
A Western blot and molecular genetic investigation of the estrogen receptor beta in giant cell arteritis

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ABSTRACT

Objective. *The epidemiology of giant cell arteritis (GCA) may indicate a pathogenetic relationship between GCA and female sex hormone metabolism; GCA is two to four times more common in women compared with men. Our previous analyses gave no support for the hypothesis that the pathogenesis of GCA should be related to somatic mutations in the estrogen receptor alpha (ER α) gene. The object of the present study was to investigate the size of the estrogen receptor beta (ER β), and the size and nucleotide sequence of the ER β gene in temporal arteries in GCA.*

Methods. *The ER β protein was analyzed by Western blot technique and the ER β gene by RT-PCR and direct sequencing of the PCR product.*

Results. *Western blot analysis revealed an ER β of normal size. There were no aberrations in size or nucleotide sequence in the ER β gene in the GCA patients.*

Conclusion. *The present observations gave no support for the hypothesis that somatic mutations in the ER β gene should be involved in the pathogenesis of GCA.*

Introduction

Giant cell arteritis (GCA) is a chronic form of vasculitis in medium size and large arteries which affects people over fifty. (1). The fact that GCA is two to four times more common in women compared with men, raises the question of whether sex hormones are involved in its pathogenesis; epidemiological reports indicate a possible relationship between GCA and female sex hormone metabolism (2, 3). The aims of the present investigation were to analyse the size of the estrogen receptor beta (ER β), and the size and nucleotide sequence of the ER β gene in GCA, using a combination of Western blot, reverse transcriptase poly-

merase chain reaction (RT-PCR) and sequencing techniques.

Materials and methods

Western blot

In the Western blot study, inflamed arteries from an 83-year-old man and two women, aged 72 and 74 who fulfilled the ACR criteria for GCA (4), were compared with non-inflamed arteries from two men, aged 72 and 74, and four women, aged 64 \pm 10 (\pm SD) who, after the biopsy was taken, proved not to have GCA. Western blot analysis of the ER β protein was performed as described elsewhere (5) with monoclonal anti ER β antibodies (Novocastra, clone EMR02; 1:300).

RT-PCR and sequencing analyses

Inflamed temporal artery tissue from four women, aged 72.5 \pm 4.4 (\pm SD), and one man aged 85 who fulfilled the ACR criteria for GCA (4), was frozen in Calor gas and liquid nitrogen. Negative temporal arterial tissue was collected from two female controls, aged 71 and 85, who later proved not to have GCA.

Total RNA was extracted from 15 x 30 μ m sections of fresh-frozen arterial tissue (Ambion's RNAqueous-4PCR Kit, Austin, USA). First-strand cDNA synthesis was performed on total RNA from GCA patients, control temporal arteries and human uterus PolyA+RNA control (BD Biosciences Clontech, USA) by using oligo dT₁₂₋₁₈ and Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences Ltd., UK). Exons 1 to 7 were amplified by PCR, using the following forward (F) and backward (B) primers: F1: 338-359/B1: 622-598, F2: 592-614/B2: 921-898, F3: 900-922/B3: 1305-1283, F4: 1254-1274/B4: 1565-1543, F5: 1504-1524/B5: 1773-1753, F6: 1724-1744/B6: 1896-1875 and F7: 1878-1900/B7: 2011-1988 (Gene bank accession number NM_001437).

The amplified fragments were purified by Micro Spin™ S-300 columns (Amersham Biosciences Ltd., UK) and subjected to direct sequencing, using Big Dye Terminator Cycle sequencing kit and an ABI PRISM™ 377 DNA sequencer (Applied Biosystem, Foster city, CA).

The study was approved by the local Research Ethics Committee at the Medical Faculty, Göteborg University (286-96).

Results

Western blot

Western blot analysis of ERβ protein showed one fragment of approximately 60 kDa, corresponding to wild-type ERβ in artery tissue from GCA patients, control artery tissue, control tissue from breast tumor and normal breast tissue (Fig. 1).

RT-PCR and sequencing analyses

RT-PCR analysis of the total RNA extracted from temporal artery tissue in GCA patients, control temporal arteries and uterus revealed the expected size of all amplified fragments (Fig. 2). In four GCA patients, the complete nucleotide sequences of the ERβ gene were analysed without finding any sequence variation. In two controls and in one more GCA patient, the whole gene, except for fragment no. 6 (nucleotides 1724-1896), was successfully analysed and found normal.

Discussion

ERα and ERβ have been identified in a number of different types of inflammatory cell and in vessel walls. Therefore, structural defects in these receptors might, theoretically, affect a number of different pathogenetic steps in GCA. However, our present investigation gave no evidence that structural defects in the ERβ should be involved. The ERβ was of normal size in GCA patients and there were no aberrations in the size or nucleotide sequence of the receptor gene. A previous study did not reveal significant aberrations in the ERα gene (6).

According to a recent report from our laboratory, three factors related to low levels of female sex hormones, i.e. early menopause, low body mass index

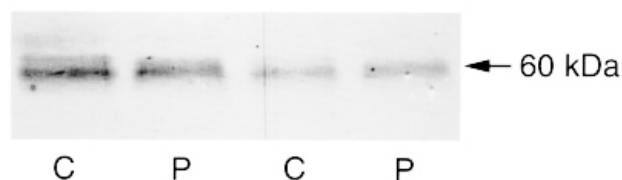


Fig. 1. Western blot analysis of ERβ in control breast tissue (C) and in inflamed temporal artery tissue from a GCA patient (P), showing normal size of the protein in both. (kDa: kilodalton).

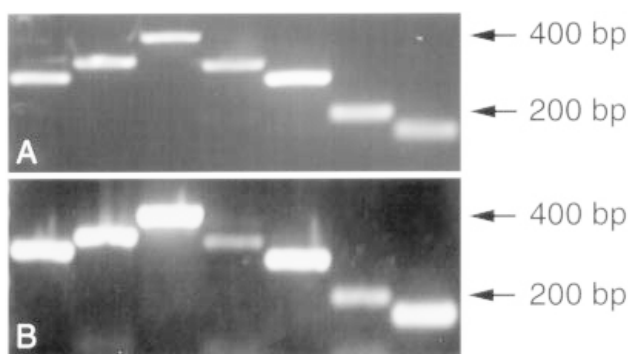


Fig. 2. RT-PCR analysis of the ERβ gene in uterine control tissue (A), and in temporal artery tissue from a GCA patient (B), showing identical fragments in both. (bp: base pair).

(BMI) and cigarette smoking were associated with an increased risk of developing GCA (3). Furthermore, Duhaut *et al.* (2) found fewer pregnancies, i.e. a shorter total hyperestrogenic period, among GCA patients than among controls, suggesting that this would imply less protection of the vascular wall and an increased risk of getting GCA.

Changes in estrogen level might influence various pathogenetic steps in GCA, and the effects mediated by ERα and ERβ might therefore be complex and manifold. In GCA, activated dendritic cells (DC) are involved in antigen presentation (7). Estrogen influences the differentiation of the DC:s, may reduce their antigen presenting capacity, and reduces the production of tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and interleukin 2 (IL-2) by mature DC:s (8). Further, estradiol influences antigen-specific T-cell expansion and IFN-γ production, two steps which are central in GCA, and it may increase as well as decrease the production of inflammatory mediators by macrophages (7, 8). Moreover, estrogen protects the cardiovascular system, partly by direct effects on the vessel walls (9, 10). It inhibits vascular smooth-muscle cell proliferation, thereby preventing neointimal thickening after experimental arterial injury, and it ameliorates atherosclerotic lesions (11, 12). In contrast, in GCA,

myofibroblast proliferation causes extensive intimal thickening, severe arterial stenosis, and ischaemic complications such as retinal necrosis and blindness (7, 13).

In conclusion, the present investigation gave no support to the contention that the pathogenesis of GCA should be related to somatic mutations in the ERβ gene. This is in accordance with our previous investigation of the ERα gene. Other pathogenetic links between estrogen metabolism and GCA should be sought for.

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