

A FAMILIAL SYNDROME OF HYPOCALCEMIA WITH HYPERCALCIURIA DUE TO MUTATIONS IN THE CALCIUM-SENSING RECEPTOR

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ABSTRACT

Background The calcium-sensing receptor regulates the secretion of parathyroid hormone in response to changes in extracellular calcium concentrations, and mutations that result in a loss of function of the receptor are associated with familial hypocalciuric hypercalcemia. Mutations involving a gain of function have been associated with hypocalcemia in two kindreds. We examined the possibility that the latter type of mutation may result in a phenotype of familial hypocalcemia with hypercalciuria.

Methods We studied six kindreds given a diagnosis of autosomal dominant hypoparathyroidism on the basis of their hypocalcemia and normal serum parathyroid hormone concentrations, a combination that suggested a defect of the calcium-sensing receptor. The hypocalcemia was associated with hypercalciuria, and treatment with vitamin D resulted in increased hypercalciuria, nephrocalcinosis, and renal impairment. Mutations in the calcium-sensing-receptor gene were identified by DNA-sequence analysis and expressed in human embryonic kidney cells (HEK-293).

Results Five heterozygous missense mutations (Asn118Lys, Phe128Leu, Thr151Met, Glu191Lys, and Phe612Ser) were detected in the extracellular domain of the calcium-sensing-receptor gene and shown to cosegregate with the disease. Analysis of the functional expression of three of the mutant receptors in HEK-293 cells demonstrated shifts in the dose-response curves so that the extracellular calcium concentrations needed to produce half-maximal increases in total inositol phosphate in the cells were significantly ($P=0.02$ to $P<0.001$) lower than those required for the wild-type receptor.

Conclusions Gain-of-function mutations in the calcium-sensing receptor are associated with a familial syndrome of hypocalcemia with hypercalciuria that needs to be distinguished from hypoparathyroidism. (N Engl J Med 1996;335:1115-22.)

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HYPOCALCEMIA is the hallmark of hypoparathyroidism, which may be inherited either as an isolated endocrinopathy or as part of an autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy or the DiGeorge syndrome, in which developmental defects of the third and fourth pharyngeal pouches result in parathyroid and thymic aplasia together with car-

diac and facial abnormalities.¹⁻³ Genetic studies have mapped the autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy syndrome and the DiGeorge syndrome loci to chromosomes 21q22.3² and 22q11,³ respectively, and studies of families with isolated hypoparathyroidism have mapped an X-linked recessive form to chromosome Xq26–q27.⁴ In two kindreds with autosomal hypoparathyroidism, mutations of the parathyroid hormone gene, located on chromosome 11p15, were identified.^{5,6} However, the majority of families with autosomal forms of isolated hypoparathyroidism do not have mutations of the parathyroid hormone gene,⁷⁻⁹ and two mutations of the calcium-sensing-receptor gene (Glu127Ala and Gln245Arg) have been reported in kindreds with autosomal dominant forms of hypocalcemia.¹⁰⁻¹²

The calcium-sensing-receptor gene is located on chromosome 3q13.3–q21 and encodes a cell-surface protein of 1078 amino acids that is expressed in the parathyroid glands and kidneys and belongs to the family of G-protein-coupled receptors.¹³⁻¹⁵ This receptor regulates the secretion of parathyroid hormone and the reabsorption of calcium by the renal tubules in response to alterations in serum calcium concentrations.^{15,16} Mutations in this calcium receptor involving a loss of function cause familial benign hypercalcemia, also known as familial hypocalciuric hypercalcemia; persons with this autosomal dominant disorder, who are generally asymptomatic, have lifelong elevations of serum calcium concentrations together with a low urinary excretion of calcium.¹⁷⁻²² The association of two mutations of the calcium-sensing-receptor gene with hypocalcemia led us to postulate that the phenotype of gain-of-function mutations may be hypocalcemia with hypercalciuria. We there-

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TABLE 1. CLINICAL AND BIOCHEMICAL FEATURES OF 20 AFFECTED MEMBERS OF SIX FAMILIES WITH HYPOCALCEMIC HYPERCALCIURIA.*

SUBJECT No.	AGE†	SERUM CALCIUM		SERUM PHOSPHATE		SERUM MAGNESIUM	SERUM PARATHYROID HORMONE		URINARY CALCIUM: URINARY CREATININE		ASSOCIATED FEATURES			
		PRETREATMENT	TREATMENT	PRETREATMENT	TREATMENT		PRETREATMENT	TREATMENT	PRETREATMENT	TREATMENT	RC	RI	OTHER	
	yr	mg/dl									NC	NL		
Family 1														
I-2	25	5.9	9.2	4.9	3.2	1.2	21 ng/liter‡	<5 ng/liter‡	NA	NA	+	-	+	S, BC
II-1	6	6.4	8.6	5.8§	5.2§	1.5	10 ng/liter‡	<5 ng/liter‡	0.29	0.37	+	-	‡¶	S
II-2	4	6.2	10.2	6.7§	4.7§	1.7	10 ng/liter‡	<5 ng/liter‡	0.20	0.81	+	+	‡¶	—
Family 2														
I-2	44	5.0	7.4	4.1	4.5	1.4	NA	1.1 pmol/liter	NA	0.26	-	+	+	CC 30 ml/min, S, BC
II-2	17	6.6	8.1	5.2	4.5	1.7	13.5 pmol/liter**	0.7 pmol/liter	NA	0.20	+	+	+	CC 28 ml/min, NX, CS
II-5	4	6.5	8.0	4.5	5.5	NA	0.6 pmol/liter¶	NA	NA	NA	+	-	+	S
II-7	22	6.0	8.5	4.8	4.0	1.7	NA	<0.7 pmol/liter	NA	NA	+	-	+	CS, P
III-1	1	7.0	6.2	9.6§	7.8§	1.5	NA	1.9 pmol/liter	0.13	0.22	-	-	-	CS
III-2	9	6.6	6.2	8.2§	7.1§	1.7	NA	0.8 pmol/liter	0.06	0.10	-	-	-	—
III-3	0.5	7.0	8.0	7.4§	5.6§	1.5	10 ng/liter‡	<10 ng/liter‡	0.29	0.73	+	+	-	S
Family 3														
I-1	48	7.6	8.1	2.9	3.8	1.8	33 ng/liter‡	NA	0.13	0.33	+	-	+	—
II-2	4	8.0	7.8	6.1§	6.5§	2.2	0.7 µg/liter‡‡	16 ng/liter‡	NA	NA	-	-	-	S
Family 4														
I-2	66	7.4	8.1	6.0	4.9	1.6	14 ng/liter‡	8 ng/liter‡	NA	0.25	-	-	+	CC 27 ml/min
II-1	35	6.8	8.0	5.1	4.5	1.3	19 ng/liter‡	10 ng/liter‡	NA	0.33	-	-	-	—
II-2	28	7.4	8.2	5.9	5.6	1.2	20 ng/liter‡	<10 ng/liter‡	0.14	NA	+	-	‡¶	CC 33 ml/min, P
III-1	4	7.6	8.3	8.8§	7.2§	1.8	14 ng/liter‡	<10 ng/liter‡	0.07	0.19	-	-	‡¶	—
Family 5														
I-1	42	6.0	8.4	3.9	3.8	1.6	12 ng/liter‡	0.3 µg/liter‡‡	0.15	0.22	-	-	+	—
II-1	1	6.8	8.8	6.6§	6.2§	NA	1.3 µg/liter‡‡	<0.1 µg/liter‡‡	0.13	0.58	+	-	+	S
Family 6														
I-1	42	7.2	NA	NA	NA	NA	NA	NA	NA	NA	-	-	-	—
II-1	14	6.2	8.6	5.9	5.6	1.7	2.8 pmol/liter¶	NA	0.21	0.85	+	-	+	CS
Normal range		8.6–10.6		2.5–4.3		1.9–2.4				<0.25				

*Affected members of all families were treated with 0.25 to 2.0 µg of 1α-hydroxycholecalciferol per day, except for Subjects I-2, II-2, and II-5 in Family 2, who were treated with ergocalciferol (50,000 to 100,000 U per day), and Subjects II-7, III-1, III-2, and III-3 in Family 2, I-1 in Family 3, and I-1 in Family 5, who were treated with calcitriol (0.5 to 2.0 µg per day). Subject I-1 in Family 6 was not treated with vitamin D. All subjects had normal renal function at the time of the initial biochemical study. To convert values for serum calcium to millimoles per liter, multiply by 0.25; to convert values for serum phosphate to millimoles per liter, multiply by 0.32; to convert values for serum magnesium to millimoles per liter, multiply by 0.41; and to convert values for the urinary calcium:creatinine ratio to millimoles per millimole, multiply by 2.83. RC denotes renal calcification, NC nephrocalcinosis, NL nephrolithiasis, RI renal impairment (serum creatinine concentration >1.5 mg per deciliter [135 µmol per liter] in adults and 25 percent above the age-corrected upper limit of normal in children), NA not available, S seizures, BC basal-ganglia calcification, CC creatinine clearance (in subjects with serum creatinine concentrations above 2.0 mg per deciliter [175 µmol per liter]), NX nephrectomy, CS symptoms of carpopedal spasm, and P symptoms of polyuria and polydipsia. Plus signs denote the presence of a condition, and minus signs its absence.

†The age at the time of the initial biochemical studies is shown.

‡The normal range is 10 to 65 ng per liter.

§Normal serum phosphate concentrations for prepubertal children range from 3.7 to 5.0 mg per deciliter (1.2 to 1.6 mmol per liter).

¶Serum creatinine returned to pretreatment values after vitamin D therapy was stopped.

||The normal range is 1.1 to 6.5 pmol per liter.

**The normal range is 5 to 32 pmol per liter.

‡‡The normal range is less than 0.1 to 8 µg per liter.

‡‡‡The normal range is less than 1.0 µg per liter.

fore investigated six kindreds with autosomal dominant hypocalcemia and hypercalciuria for mutations involving the calcium-sensing-receptor gene.

METHODS

Patients

We studied six kindreds with hypocalcemia in which isolated hypoparathyroidism had been diagnosed but in which hypocalcemia was associated with normal serum parathyroid hormone con-

centrations.^{1,7} Clinical and biochemical studies revealed 20 affected (Table 1) and 17 unaffected family members. Biochemical measurements were performed as described previously,²³ and statistical analyses were performed with Student's t-test and analysis of variance.

Preparation of Genomic DNA and Characterization of Mutations in the Calcium-Sensing-Receptor Gene

Samples of venous blood were obtained from the 20 affected and 17 unaffected members of the six families. DNA was extract-

ed from leukocytes²⁴ and amplified with 12 pairs of oligonucleotide primers specific for the calcium-sensing-receptor gene to examine the 6 coding exons and 9 of the 12 splice sites by the polymerase chain reaction (PCR), as previously described.^{17,20} DNA-sequence analysis of the resulting PCR products with a semiautomated laser detection system (Sequencer 373a, Applied Biosystems, Foster City, Calif.) was performed for the proband of each family, and the results were compared with the normal DNA sequence (GenBank number X81086), as previously described.²⁰ The DNA-sequence abnormalities in the probands were confirmed either by restriction-endonuclease analysis or by hybridization to sequence-specific oligonucleotides, as previously described.²⁰ In addition, each abnormality was demonstrated to cosegregate with the disorder and to be absent, and therefore not a neutral polymorphism, in the DNA obtained from 55 unrelated normal subjects.

Analysis of Single-Strand Conformational Polymorphisms

Genomic DNA obtained from the probands in whom mutations had been identified and 10 unrelated normal subjects was amplified by PCR with appropriate primers,^{17,20} and the products were subjected to analysis of single-strand conformational polymorphisms (SSCPs) with the Phast electrophoresis system (Pharmacia LKB, Uppsala, Sweden), as previously described.²⁰ In order to detect mutations in exon 3 (Asn118Lys, Phe128Leu, and Thr151Met), we used a temperature of 10°C and a run length of 240 volt-hours. The results were scored by two observers who were unaware of the identity of the samples.

Expression of Wild-Type and Mutant Calcium-Sensing Receptors

Restriction fragments of complementary DNA (cDNA) from the wild-type human calcium-sensing-receptor gene (HuPCaR4.0)¹⁴ were ligated into pBluescript SK(-) (Stratagene, La Jolla, Calif.), and three mutations (Phe128Leu, Thr151Met, and Glu191Lys) were successfully produced by site-directed mutagenesis.²⁵ The Phe612Ser mutation could not be produced because it was too close to the 3' end of one of the restriction fragments used for site-directed mutagenesis, and the Asn118Lys mutation was not studied. Mutant clones, which were verified by DNA sequencing of both strands, were ligated into the construct of the full-length receptor cDNA in the mammalian expression vector pcDNA3 (Invitrogen, San Diego, Calif.). One microgram of the wild-type or mutant calcium-sensing-receptor cDNA was bound to lipofectamine (GIBCO BRL, Gaithersburg, Md.) and transfected into human embryonic kidney cells (HEK-293, American Type Culture Collection number CRL-1573) that had been grown to 90 percent confluence in Dulbecco's modified Eagle's medium (GIBCO BRL) supplemented with 10 percent heat-inactivated fetal-calf serum (Hyclone, Logan, Utah). Forty-eight hours after transfection, the cells were labeled for 18 hours with 30 μ Ci of [³H]inositol per milliliter (New England Nuclear, Boston), washed, and incubated for 30 minutes in Dulbecco's modified Eagle's medium (free of bicarbonate, calcium, and magnesium), supplemented with 20 mM HEPES buffer (pH 7.45), 0.2 percent bovine serum albumin, 10 mM lithium chloride, 0.5 mM magnesium chloride, and various concentrations of calcium chloride (0.5, 1.0, 1.5, 2.0, 3.0, and 5 mM).²⁶ Each calcium concentration was studied in a total of four transfection experiments performed independently on two days, and the total cellular inositol phosphate that accumulated (i.e., IP + IP₂ + IP₃ + IP₄) was measured by ion-exchange chromatography,²⁷ with the value normalized on the basis of cellular protein by measurement of the total protein content (micro BCA protein assay, Pierce, Rockford, Ill.). Inositol phosphate values are reported as means \pm SE. The effective extracellular calcium concentration required for a half-maximal inositol phosphate response for each clone was derived from the mean of the four transfection experiments.

RESULTS

Clinical and Biochemical Studies

Twenty of the 37 family members studied had hypocalcemia, of whom 11 had carpopedal spasm or childhood seizures. Two subjects, Subject I-2 in Family 1 and Subject I-2 in Family 2, had calcification of basal ganglia and seizures, and in Subject I-2 in Family 1, the seizures continued into adult life. The remaining 9 affected subjects had asymptomatic hypocalcemia, and 16 subjects also had hypomagnesemia (Table 1). In addition, urinary calcium excretion was either inappropriately within the normal range or high at the time of the initial diagnosis. The mean (\pm SE) ratio of urinary calcium to urinary creatinine (expressed as milligrams of calcium per milligram of creatinine [and as millimoles of calcium per millimole of creatinine]) before treatment in the 11 affected subjects in whom it was measured was significantly higher than that reported in 10 untreated subjects with idiopathic or postoperative hypoparathyroidism²⁸ (0.16 ± 0.02 vs. 0.07 ± 0.02 [0.5 ± 0.1 vs. 0.2 ± 0.1], $P = 0.004$), despite the presence of similar levels of hypocalcemia (6.8 ± 0.2 vs. 6.9 ± 0.4 mg per deciliter [1.7 ± 0.04 vs. 1.7 ± 0.1 mmol per liter]).²⁸

Nineteen subjects were treated with oral preparations of vitamin D; serum parathyroid hormone concentrations became low or undetectable in 16, and 9 had hypercalciuria (ratio of urinary calcium to urinary creatinine before treatment, 0.17 ± 0.03 [0.5 ± 0.1]; during treatment, 0.44 ± 0.09 [1.2 ± 0.2]; $P = 0.005$). Renal calcification developed in eight of these nine subjects, and renal impairment in seven (Table 1). Renal calcification and renal impairment also developed in seven other subjects during vitamin D therapy; in three of these subjects the ratios of urinary calcium to urinary creatinine were in the high-normal range (0.19 to 0.22 [0.5 to 0.6]; normal, <0.25 [<0.7]).^{29,30} Urinary measurements were not done in the remaining four subjects.

The bone mineral density of the lumbar spine, as assessed by dual-emission x-ray absorptiometry, was normal in four affected subjects (Subjects II-2 and III-1 in Family 4 and Subjects II-1 and II-2 in Family 1), but increased (2.4 to 9.0 SD above the age-adjusted normal mean values) in three others (Subjects I-2, II-5, and II-7 in Family 2) (Table 1).

Analysis of Mutations

An analysis of the DNA sequence of the entire 3234-bp coding region of the calcium-sensing-receptor gene from each proband revealed heterozygous missense mutations involving the extracellular domain of the receptor in five of the six families (Table 2). Three of these mutations predicted the substitution of leucine (Leu) for phenylalanine (Phe) at codon 128 (TTC to CTC) in Family 2 (Fig. 1), the substitution of methionine (Met) for threonine (Thr)

TABLE 2. MUTATIONS IDENTIFIED IN FIVE FAMILIES WITH HYPOCALCEMIC HYPERCALCIURIA.

FAMILY No.	EXON	CODON	BASE CHANGE	AMINO ACID CHANGE	METHOD OF IDENTIFICATION*
1	3	118	AAC→AAA	Asn→Lys	SSO
2	3	128	TTC→CTC	Phe→Leu	<i>AluI</i>
3	3	151	ACG→ATG	Thr→Met	<i>NcoI</i>
4	4	191	GAG→AAG	Glu→Lys	SSO
5	7	612	TTT→TCT	Phe→Ser	<i>MnII</i>

*The mutations were identified by restriction-enzyme or sequence-specific-oligonucleotide analysis (SSO).

at codon 151 (ACG to ATG) in Family 3, and the substitution of serine (Ser) for phenylalanine (Phe) at codon 612 (TTT to TCT) in Family 5. These three mutations were associated with the alteration of a restriction-enzyme site (Table 2), which allowed the demonstration of the cosegregation of the mutations with hypocalcemia in these families (Fig. 1).

The other two mutations predicted the substitution of lysine (Lys) for asparagine (Asn) at codon 118 (AAC to AAA) in Family 1 and the substitution of lysine (Lys) for glutamate (Glu) at codon 191 (GAG to AAG) in Family 4 (Fig. 2). These two mutations were not associated with altered restriction-enzyme sites, and the technique of sequence-specific oligonucleotide hybridization²⁰ was therefore used to confirm their cosegregation with hypocalcemia. Each of the five mutations was absent in 110 alleles from 55 unrelated subjects with normal serum calcium concentrations, thereby demonstrating that it was not a neutral polymorphism occurring in more than 1 percent of the population.

All five mutations found by direct DNA-sequence analysis were correctly identified by SSCP analysis. Similar analysis of 244 individual PCR products of the calcium-sensing-receptor gene did not consistently detect any other abnormal bands,²⁰ thereby indicating an absence of false positive results. Thus, SSCP analysis reliably detected all mutations, a result consistent with our experience in the detection of calcium-sensing-receptor mutations in familial benign hypercalcemia.²⁰

Functional Characterization of Mutant Calcium-Sensing Receptors

Functional expression of the wild-type calcium-sensing-receptor cDNA in HEK-293 cells, assessed in terms of the inositol phosphate response, was maximal at an extracellular calcium concentration of 5.0 mM (Fig. 3). In contrast, in cells transfected with the mutant receptors (Phe128Leu, Thr151Met, and Glu191Lys), the responses were maximal at extracellular calcium concentrations between 1.5 and 2.0

mM (Fig. 3). The inositol phosphate concentrations were significantly higher at one or more calcium concentrations in cells transfected with the mutant receptors than in cells transfected with the wild-type receptors. The half-maximal responses of the mutant receptors were also decreased (1.3 mM for the Phe128Leu mutation, 1.2 mM for the Thr151Met mutation, and <1.0 mM for the Glu191Lys mutation), as compared with the response of 2.9 mM for the wild-type receptor (the latter may be an underestimate, because the effects of higher extracellular calcium concentrations were not tested). These results demonstrate a leftward shift in the dose-response curve for extracellular-calcium-activated accumulation of inositol phosphate in cells transfected with mutant calcium-sensing receptors. Thus, these mutant receptors are active at lower extracellular calcium concentrations than the wild-type receptor, which is consistent with their gain of function and the hypocalcemia in affected family members.

DISCUSSION

We have identified five novel mutations of the calcium-sensing-receptor gene in families with hypocalcemia and hypercalciuria, thereby providing evidence of the role of abnormal calcium receptors in the cause of this syndrome. The five missense mutations, which result in structurally important changes in amino acids, were confined to the extracellular domain of the receptor, whereas in subjects with familial benign hypercalcemia, mutations have been detected in both the extracellular and transmembrane domains of the receptor.^{17,19-22} Activating mutations of other G-protein-coupled receptors — for example, the thyrotropin or luteinizing hormone receptors, which result in follicular thyroid adenomas³¹ and familial precocious male puberty,³² respectively — involve mutations in the transmembrane domains that render these receptors constitutively hyperactive. In one family (Family 6), no DNA-sequence abnormalities were detected, a finding similar to that in a previous report of a family with autosomal dominant hypocalcemia.^{10,33} These families may have a mutation within the promoter region of the receptor, or there may be genetic heterogeneity, as found in familial benign hypercalcemia.^{34,35}

The hypocalcemia in the families with hypocalcemia and hypercalciuria was initially attributed to hypoparathyroidism^{1,7} because it was associated with serum parathyroid hormone concentrations in the low-normal range.^{36,37} However, it is important to differentiate patients with familial hypocalcemic hypercalciuria from those with hypoparathyroidism,^{1,4-9} because treatment with vitamin D to correct the hypocalcemia in the former may lead to hypercalciuria, nephrocalcinosis, and renal impairment. We suggest that asymptomatic patients with familial hypocalcemic hypercalciuria should not routinely receive vita-

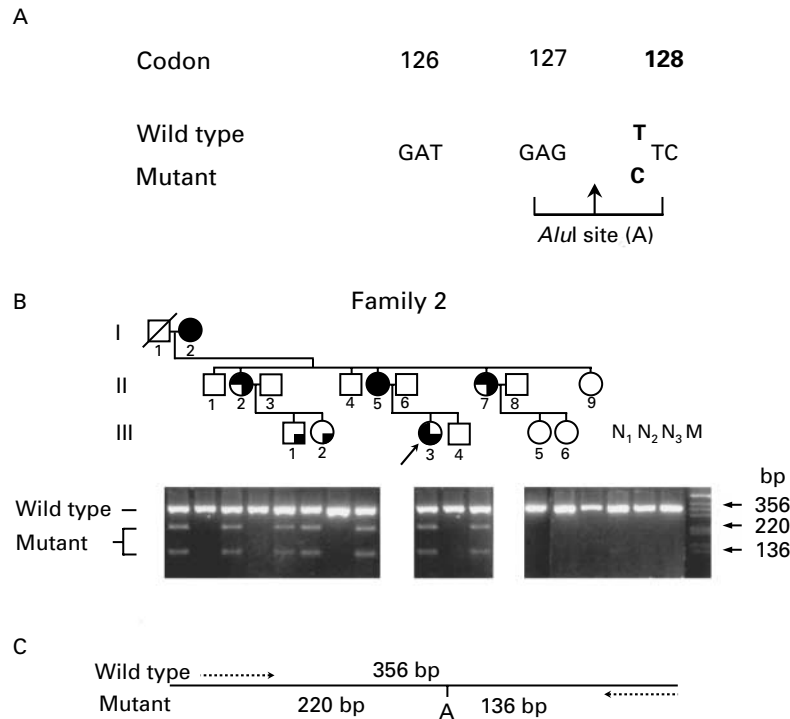


Figure 1. Missense Mutation in Exon 3 of the Calcium-Sensing-Receptor Gene in Family 2. Analysis of the DNA sequence of Subject III-3 revealed the substitution of C for T at codon 128 (Panel A). Panel B shows the pedigree. Squares denote male family members, circles female family members, symbols with a slash deceased family members, symbols with a solid lower right quadrant subjects with hypocalcemia, symbols with a solid upper right quadrant subjects with renal impairment, symbols with a solid upper left quadrant subjects with nephrocalcinosis or nephrolithiasis, and symbols with a solid lower left quadrant subjects with seizures. The proband is indicated by the arrow. Panel C shows the restriction-enzyme map of the PCR product. Restriction-enzyme analysis was used to demonstrate cosegregation of this mutation with hypocalcemia. At codon 128 the wild-type sequence is TTC, encoding a phenylalanine residue, whereas the mutant sequence is CTC, encoding a leucine. This missense mutation resulted in the formation of an *AluI* restriction-enzyme site (A) (AG/CT). Amplification with the PCR and digestion with *AluI* result in one product of 356 bp from the normal (wild type) sequence but two products of 220 bp and 136 bp from the mutant sequence. The cosegregation of this Phe128Leu mutation with hypocalcemia and its heterozygosity in affected members are revealed by the analysis, and the absence of this mutation in 110 alleles from 55 unrelated subjects with normocalcemia (N_1 , N_2 , and N_3 are shown) indicates that it is not a common DNA-sequence polymorphism. M denotes the DNA size markers.

min D; such treatment should be reserved for symptomatic patients and given to them with the aim not of restoring normocalcemia, but of maintaining a serum calcium concentration just sufficient to alleviate the symptoms.

Familial hypocalcemic hypercalciuria may be difficult to distinguish from hypoparathyroidism on the basis of measurements of serum parathyroid hormone and urinary calcium. However, the identification of mutations in the calcium-sensing-receptor gene will help in making this distinction and in facilitating early recognition of patients with hypocalcemic hypercalciuria, but the mutational diversity of the gene¹⁷⁻²² makes screening for the disorder arduous and time consuming. The use of the SSCP tech-

nique for rapid molecular genetic screening has so far allowed detection of all the mutations in the extracellular domain of the calcium-sensing receptor, suggesting that SSCP analysis should be helpful in differentiating familial hypocalcemic hypercalciuria from other causes of hypocalcemia. Thus, a finding of hypocalcemia that is not associated with an undetectable or very low serum parathyroid hormone concentration and markedly reduced urinary calcium excretion should suggest a diagnosis of hypocalcemic hypercalciuria, which can be confirmed by analysis of mutations in the calcium-sensing-receptor gene.

Studies of the expression of three of the mutant calcium-sensing receptors associated with hypocalcemia revealed a gain of function that led to a leftward

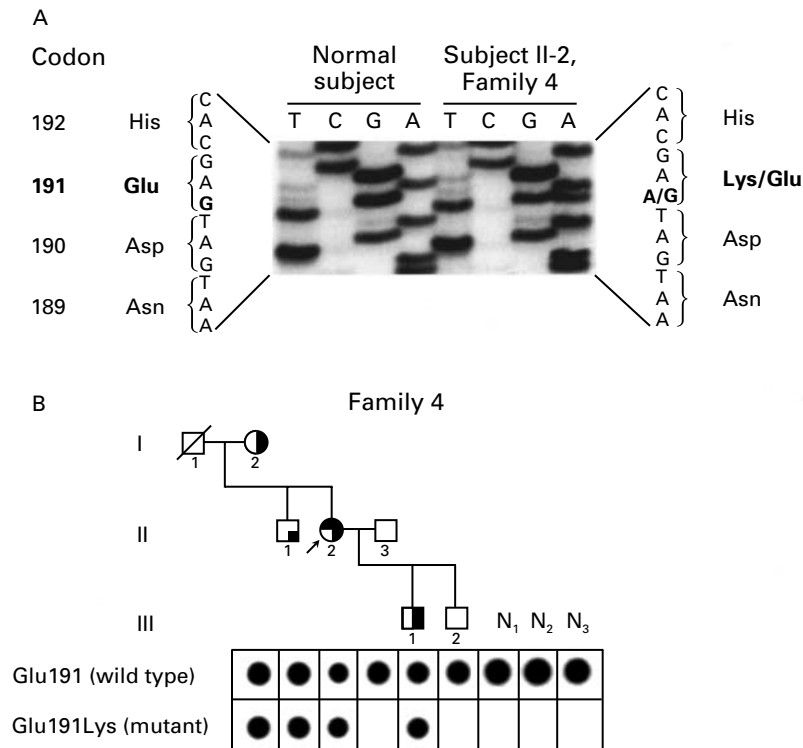


Figure 2. Missense Mutation in Exon 4 of the Calcium-Sensing-Receptor Gene in Family 4. The DNA sequences from codons 189 to 192 of Subject II-2 and a normal subject are shown in Panel A. The replacement of the wild-type G with A in one allele at the first position of codon 191 results in a change from glutamate to lysine. Panel B shows the pedigree and the results of sequence-specific oligonucleotide analysis of dot blots. The symbols used for the pedigree members are as indicated in Figure 1, and the proband is indicated by the arrow. The cosegregation of the mutation (Glu191Lys) with hypocalcemia in Family 4 and its absence in 55 subjects with normocalcemia (N₁, N₂, and N₃ are shown) were demonstrated by sequence-specific oligonucleotide hybridization analysis¹³ because it was not associated with an alteration of a restriction-enzyme site. Thus, the unrelated normal subjects and the unaffected Subjects II-3 and III-2 were homozygous for the wild-type sequence. However, all the affected family members had both the wild-type and the mutant sequence.

shift in calcium-activated stimulation of inositol phosphate accumulation. This is consistent with activation of the calcium-sensing receptors, which may be due to either an increased affinity for calcium or a greater basal activity of the receptor.¹⁰ This, in turn, would suppress the secretion of parathyroid hormone and increase renal calcium excretion at inappropriately low levels of serum calcium, thereby leading to stable hypocalcemia; this situation is the converse of that in familial benign hypercalcemia, in which the parathyroid glands and kidney are “resistant” to the elevations in serum calcium and thereby increase the secretion of parathyroid hormone and decrease the excretion of urinary calcium, respectively, at any extracellular calcium concentration. However, the underlying mechanism responsible for the hypercalciuria and nephrocalcinosis that occur during vitamin D therapy in patients with hypocalcemic hypercalciuria is not known. It may be due to a decrease in renal calcium

reabsorption due to the inhibition of parathyroid hormone secretion when the serum calcium concentration is increased by vitamin D therapy. Alternatively, it may reflect a greater degree of activation of the mutant calcium-sensing receptors in the distal tubules that are involved in regulating renal calcium reabsorption^{15,38} than that which occurs in patients with hypoparathyroidism when their serum calcium concentrations are increased. This situation contrasts with that in familial benign hypercalcemia, in which the mutant calcium-sensing receptors have decreased function, so that hypercalcemia-induced increases in urinary calcium excretion are markedly reduced, even after total parathyroidectomy.^{39,40} In addition, polyuria and polydipsia develop at normal serum calcium concentrations in some subjects with hypocalcemic hypercalciuria, perhaps due to increased activity of the mutant receptors in the collecting duct; this also contrasts with familial benign hypercalcemia, in

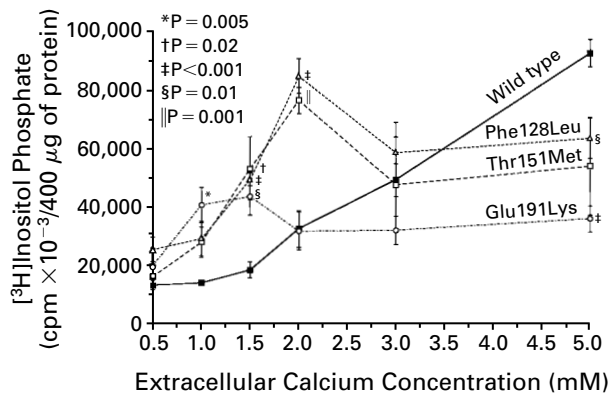


Figure 3. Functional Expression in HEK-293 Cells of the Wild-Type Calcium-Sensing Receptor and Three Mutant Receptors — Phe128Leu, Thr151Met, and Glu191Lys — Involving a Gain of Function.

The accumulation of total tritiated inositol phosphates (adjusted for the total cellular protein) in cells transfected with the wild-type receptor and each of the mutant receptors was measured after the transfected cells were incubated in medium containing various extracellular calcium concentrations. The results are the mean (\pm SE) values from four separate transfection experiments. The maximal inositol phosphate response in the cells transfected with the wild-type receptor occurred at a calcium concentration of 5.0 mM, whereas the maximal responses in the cells transfected with the three mutant receptors occurred at concentrations ranging from 1.5 to 2.0 mM. In each case the P value is for the comparison with the wild-type receptor.

which hypercalcemia does not impair urinary concentrating ability.⁴¹ Thus, the combined effects of hypercalciuria and dehydration may make subjects with hypocalcemic hypercalciuria particularly susceptible to nephrocalcinosis and renal impairment.

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