Calcitonin receptor (CTR) gene polymorphism in a Turkish population using PCR-RFLP assay

Turgut Ozturk, Pinar Okyay, Fevzi Bardakci

Abstract

C/T polymorphism at the nucleotide position 1340 (codon position 447) in the CTR gene has been associated with osteoporosis and kidney stones. This study is set to determine the frequency of such an important polymorphism in the CTR gene in a Turkish population to determine their possible susceptibility to the diseases that have been associated in previous studies. CTR gene was amplified by Polymerase Chain Reaction (PCR) from 489 healthy blood donors from Aydin province where is located on the Southern Aegean Region of Turkey. To detect C/T polymorphism in CTR gene, a PCR product of 228 bp was digested with restriction enzyme Alu I. Data obtained revealed three genotypes so called C/C, C/T and TT of which frequencies were 16.35%, 47.44% and 36.21%, respectively. The frequency of the mutant T allele was 0.5992 in the Turkish population. The Turkish population showed a close similarity with Italian population with regard to genotype and allele frequencies of CTR gene. The population studied was in Hardy-Weinberg equilibrium suggesting that there is no strong selection in favor of any genotype. Frequency of heterozygote genotype, which has been suggested being less prone to osteoporosis, was higher than Japanese and Asian populations.

Keywords: Osteoporosis, Calcitonin receptor, polymorphism, Turkish.

Introduction

Calcitonin (CT), discovered by Copp et al. in 1962 [1], reduces blood concentration of calcium and secreted by parafollicular or C cells of thyroid gland [2]. CT is a 3418 Da weight and 32 amino acid length peptide hormone inhibits bone resorption and stimulates renal calcium excretion [3, 4]. Because of the ability of inhibition bone resorption, CT is used in the clinical treatment of osteoporosis [5, 6], paget’s disease [7, 8] and hypercalcemia [9]. CT acts its target cells through binding its receptor so that calcitonin receptor (CTR) is activated. CTR contains seven potential transmembrane domains located on the surface of osteoclasts [10] and also occurs on the other cell types such as kidney [11], ovary [12], brain [13] lung [14] and testis [15]. Activation of CTR induces adenylcyclase and causes a decline in the osteoelastic bone resorption [10]. Human CTR gene located on 7q 21.3 [17] has several splice variant, two of that known as CTR-1 and CTR-2 [18]. CTR-2 has a single nucleotide polymorphism at 1340 position (C→T) which lead to amino acid change (Pro→Leu) at codon 447 [19]. This base mutation in CTR-2 is correspond to at 1377 nucleotide position and in 463 codon position of amino acid sequence in splice variant CTR-1 [18]. CTR polymorphisms have been used for many association studies due to its occurrence in the coding region [20, 18, 21]. A number of studies have also been carried out on allelic frequencies in different populations [22, 21]. However, to our knowledge, no information exists on polymorphism and distribution of the CTR gene in Turkey. The present work aimed to determine the allelic frequencies of the CTR gene in the Turkish population that could provide some valuable information for determination of its susceptibility to the diseases associated with them.

Materials and Methods

Study Population

Cross-sectional and Community based study was performed in Aydin province, a major city in the Aegean region of Turkey (western Anatolia). The sample size was calculated as 384 on a prevalence of 50%, d = 0.05, at a confidence level of 95%. A number design of 1.5 was used to allow for multistage sampling. The calculated study population size was finally 576. Multistage sampling was used in the selection of the study sample. Aydin was separated into five regions according to socio-economic and health data taken from the Directory of Health. When a volunteer applied to the blood donation center, a detailed physical examination was performed by the physician of the center. Healthy volunteers were asked to join the study, and a face-to-face questionnaire was performed. The exclusion criteria were: (1) Not being...
accepted as a blood donor as a result of the physical examination; and (2) Only one randomly selected person was included from a family.

The current study was one of two studies to be performed using blood samples from the same population. The study protocol was approved by the Ethical Committee of the Medical School of Adnan Menderes University. Permission for the field study was given by the Turkish Red Crescent. Finally, written informed consent was provided by each participant.

Polymerase Chain Reaction
Blood samples of 2.5 ml were collected in blood collection tubes with EDTA. Genomic DNA was extracted using a Rapid DNA Purification kit, according to the manufacturer’s protocol (Epicentre Biotechnologies, USA). To determine polymorphisms, a region of 228 bp in CTR was amplified using the PCR primer sets [21]:

- F: 5'-CTCAGTGATCACGATACTGTG-3'
- R: 5'-ATTCAGTTGAAACCAGCTTG-3'

PCR was performed in a 25M l mixture of (100–150 ng/ml) DNA, 1X PCR buffer, 1.5 mM MgCl2, 0.1 mM dNTPs, 0.1 uM each primer, and 0.5 U Taq DNA polymerase. The amplification conditions were 5 min at 95 °C, 30 s at 95 °C, 30 s annealing at 55 °C, 30 s at 72 °C, and finally 10 min at 72 °C, for 35 cycles.

PCR-RFLP Assay
For detection of the C/T polymorphism in CTR, a PCR product of 228 bp was digested with restriction enzyme AluI (Fermentas, Vilnius, Lithuania), which has a recognition site in this region. Eight microliters of PCR product was digested by AluI (1 unit) in a 25 ul reaction according to manufacturer protocol incubated at 37 °C overnight. Individual genotypes were determined after electrophoresis of digested PCR products in 3% agarose gel stained with ethidium bromide.

Statistical Analysis
Observed-expected genotype frequencies and deviation from Hardy–Weinberg equilibrium for CTR gene at 1340th allelic site were assessed using Chi-square analyses. The value of 0.05 is considered statistically significant.

Results and Discussions
In this study, 54 females and 435 males (total 489 volunteers) were screened for CTR/C gene at 1340th allelic site. The allele frequency determined for 489 volunteers was 0.4008 for C and 0.5992 for T. The genotype frequency of C/C, C/T, and T/T was found 16.35%, 47.44% and 36.21%, respectively. Evaluating males and females allele and genotype frequencies separately, T/T genotype frequency in females was found relatively higher than males genotype frequency. Of 54 females screened for C/T base substitution, genotype frequencies were as follow: C/C, 12.96%, C/T, 46.42% and T/T, 40.62. On the other hand, the genotype frequencies C/C, C/T and T/T in 435 males were 16.78%, 47.35% and 35.87%, respectively. Distribution of genotypes of CTR gene in the studied population was in Hardy-Weinberg equilibrium using Chi-square analyses. The population was in Hardy-Weinberg equilibrium using Chi-square analyses. suggesting that there is no strong selection in favor of any genotype at least until at the age of maturity.

There have been a number of genetic association studies with osteoporosis [21, 24] and calcium oxalate urolithiasis [22, 25] in various populations. The study performed by Masi et al. [21] showed that Italian osteoporotic women have less frequently CC genotype with respect to healthy women. Taboulet et al. [20] have found association between CTR polymorphism and higher bone density in French women after menopause. Similarly there is another study has been carried out to determine correlation with body mass and bone mineral density in Japanese women [26]. According to their results, C allele was predominant in Japanese population and CTR allele has been associated with body weight in Japanese women. As regards calcium oxalate urolithiasis, Chen et al. [22] have found that frequency of TT genotype was higher in patients suffering from calcium oxalate stones than those had no health problems. On the other hand, Bid et al. [27] have found C allele more frequent in pediatric nephrolithiasis group. Mittal et al. [23] found no association between the C/T polymorphism and urinary stone disease. Even though there are some controversies, CTR C/T polymorphism seems to have potential effect on the susceptibility of polygenic disease like osteoporosis and kidney stones.

Table 1: Allelic and genotype frequencies of C/T polymorphism at 1340th allelic site of CTR gene in the Turkish population

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Allele</th>
<th>Frequency</th>
<th>Genotype</th>
<th>Frequency</th>
<th>χ²</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All volunteers n: 489</td>
<td>C</td>
<td>0.4008</td>
<td>C/C</td>
<td>80 (16.35%)</td>
<td>0.086285</td>
<td>0.76895</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>0.5992</td>
<td>C/T</td>
<td>232 (47.44%)</td>
<td>0.76895</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T/T</td>
<td>177 (36.21%)</td>
<td>0.14475</td>
<td>0.703604</td>
</tr>
<tr>
<td>Females n: 54</td>
<td>C</td>
<td>0.3704</td>
<td>C/C</td>
<td>7 (12.96%)</td>
<td>0.028665</td>
<td>0.865555</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>0.6296</td>
<td>C/T</td>
<td>26 (46.42%)</td>
<td>0.028665</td>
<td>0.865555</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T/T</td>
<td>21 (40.62%)</td>
<td>0.14475</td>
<td>0.703604</td>
</tr>
<tr>
<td>Males n: 435</td>
<td>C</td>
<td>0.4046</td>
<td>C/C</td>
<td>73 (16.78%)</td>
<td>0.028665</td>
<td>0.865555</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>0.5954</td>
<td>C/T</td>
<td>206 (47.35%)</td>
<td>0.028665</td>
<td>0.865555</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T/T</td>
<td>156 (35.87%)</td>
<td>0.14475</td>
<td>0.703604</td>
</tr>
</tbody>
</table>

*Hardy Weinberg conformity, P value of 0.05 is considered statistically significant.
A number of studies have also investigated CTR allele variations in different ethnic groups (Table 2). With respect to genotype and allele frequencies, Asian and Japanese populations are the most different ones due to the lower TT and higher CC genotype frequencies. Both populations have higher frequencies of C allele than T relative to other ethnic populations. The American-Caucasian and French-Caucasian populations have lower frequency of CC genotype while in Hispanic and African-American populations both homozygous genotypes are almost equal frequencies and high heterozygous genotypes. When we compared our findings to those reported studies only an Italian populations was close to the Turkish allele frequencies and almost equal genotype distributions. In conclusion, frequency of T allele was found to be close to the C allele in Turkish population giving higher frequency of heterozygote genotype which has been suggested to be less prone to osteoporosis.

**Table 2. Genotype and allele frequencies of CTR C→T polymorphism in some ethnic populations including the Turkish population**

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Genotype Sample size (%)</th>
<th>Allele Sample size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT</td>
<td>TC</td>
</tr>
<tr>
<td>American-Caucasian (24)</td>
<td>104(59.09)</td>
<td>64 (36.36)</td>
</tr>
<tr>
<td>African-American (24)</td>
<td>23 (25.06)</td>
<td>41 (47.7)</td>
</tr>
<tr>
<td>Asian (24)</td>
<td>0 (0)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Hispanic (24)</td>
<td>10 (20)</td>
<td>26 (52)</td>
</tr>
<tr>
<td>Italian (21)</td>
<td>254 (41.4)</td>
<td>136 (44.3)</td>
</tr>
<tr>
<td>French-Caucasian (20)</td>
<td>105 (48.8)</td>
<td>96 (44.7)</td>
</tr>
<tr>
<td>Japanese (18)</td>
<td>1 (0.9)</td>
<td>22 (18.8)</td>
</tr>
<tr>
<td>North Indian (23)</td>
<td>6 (5.7)</td>
<td>73 (69.5)</td>
</tr>
<tr>
<td>The present study</td>
<td>177 (36.2)</td>
<td>232 (47.4)</td>
</tr>
</tbody>
</table>

**Acknowledgements**

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**References**


