, including diffuse goiter, ophthalmopathy, elevated radioactive iodine uptake, and thyroid hormone levels. HT patients had documented clinical and biochemical hypothyroidism requiring thyroid hormone replacement therapy and showed autoantibodies against thyroid peroxidase with or without antibodies against thyroglobulin. One hundred and ninety unrelated Japanese women without clinical evidence or family history of any autoimmune diseases were selected as normal control subjects. The research protocol was approved by the ethics committee of our hospital, and informed consent was obtained from all patients and controls.

Determination of microsatellite polymorphism by polymerase chain reaction

Genomic DNA was isolated from whole blood with a Genomix kit (Talent, Trieste, Italy). Microsatellite marker loci were typed with fluorescence-based methods [23,24]. The polymerase chain reaction (PCR) was performed with oligonucleotide primers designed to amplify a polymorphic (TA)_n repeat at 1174-base pair upstream of the human ER α gene [18]. PCR was performed in a total volume of 20 μ l of the following mixture: 100 ng of human genomic DNA; 5 pmol of each primer (Cy-5'-GACGCATGATATACTTCACC-3' and 5'-GCAGAATCAAATATCCAGATG-3'); 200 μ M of each dNTPs; 2 μ l of 10 \times reaction buffer (Takara Shuzo Co., Kyoto, Japan); and 1 unit of *Taq* DNA polymerase (Takara Shuzo Co.). Thirty PCR cycles of 2 min at 94°C, 1 min at 58°C, and 1 min at 74°C were performed. The amplified fragments were analysed by electrophoresis with an automatic DNA sequencer using 8% or 6% polyacrylamide gels containing 7 M Urea (Amersham Pharmacia Biotech, Buckinghamshire, UK). The raw data were converted to dinucleotide repeat polymorphic band patterns with the use of a software program (Amersham Pharmacia Biotech.). The length of TA repeat in each amplified product was determined by comparison with a ladder of control DNAs.

Statistical Analyses

Comparisons between the various alleles in patients with AITDs and in controls were made with the X²-test, and $p < 0.05$ was considered significant. Fisher's exact test was used when necessary. The relative risk was calculated by Woolf's method [25].

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