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# Chromosomal findings and sequence analysis of target exons of calcium-sensing receptor (*CaSR*) gene in patients with Sagliker syndrome

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**Background/aim:** Sagliker syndrome (SS) develops as a continuation of chronic kidney disease and secondary hyperparathyroidism conditions. It was thought that there are some genetic predisposition factors leading to SS. The calcium-sensing receptor (CaSR) is essential for calcium homeostasis in the body. We aimed to examine SS patients for chromosome aberrations (CAs) and *CaSR* gene abnormalities in exons 2 and 3.

**Materials and methods:** Twenty-three patients and 23 control subjects were admitted to Balcalı Hospital of the Medical Faculty of Çukurova University in Turkey between 2009 and 2011. Chromosomal analysis was performed according to standard cytogenetic methods. Full sequencing of exons 2 and 3 of the *CaSR* gene was done.

**Results:** We found base alterations and deletions in exons 2 and 3 of the *CaSR* gene. We also found a statistically significant increase in the rate of CAs in patients compared to controls. In total we evaluated 639 metaphase plaques in 23 patients and found 241 CAs, of which 88% were structural and 12% were numerical abnormalities.

**Conclusion:** There is no relation between the etiology of SS and nucleotide alterations that we could find in exons 2 and 3 of the *CaSR* gene. Our data suggest that there may be a correlation between CAs and the progression of SS.

**Key words:** Sagliker syndrome, *CaSR* gene, chromosomal abnormalities, chronic kidney disease, nucleotide alterations

## 1. Introduction

Sagliker syndrome (SS), as a new entity, was named by Sagliker et al. in 2004 (1). This syndrome starts and develops particularly before puberty while chronic kidney disease (CKD) reaches the stage III level and as a continuation of secondary hyperparathyroidism. Defining prominent features of CKD, including uglifying human facial appearance, short stature, extremely severe maxillary and mandibulary changes, soft tissues in the mouth, teeth-dental abnormalities, fingertip changes, neurologic manifestations, audiological findings, and severe psychological problems (1–13) (Figures 1 and 2), are also important findings for this unique syndrome. The etiology of SS is not known. In CKD patients having bad prognosis and developing SS, it is plausible to think that there must be some genetically predisposing factors. Genetic studies need to be done in order to clarify this syndrome. There is a proposal about a cytogenetic definition in the etiology of CKD patients. This was based on reports that papillary renal cell carcinomas have gains of chromosomes 7, 12, 16, 17, and 20, whereas small papillary renal tumors have only gains of chromosomes 7 and 17 and loss of the Y chromosome (14–17) . Gains of chromosomes 7 and 17 and loss of the Y chromosome in renal papillary adenoma were also reported (18). Gains of additional chromosomes, most frequently of chromosomes 12, 16, and 20, were suggested as responsible for the progression to papillary carcinoma. It has been shown that the most common chromosome aberration (CA) in renal oncocytomas is the loss of chromosome 1 or 1p, which was detected in 32% of tumors (19). Monosomy 3 was only observed in high-grade and/or advanced renal cell carcinomas. Trisomy 17 was only detectable in papillary renal cell tumor subtypes, irrespective of tumor state, showing increased copies with tumor growth.

The calcium-sensing receptor (CaSR) is a G-protein-coupled receptor that plays a central role in the regulation

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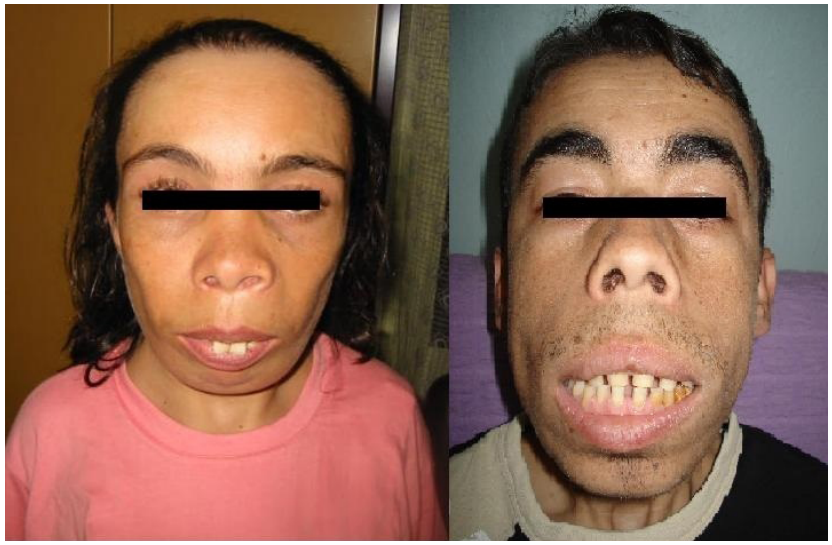

**Figure 1.** Pictures showing unique uglifying human facial appearances in two patients with chronic kidney disease and Sagliker syndrome.

**Figure 2.** Left picture showing height differences in sisters. Right older and shorter girl is the patient with Sagliker syndrome who is shorter than her younger and sister. Right picture showing height differences in siblings. The girl who is on the left is the patient with Sagliker syndrome. She is older but quite shorter than her brother.

of parathyroid hormone secretion by calcium (20). CaSR in the parathyroid, thyroid, and kidneys is essential for calcium homeostasis. CaSR, located in the plasma membrane of the cell, detects changes in extracellular calcium concentration. When the extracellular calcium concentration rises, CaSR activates the G-protein signaling pathway, which reduces transcription of the *PTH* gene and secretion of the PTH hormone, resulting in a decrease in free calcium concentration. Human CaSR is encoded by six exons (exons 2–7) (21–23), located on chromosome 3q13.3–21 (24) with exon 2 encoding 242 nucleotides of the 5'-untranslated region, followed by the translation start site. Many mutations have been identified to date in the coding region of extracellular CaSR that are

associated with inherited human hypo- and hypercalcemic disorders. In particular, the recently cloned extracellular CaSR has provided key insights into the pathogenesis of inherited human hypo- and hypercalcemic disorders (20,25). Mutations of *CaSR* have been reported to disturb the tight relationship between blood calcium and PTH secretion and to result in several diseases with abnormal blood calcium levels (21). *CaSR* mutations that result in a loss of function are associated with familial benign hypercalcemia. However, *CaSR* mutations that result in a gain of function lead to hypocalcemia with hypercalciuria.

We aimed to evaluate the association between late secondary hyperparathyroidism and *CaSR* gene polymorphisms and CAs in SS patients. The present study is a continuation of the study that analyzed the *GNAS1* gene exons 1, 4, 10, 4 in patients with SS and was published in 2012 (12).

## 2. Materials and methods

### 2.1. Cytogenetic analysis

Peripheral blood was taken from each subject, 23 patients and 23 controls, for culture study. Controls were selected from among healthy persons who had no important medical problems in their past. Each sample was examined for CAs in the cytogenetic laboratory of the Department of Medical Biology and Genetics, Faculty of Medicine, Çukurova University, Adana, Turkey. Standard cytogenetic techniques were used for culturing, harvesting, and slide preparation. Fifty metaphases were scored for each assay. The classification of CAs was done according to the nomenclature established in human gene mapping HGM11 (26). A nonparametric Fisher exact test was used to determine the difference in frequency of CAs observed in the patient and control groups.

### 2.2. *CaSR* genotyping

Blood samples were collected from 23 patients with SS. Written informed consent from all subjects and ethical consent from the Ethics Committee of the Medical School of Çukurova University were received. Genomic DNA was isolated from whole blood using the salt precipitation method. At the beginning, we aimed to sequence all six coding exons of the *CaSR* gene (exons 2–7). However, because of inadequate financial support gained for the study, we could not manage to sequence all exons. We chose to sequence exons 2 and 3 of the *CaSR* gene, because these exons code the extracellular part of the CaSR protein and in terms of Ca sensing the extracellular part of the CaSR protein is important. For sequencing of exons 2 and 3 of the *CaSR* gene, the following primer sets were used: exon 2, forward TAAATGGAAATTCTGAGCCACCTTAG and reverse TGATTACCAATCAATCACTATTC; exon 3, forward ACTCTGGACCTCAAGTGATCCACC and reverse TGGTCATGATACAGCATATCAACT.

The first PCR was performed in a final volume of 25 µL containing 12.5 µL of AmpliTaq Gold PCR Master Mix (2X), 2 µL of 2 pmol/µL reverse and 2 µL of 2 pmol/µL forward primers, 1 µL of 40–413 ng/µL DNA, and 7.5 µL water. For exon 2, PCR cycle conditions were 95 °C for 10 min, followed by 95 °C for 30 s, 60 °C for 1.5 min, and 72 °C for 1 min (40 cycles) and then 72 °C for 7 min. For exon 3, PCR cycle conditions were 95 °C for 10 min, followed by 95 °C for 30 s, 63 °C for 1 min, and 72 °C for 1 min (40 cycles) and then 72 °C for 7 min. For the purification of PCR product, ExoSap Purification was performed using a cycle condition protocol of 37 °C for 30 min and 80 °C for 15 min. The second PCR (BigDye reaction) was performed in a final volume of 10 µL containing BigDye Cycle Sequencing v3.1 (2 µL), 5X Sequencing Buffer (2 µL), forward primer (2 µL), PCR product (2 µL), and water (2 µL). PCR conditions were 96 °C for 1 min (activation), followed by 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min (25 cycles for amplification). Obtained PCR products were then purified using the Sephadex purification protocol. Finally Sephadex-purified PCR products were loaded onto an ABI 3130 machine for capillary electrophoresis and results were evaluated using SeqScape software.

### 2.3. Statistical analysis

CA frequencies of patients and healthy control subjects were compared using the nonparametric Fisher exact test and SPSS 15.0.

## 3. Results

### 3.1. Cytogenetic findings

The numerical and structural CAs of peripheral blood leukocytes from 23 patients with SS are summarized in Table 1. A total of 18 (78.2%) patients out of 23 revealed predominantly numerical and structural aberrations. In total we evaluated 639 metaphase plaques in 23 patients and we found 241 CAs (38% of all metaphases), of which 88% were structural and 12% were numerical abnormalities. In the control group, we examined 1150 metaphase plaques and we found 182 (15.8%) CAs. We found a statistically significant increase in the rate of CAs in patients compared to controls ($P < 0.001$). In SS patients, a total of 212 cells revealed a structural abnormality in at least one metaphase plaque or more. Structural aberrations predominated and usually consisted of deletions, translocations, breaks, and fragilities in various chromosomes (Figure 3). Important structural CAs that we found are related to chromosomes 2, 4, 5, 6, 7, 9, 10, 11, 13, X, and Y (Table 1; Figure 3).

### 3.2. Molecular findings

Sequence analysis was performed for exons 2 and 3 of the *CaSR* gene in 23 SS patients. For exon 2, no mutation was found in 16 patients. In three patients, adenine (A) base deletions at position 70239 were found. Because the 70239 position is outside of the boundaries of the coding region,

**Table 1.** Frequencies of cytogenetic findings in Sagliker syndrome patients.

| Karyotype | Total cells | Freq. in all cells (%) | Freq. in all anomalies (%) | Karyotype | Total cells | Freq. in all cells (%) | Freq. in all anomalies (%) |
|---|---|---|---|---|---|---|---|
| 46,XY | 275 | 43 | - | 13ps+ | 12 | 1.87 | 4.97 |
| 46,XX | 154 | 24 | - | chtb(13q31.1) | 1 | 0.15 | 0.41 |
| Total | 429 | 67 | - | chtb(16q22) | 1 | 0.15 | 0.41 |
| Structural abnormalities | | | | chtb(17q23) | 1 | 0.15 | 0.41 |
| t(1;3)(p32;q25) | 1 | 0.15 | 0.41 | chrb(18q22) | 1 | 0.15 | 0.41 |
| chtb(1p33) | 1 | 0.15 | 0.41 | del(X)(p22.1-pter) | 1 | 0.15 | 0.41 |
| chtb(2q23) | 1 | 0.15 | 0.41 | del(X)(q13.2-qter) | 1 | 0.15 | 0.41 |
| chtb(2p31) | 1 | 0.15 | 0.41 | Yq+ | 12 | 1.87 | 4.97 |
| fra(2p23) | 1 | 0.15 | 0.41 | Total | 212 | 33.17 | 87.96 |
| gap(2q23) | 17 | 2.66 | 7.05 | Numerical abnormalities | | | |
| t(3;7)(p23;p22) | 1 | 0.15 | 0.41 | 45,XY,-1 | 1 | 0.15 | 0.41 |
| del(4)(q22-qter) | 1 | 0.15 | 0.41 | 45,XX,-3 | 1 | 0.15 | 0.41 |
| chtb(4q31) | 1 | 0.15 | 0.41 | 45,XY,-8 | 1 | 0.15 | 0.41 |
| t(5;17)(p13.3-p13) | 1 | 0.15 | 0.41 | 45,XY/XX,-10 | 3 | 0.47 | 1.24 |
| chtb(5q31) | 1 | 0.15 | 0.41 | 45,XX,-12 | 2 | 0.31 | 0.83 |
| fra(5q31) | 1 | 0.15 | 0.41 | 45,XY/XX,-13, | 3 | 0.47 | 1.24 |
| gap(5q31) | 1 | 0.15 | 0.41 | 47,XY,+15 | 1 | 0.15 | 0.41 |
| fra(6q22) | 1 | 0.15 | 0.41 | 45,XY,-15 | 1 | 0.15 | 0.41 |
| gap(6q21) | 5 | 0.78 | 2.07 | 45,XX,-16 | 1 | 0.15 | 0.41 |
| fra(7q22) | 1 | 0.15 | 0.41 | 46,X,+17,-X | 1 | 0.15 | 0.41 |
| gap(7q22) | 1 | 0.15 | 0.41 | 45,XY,-17 | 1 | 0.15 | 0.41 |
| 9qh+ | 137 | 21.43 | 56.84 | 45,XY/XX,-19 | 5 | 0.78 | 2.07 |
| del(10)(p13-pter) | 1 | 0.15 | 0.41 | 45,XY/XX,-20 | 2 | 0.31 | 0.83 |
| del(10p15) | 1 | 0.15 | 0.41 | 45,XY,-22 | 3 | 0.47 | 1.24 |
| gap(10q22) | 2 | 0.31 | 0.83 | 45,X,-Y | 3 | 0.47 | 1.24 |
| del(11)(p13-pter) | 1 | 0.15 | 0.41 | Total | 29 | 4.53 | 12.03 |
| chtb(11p11.2) | 1 | 0.15 | 0.41 | General total | 241 | 37.7 | - |
| der(12p?) | 1 | 0.15 | 0.41 | | | | |
| t(13;15)(q34-q21) | 1 | 0.15 | 0.41 | | | | |

this alteration does not have any effect on the gene product. A > G heterozygote alterations were found at position 70471 in 2 patients. Although position 70471 is within the boundaries of exon 2, because of this region corresponds to the upstream region of the ATG start codon, these alterations also do not have any effect on the gene product. T > C heterozygote alteration was found at position 70622 in one patient. Because codon 38 is in the coding region of exon 2, codon changing takes place and a TTT ® TTC transition occurs. Both the TTT and TTC codons code for phenylalanine amino acid in the translation process, so the final gene product does not change because of this

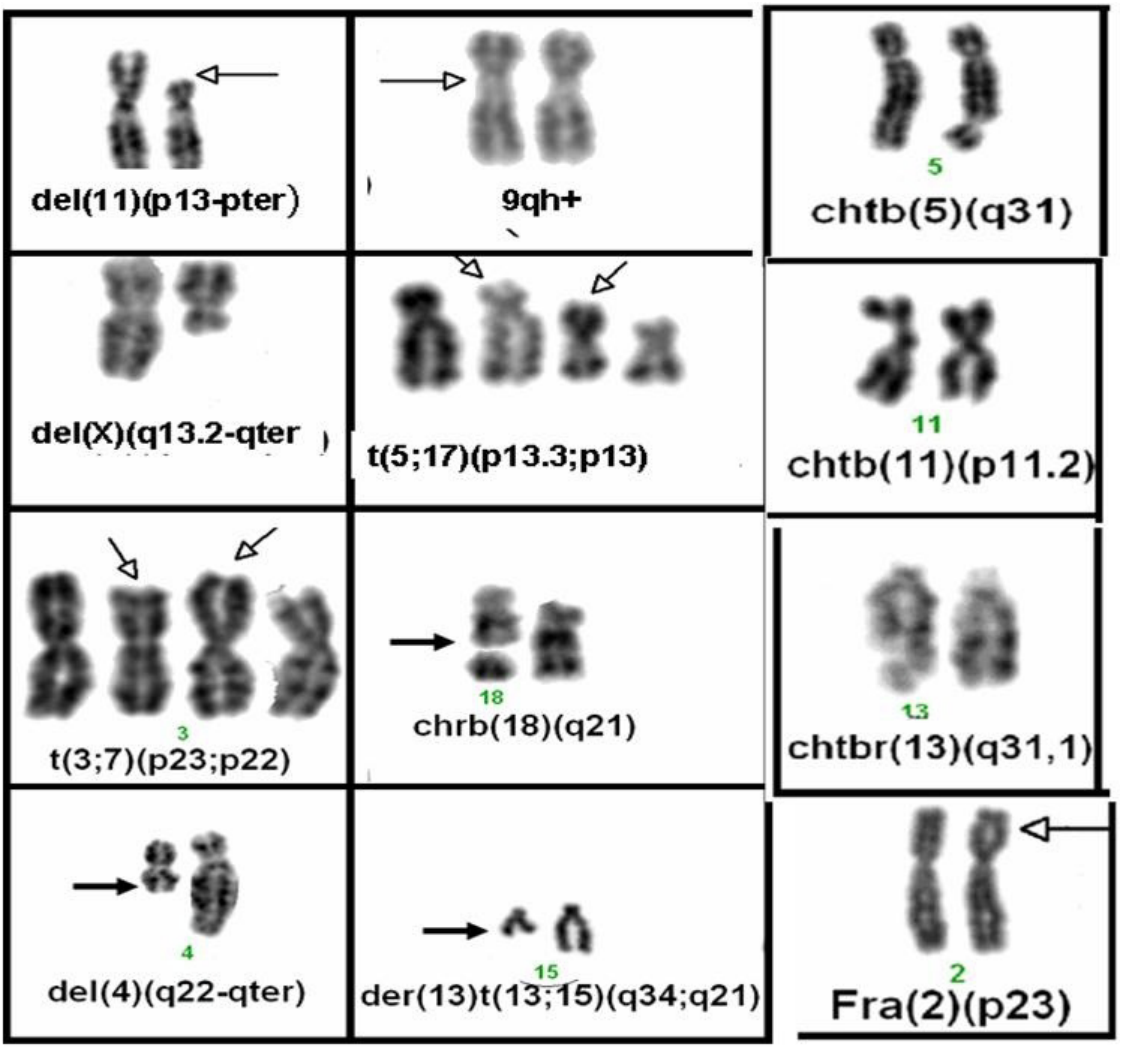


**Figure 3.** Structural chromosomal aberrations showed in the shape of partial karyotypes of SS patients.

transition (Table 2). For exon 3, no mutation was found in 8 patients. At position 73724, G > A homozygote and G > A heterozygote alterations were found in 11 patients and 1 patient, respectively. Position 73724 is outside of the boundaries of exon 3, so a change in the gene product does not take place (Table 2; Figure 4).

**4. Discussion**

In the present study, a total of 18 (78%) patients revealed predominantly numerical and structural aberrations (88% of the all aberrations were structural aberrations, and only 12% were numerical). The most common abnormalities were an increased incidence of autosomal aneuploidy, deletions, and chromatid breaks (Table 1). Aneuploidy, a sign of genomic instability, was observed in some of the patients in this study, along with other chromosomal changes. Autosomal monosomies and loss of Y were observed in our patients. In other studies, cytogenetic studies of papillary renal cell carcinoma and papillary adenoma showed frequent gains of chromosomes 7 and 17 and loss of the Y chromosome. It has been demonstrated that multiple genetic abnormalities, which included consistent losses of chromosomes 1, 4, 6, 8, 9, 13, 14, 15, and 22, are frequently encountered in cases of papillary renal cell carcinoma and papillary adenoma (27). It was indicated that the rate of loss of chromosome 16 from renal epithelial cells far exceeded that of chromosome 1 for mentioned cases (28). It is also known that the resulting gene dosage imbalance of aneuploidies has a noticeable effect on the phenotype. We think that the aneuploidies of chromosomes 1, 3, 8, 10, 12, 13, 15, 16, 17, 19, 20, and 22 that we found in our patients could also be of great importance in terms of early diagnosis and evaluation of the prognosis of SS.

**Table 2.** The observed sequence alterations of exons 2 and 3 of the *CaSR* gene.

| Exon 2 | | | Exon 3 | |
|---|---|---|---|---|
| Patient No. | Sequence alterations | Position | Sequence alterations | Position |
| 3 | T>C heterozygote | 70622 | | |
| 6 | | | G>A homozygote | 73724 |
| 7 | Deletion of adenine | 70239 | | |
| 8 | | | G>A homozygote | 73724 |
| 9 | A>G heterozygote | 70471 | G>A homozygote | 73724 |
| 10 | Deletion of adenine | 70239 | | |
| 11 | | | G>A heterozygote | 73724 |
| 12 | | | G>A homozygote | 73724 |
| 13 | Deletion of adenine | 70239 | | |
| 14 | | | G>A homozygote | 73724 |
| 15 | A>G heterozygote | 70471 | G>A homozygote | 73724 |
| 16 | | | G>A homozygote | 73724 |
| 17 | | | G>A homozygote | 73724 |
| 20 | | | G>A homozygote | 73724 |
| 21 | | | G>A homozygote | 73724 |
| 22 | | | G>A homozygote | 73724 |

Seventeen gaps, two chromatid breaks, and FS at bands q23x17, p31, q23, and p23 on chromosome 2 were also seen in four patients in our study. According to the previous study, a gene locus for nephronophthisis type I (NPH1) has been mapped by linkage analysis to chromosome 2q13. On the contrary, a known familial NPH locus on chromosome 2q13 and autosomal recessive polycystic kidney disease locus on chromosome 6p21.1-p12 were excluded by genetic linkage analysis (29–33). Therefore, we supposed that the chromosome region of 2p-q could play a role in the pathogenesis of SS. In another study, the 10p region was reported to include and harbor the susceptibility genes for African American individuals with early-onset nondiabetic etiologies of end-stage renal disease, and this was confirmed in diabetic families, as well (34). We also found both deletions and gaps at bands del(10)(p13-pter), del(10)(p15), and gap(10q22)×2 in four metaphases. These results show that both numerical and structural aberrations of chromosome 10 might be accepted as important in the evaluation of SS patients.

CaSR has important effects on extracellular calcium homeostasis. This is well known from studies of hypercalcemic and hypocalcemic humans harboring mutations (either inactivating or activating mutations, respectively) in the *CaSR* gene (21,35). Loss of CaSR function occurs in the inherited disorder neonatal severe hyperparathyroidism because of homozygous inactivation of the *CaSR* gene (36) or in cases in which heterozygous inactivation of the *CaSR* gene causes familial hypocalciuric hypercalcemia with atypical hyperparathyroidism (37) or familial isolated hyperparathyroidism (38). SS is also related to late secondary hyperparathyroidism, hypocalcemia, and hyperphosphatemia.

Some studies have identified an association of kidney stone formation with *CaSR* polymorphisms. Severe uremic secondary hyperparathyroidism shows reduced CaSR expression (39–43). In addition, mutations in growth-regulating genes may secondarily alter the calcium set-point by decreasing expression of CaSR (44,45). However, the specific genes and precise mechanisms involved in downregulation of parathyroid CaSR expression are not known. Hence, we wanted to describe the alterations of the *CaSR* gene in patients affected by SS. We found four sequence variations in SS patients. Among these variations, only one sequence variation (TTT ® TTC transition), found at position 70622 in exon 2, showed a different silent mutation in codon 38, and this nucleotide change does not lead to an amino acid change and


**Figure 4.** Complete sequences of exons 2 and 3 of the *CaSR* gene. Exon sequences and primers sequences are illustrated with red letters whereas base alterations are illustrated with blue capital letters.

results in no protein sequence alteration. Other sequence variations at positions 70239, 70471, and 73724 are outside of the boundaries of the coding regions, so these alterations do not have any effect on the gene product. Our findings show that hyperparathyroidism, hypocalcemia, and hyperphosphatemia were not associated with *CaSR* polymorphisms in these SS patients. Mutations in exons 2 and 3, which are postulated to encode the large extracellular domain of this protein, produce two different phenotypes. While most of the mutations identified in this region cause *FHH* and *NSHPT*, one mutation, 128Glu-Ala, results in autosomal dominant hypocalcemia (25). The divergent phenotypes associated with these mutations suggest that the extracellular domain is critical for determining the receptor's affinity or interaction with $Ca^{2+}$; mutations within this region appear to reset (up or down) ion binding and receptor activation.

In conclusion, our findings regarding a statistically significant increase in the rate of CAs in patients compared to controls ($P < 0.001$) confirm that chromosomal damages may be important tools to more clearly define the role of genomic factors in CKD susceptibility. However, exons 2 and 3 of *CaSR* gene variants do not seem to predispose to the development of SS. Further studies are needed to supplement the current data with newly diagnosed patients nationwide, in addition to existing patients.

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