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A Novel Mutation in Ca^{2+}-Sensing Receptor Gene in Familial Hypocalciuric Hypercalcemia

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Missense mutations in the calcium-sensing receptor (*CaSR*) gene have previously been identified in patients with familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism. We identified a newborn with hypercalcemia in our hospital by mass screening. The family members were studied, and we found a novel *CaSR* missense mutation with polymerase chain reaction single-strand conformational polymorphism analysis. The mother, grandmother, and aunt of the baby all had FHH. A heterozygous missense mutation in exon 6 that substitutes a glutamic acid for the glycine at codon 557 (Gly557Glu), which corresponds to the extracellular domain of *CaSR*, was identified and shown to cosegregate with the disease. Identification of the mutation responsible for the FHH phenotype in this family may facilitate rapid testing of individuals at risk for FHH.

Key Words: Hypercalcemia; gene; familial; hypocalciuric; receptor; mutation.

Introduction

Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant disorder characterized by modest elevation of serum calcium concentration, relative hypocalciuria, and inappropriately normal levels of parathyroid hormone (PTH) *(1–3)*. The condition is inherited as an autosomal dominant trait. Whereas the penetrance of FHH appears to be ≥90%, affected individuals do not typically exhibit the morbidity associated with hypercalcemia. Physiologic and biochemical studies of individuals with FHH have revealed abnormal responses of both the kidney and parathyroid gland in sensing blood calcium levels. The mechanism by which the parathyroid gland senses extracellular calcium was recently clarified when a calcium-sensing receptor (*CaSR*) expressed in both parathyroid and kidney was cloned and characterized *(4)*. This calcium receptor, which is a member of the G-protein-coupled receptor superfamily, responds to increased levels of extracellular calcium by triggering a phospholipase C pathway and elevating intracellular calcium levels. This inhibits secretion of PTH from the parathyroid gland. Inactivating mutations of *CaSR* result in FHH *(5)*, whereas mutations that make the receptor more sensitive to extracellular calcium result in autosomal dominant and sporadic hypocalcemia *(6)*.

Inheritance of a single copy of a mutated gene causes FHH, and homozygous individuals who inherit two copies of the mutated gene may have neonatal severe primary hyperparathyroidism (NSHPT) *(7)*, which is characterized by marked hypercalcemia, skeletal demineralization, and parathyroid hyperplasia. In addition, without parathyroidectomy *(8)*, it is usually fatal *(9)*, although some less severely affected neonates have been medically managed successfully *(10)*.

More than 30 mutations have been identified in the coding region of the *CaSR* gene. We scanned for mutations in all exons of the *CaSR* gene by polymerase chain reaction single-strand conformational polymorphism (PCR-SSCP) analysis in a Japanese family with FHH that was identified through a newborn infant with hypercalcemia.

Case Report

A preterm male infant weighing 2190 g was born by spontaneous labor at 37 wk of gestation. He was admitted for the treatment of low body weight on February 25, 2000. He is the mother's first child. His apgar scores were 8 and 10 at 1 and 5 min, respectively. Laboratory tests indicated mild hypercalcemia and acidosis. His serum Ca level at birth was 11.8 mg/dL. Physical examination revealed no abnormalities. As shown in Table 1, the infant's serum level of intact PTH was normal (43 pg/mL) despite mild hypercalcemia. His fractional calcium excretion was compared to the criteria of FHH (<1%). He recovered with nutritional supplement, but the etiology of his hypercalcemia remained unknown. To confirm FHH, we measured serum Ca concentrations in his parents and found that his 21-yr-old mother

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Table 1
Clinical Data of Family Members[a]

		Individual					
		I-2	II-1	II-2	II-3	II-4	III-1
Age	(yr)	48	28	25	21	21	0
Serum calcium	(mg/dL)	10.9	9.9	10.9	9.3	11.3	11.9
Serum phosphorus	(mg/dL)	2.7	2.8	2.7	4.2	3.0	4.4
Serum creatinine	(mg/dL)	0.58	0.84	0.51	0.77	0.63	0.21
C-terminal PTH	(ng/mL)	0.4	<0.2	0.3	<0.2	0.3	0.3
Intact PTH	(pg/mL)	59	28	38	30	47	43
FECa	(%)	1.0	2.0	0.9	1.0	0.2	0.2

[a] PTH, parathyroid hormone; FECa, fractional excretion of calcium.
C-terminal PTH (ng/mL): normal range: <0.5. Intact PTH (pg/mL): Allegro, normal range: 15–50.

had asymptomatic hypercalcemia, with a serum Ca level of 10.9 mg/dL. His father was normocalcemic. Oddly, the mother also had normal serum PTH levels despite hypercalcemia. Subsequently, we evaluated all family members (Table 1). There were no consanguineous marriages in this family. The findings strongly suggested that the newborn infant (III-1), his mother (II-4), aunt (II-2), and grandmother (I-2) had FHH. Informed consent was obtained and subsequent DNA analyses were performed for all members of this family with the exception of the infant's grandfather, who was unavailable owing to divorce. The grandmother had a history of gastric ulcer at the age of 20. None of the other family members had histories of symptoms associated with hypercalcemia, such as urinary tract stones or pancreatitis. For longer than 1 yr, the serum Ca concentration in the proband has remained at the same high level as at birth.

Clinical Laboratory Assays

Blood and urine samples were collected from the proband and his parents. Second voiding urine was collected during a visit to our hospital. Serum and urine concentrations of calcium, creatinine, and inorganic phosphorus were determined with an automated clinical chemistry analyzer. Plasma C-terminal PTH levels (Eiken, normal: <0.5 ng/mL) were quantified by radioimmunoassay, and intact PTH levels (Allegro, normal range: 15–50 pg/mL) were determined by immunoradiometric assay. The inter- and intraassay variations and sensitivity of C-terminal PTH were 10.3%, 9.2%, and 0.2 ng/mL, respectively. The inter- and intraassay variations and sensitivity of intact PTH were 5.50%, 5.80%, and 3 pg/mL, respectively.

Results

PCR-SSCP Analysis

The initial SSCP screening showed that the PCR product of exon 6 of *CaSR* from the proband (III-1), his mother (II-4), his maternal aunt (II-2), and his grandmother (I-2) exhibited mobility patterns that differed from those of normal controls (Fig. 1). No difference was observed in the mobility patterns of the PCR products of the remaining exons.

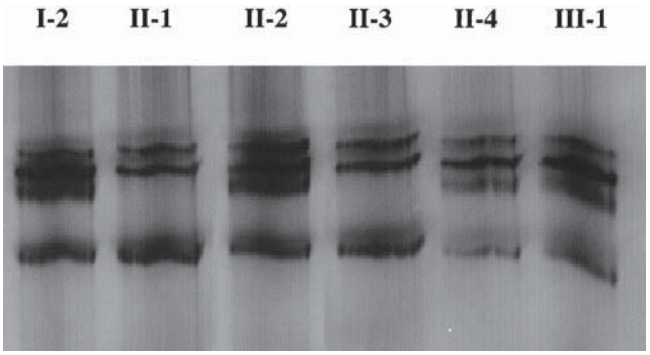

Fig. 1. PCR-SSCP analysis.

Sequencing Analysis

Automated DNA sequencing revealed a heterozygous mutation at the same position in both the proband and his mother (codon 557; GGG to GAG; *see* Fig. 2). This mutation causes a glycine to glutamic acid substitution at position 557 (G557E), which is missense mutation within the extracellular domain of *CaSR*. No other mutations were identified.

PCR Restriction Fragment Length Polymorphism Analysis

Genomic DNAs from members of the proband's family were screened for the mutation by PCR-restriction fragment length polymorphism (RFLP) using *Ear*I after PCR amplification with primers MF and MR. The G557E mutation was found in the DNA of affected individuals in the family and was absent in the DNA from unaffected family members (Fig. 3). The G557E mutation was not found in 100 healthy subjects.

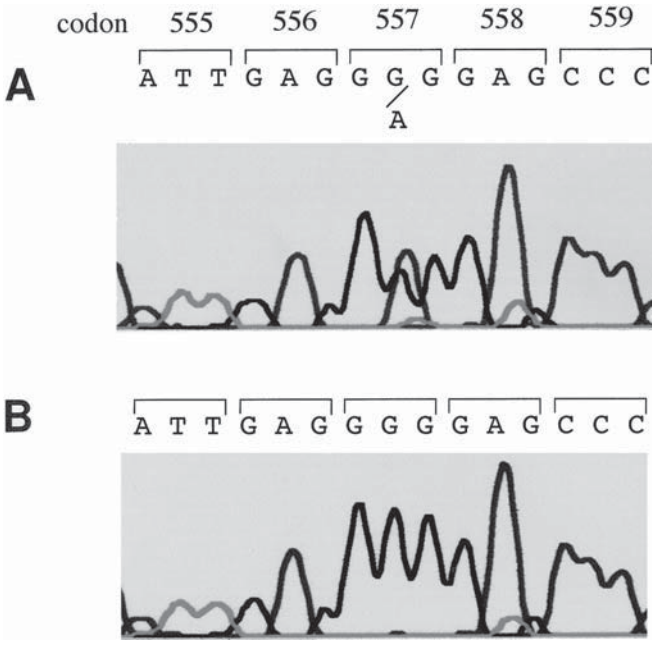

Fig. 2. DNA sequencing analysis. (**A**) Nucleotide sequence of proband (newborn infant); (**B**) nucleotide sequence of proband's normal father.

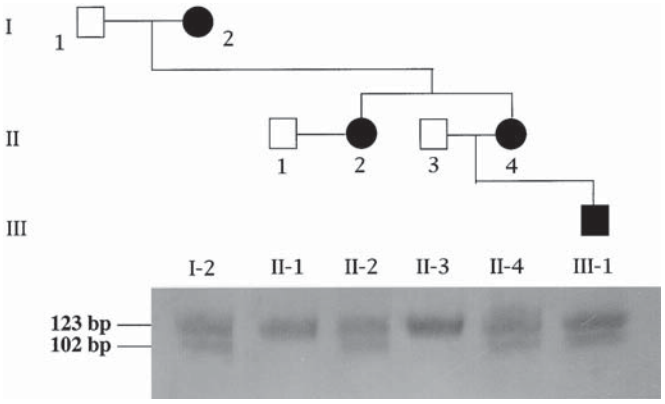

Fig. 3. PCR-RFLP analysis. The missense mutation was detected by the presence of an *Ear*I restriction site in PCR products. The I-1 individual was not tested.

Discussion

FHH is characterized by an autosomal dominant pattern of inheritance and lifelong hypercalcemia without hypercalciuria, which is often misdiagnosed as classical primary hyperparathyroidism. Recently, it was shown that FHH is caused by mutations in the *CaSR* gene located on chromosome 3q21-24 *(5)*. There are two additional loci associated with FHH that localized to 19p13.3 *(11)* and 19q13 *(12)*. Although patients with FHH owing to mutations in the *CaSR* gene appear to have normal serum levels of PTH, one kindred from Oklahoma with FHH was linked to 19q13 that was associated with a developmental elevation in serum PTH levels *(13)*. The contributions of the two additional loci to pathophysiology have not been clarified. Thus, determination and evaluation of mutations in the *CaSR* gene are important to understand the mechanism of FHH and to develop treatments.

The human *CaSR* gene comprises seven exons encoding 1078 amino acids, including an initial 20 amino acids as a signal peptide *(5)*. Exon 1 and the 3' end of exon 7 are untranslated; exons 2 through the 5' end of 7 encode the 612 amino acids of the extracellular and, presumably, ligand-binding domain (LBD). Exon 7 encodes the seven putative transmembrane-spanning regions, the extracellular and intracellular loops, and the COOH-terminal intracellular tail. Several missense mutations and one nonsense mutation scattered throughout the *CaSR* gene have been found (Table 2). Mutations in the LBD would be expected to interfere with the function of *CaSR*. Mutations in the *CaSR* gene have been classified as activating or inactivating, as determined by examining the responses of *Xenopus laevis* oocytes injected with mutated cRNAs. Fourteen activating and 30 inactivating mutations have been described to date.

Exons 2–5 encode the region thought to be involved in calcium binding, and mutations in this region would be expected to interfere with this function. Neither activating nor inactivating mutations have been found in exon 5. Furthermore, only one mutation has been found in exon 6. Polymorphisms in the coding region have been reported *(33)*. In the present family, we identified a novel genetic variant in affected members; this change in exon 6 is thought to be an inactivating mutation. If the serum calcium concentrations had not been evaluated, the FHH in this family would not have been diagnosed because none of the affected members had remarkable symptoms owing to hypercalcemia. This suggests that measurements of serum and urinary calcium levels are important to identify FHH patients.

In summary, we describe a novel missense mutation in exon 6 of the *CaSR* gene in a family with FHH. Structure-function studies of mutations in the *CaSR* gene improve our understanding of normal *CaSR* function and of the role of the receptor in the pathogenesis of FHH and NSHPT.

Materials and Methods

Single-Strand Conformational Polymorphism

Genomic DNA was isolated from peripheral leukocytes by a standard method *(34)*. For initial screening, DNA from the proband, his mother, and two normal subjects, including his father, were analyzed with PCR-SSCP analysis of the coding exons of *CaSR* with the use of a DNA fragment analysis kit (GenePhor System, Pharmacia Biotech, Tokyo, Japan) according to the manufacturer's instructions. Exons 2, 3, 5, and 6 of *CaSR* were amplified with a single PCR reaction, whereas exons 4 and 7 were amplified with two PCR reactions that produced two overlapping PCR products. The primers used are given in Table 3 *(14)*. PCR was performed with a GeneAmp PCR system 9700 (Perkin-Elmer, Norwalk, CT) with the following protocol: an initial

Table 2
Mutations of *CaSR* Gene

Mutation	Type	Exon	Domain[a]	Reference
Lys47Asn	Activating	2	Extracellular	Okazaki R, 1999 *(14)*
Ala116Thr	Activating	3	Extracellular	Baron J, 1996 *(15)*
Asn118Lys	Activating	3	Extracellular	Pearce SHS, 1996 *(16)*
Glu127Ala	Activating	3	Extracellular	Pollak MR, 1994 *(7)*
Phe128Leu	Activating	3	Extracellular	Pearce SHS, 1996 *(16)*
Thr151Met	Activating	3	Extracellular	Pearce SHS, 1996 *(16)*
Glu191Lys	Activating	4	Extracellular	Pearce SHS, 1996 *(16)*
Gln245Arg	Activating	4	Extracellular	Perry YM, 1994 *(17)*
Phe612Ser	Activating	7	Extracellular	Pearce SHS, 1996 *(16)*, Mancilla EE, 1998 *(18)*
Leu616Val	Activating	7	TMI	Stock JL, 1999 *(19)*
Gln681His	Activating	7	TM2–3	Baron J, 1996 *(15)*
Leu773Arg	Activating	7	TN4–5	Deluca F, 1997 *(20)*
Phe788Cys	Activating	7	TM5	Watanabe T, 1998 *(21)*
Phe806Ser	Activating	7	TM5–6	Baron J, 1996 *(15)*
Ser895deletion	Activating	7	Intracellular	Lienhardt A, 2000 *(22)*
Gln27Arg	Inactivating	2	Extracellular	Chikatsu N, 1999 *(23)*
Pro39Ala	Inactivating	2	Extracellular	Aida K, 1995 *(24)*
Ser53Pro	Inactivating	2	Extracellular	Heath H, 1996 *(25)*
Pro55Leu	Inactivating	2	Extracellular	Pearce SHS, 1995 *(26)*
Arg62Met	Inactivating	2	Extracellular	Chou Y-HW, 1995 *(27)*
Arg66Cys	Inactivating	3	Extracellular	Chou Y-HW, 1995 *(27)*
Thr138Met	Inactivating	3	Extracellular	Chou Y-HW, 1995 *(27)*
Gly143Glu	Inactivating	3	Extracellular	Chou Y-HW, 1995 *(27)*
Leu174Arg	Inactivating	4	Extracellular	Ward BK, 1997 *(28)*
Asn178Asp	Inactivating	4	Extracellular	Pearce SHS, 1996 *(16)*
Arg185Gln	Inactivating	4	Extracellular	Pollak MR, 1993 *(5)*
Arg185stop	Inactivating	4	Extracellular	Kobayashi M, 1997 *(29)*
Asp215Gly	Inactivating	4	Extracellular	Heath H, 1996 *(25)*
Tyr218Ser	Inactivating	4	Extracellular	Pearce SHS, 1995 *(26)*
Arg220gln	Inactivating	4	Extracellular	Pearce SHS, 1996 *(16)*
Pro221Ser	Inactivating	4	Extracellular	Pearce SHS, 1996 *(16)*
Arg227Leu	Inactivating	4	Extracellular	Pearce SHS, 1995 *(26)*
Arg221Gln	Inactivating	4	Extracellular	Chou Y-HW, 1995 *(27)*
Glu297Lys	Inactivating	4	Extracellular	Pollak MR, 1993 *(5)*
Gly553Arg	Inactivating	6	Extracellular	Schwarz P, 2000 *(30)*
Gly557Glu	Inactivating	6	Extracellular	Our case
Cys582Tyr	Inactivating	7	Extracellular	Pearce SHS, 1995 *(26)*
Ser607stop	Inactivating	7	Extracellular	Pearce SHS, 1995 *(26)*
Ser657Tyr	Inactivating	7	TM2	Heath H, 1996 *(25)*
Gly670Arg	Inactivating	7	TM2	Pearce SHS, 1995 *(26)*
Gly670Glu	Inactivating	7	TM2	Kobayashi M, 1997 *(29)*
Arg680Cys	Inactivating	7	TM2–3	Pearce SHS, 1995 *(26)*
Pro747frameshift	Inactivating	7	TM4–5	Pearce SHS, 1995 *(26)*
Pro748Arg	Inactivating	7	TM4–5	Heath H, 1996 *(25)*
Arg795Trp	Inactivating	7	TM5–6	Pollak MR, 1993 *(5)*
Val817Ile	Inactivating	7	TM6	Pearce SHS, 1995 *(26)*
Thr876Alu	Inactivating	7	Intracellular	Janicic N, 1995 *(31)*
Phe881Leu	Inactivating	7	Intracellular	Carling T, 2000 *(32)*

[a] TM, transmembrane domain.

Table 3
Primers Used in SSCP Analysis of *CaSR* Gene

Target region	Forward primer	Reverse primer
Exon 2	5'-atcccttgccctggagagacggc	5'-agagaagagauggcagattaggcc
Exon 3	5'-agcttcccatttttcttccacttctt	5'-cccgtctgagaaggcttgagtacct
Exon 4	5'-actcattcaccatgttcttggttct	5'-gctgttgctaaacctgtcgc
Exon 4	5'-cccaggaagtctgtccacaatg	5'-cccaactctgctttattatacagca
Exon 5	5'-ggcttgtactcattctttgctcctc	5'-gacatctggttttctgatggacagc
Exon 6	5'-caaggacctctggacctccctttgc	5'-gaccaagccctgcacagtgcccaag
Exon 7	5'-agtctgtgccacacaataactcactc	5'-cttgttgaagaagatgcacgcca
Exon 7	5'-tgctcatcttcttcatcgtctgg	5'-ctctctgcattctccctagcccagt

denaturation at 96ºC for 180 s followed by 35 cycles of 96ºC for 25 s, 63ºC for 30 s, and 72ºC for 60 s and a final elongation at 72ºC for 10 min. Each PCR product was subjected to SSCP analysis. PCR products were separated by electrophoresis on 10% precast polyacrylamide gels (Pharmacia Biotech) at 5ºC for 1.5 h and then subjected to silver staining (Dai-ichi Kagaku).

Sequencing Analysis

PCR products for exon 6 from the proband, his mother, and the two normal control subjects were gel purified and subjected to automated DNA sequencing analyses with fluorescence-labeled dideoxyterminators (ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit; Perkin-Elmer) according to the manufacturer's instructions (ABI PRISM 310 Genetic Analyzer; PE Applied Biosystems, Foster City, CA) *(35)*. Sequencing was performed for both strands.

PCR-RFLP Analysis

Primers MF and MR for exon 6 of the *CaSR* gene were prepared to screen for the presence of the missense mutation (Gly557Glu) in other family members: the sense primer including mutation (MF) was 5'-gggaccaggaaaggg atcattgaag-3' (nucleotides 1645–1669; underscore indicates the mutation), and the antisense primer (MR) was 5'-gaccaa gccctgcacagtgcccaag-3' *(14)*. PCR cycling conditions were the same as those for PCR-SSCP analysis. PCR products were digested with *Ear*I (New England Biolabs) and then subjected to electrophoresis through 15% polyacrylamide gels to determine whether the DNA contained the restriction site created by the point mutation. Digestion of the wild-type allele yields a 123-bp band, whereas digestion of the mutant allele yields bands of 102 and 21 bp.

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References

1. Foley, T. P. Jr., Harrison, H. C., Arnaud, C. D., and Harrison, H. E. (1972). *J. Pediatr.* **81,** 1060–1067.
2. Marx, S. J., Attie, M. F., Levine, M. A., Spiegel, A. M., Downs, R. W. Jr., and Lasker, R. D. (1981). *Medicine* **60,** 397–412.
3. Law, W. M. Jr. and Heath, H. III. (1985). *Ann. Intern. Med.* **102,** 511–519.
4. Brown, E. M., Gamba, G., Riccardi, D., Lombardi, M., Butters, R., Kifor, O., Sun, A., Hediger, M. A., Lytton, J., and Hebert, S. C. (1993). *Nature* **366,** 575–580.
5. Pollak, M. R., Brown, E. M., Chou, Y.-H. W., Hebert, S. C., Marx, S. J., Steinmann, B., and Levi, T. (1993). *Cell* **75,** 1297–1303.
6. Pollak, M. R., Brown, E. M., Estep, H. L., McLaine, P. N., Kifor, O., Park, J., Hebert, S. C,. Seidman, C. E., and Seidman, J. G. (1994). *Nat. Genet.* **8,** 303–307.
7. Pollak, M. R., Chou, Y. H., Marx, S. J., Steinmann, B., Cole, D. E., Brandi, M. L., Papapoulos, S. E., Menko, F. H., Hendy, G. N., and Brown, E. M. (1994). *J. Clin. Invest.* **93,** 1108– 1112.
8. Pratt, E. L., Beren, B. B., and Neuhauser, E. B. D. (1947). *J. Pediatr.* **30,** 388–399.
9. Goldbloom, R. B., Gillis, D. A., and Prasad, M. (1972). *Pediatrics* **49,** 514–523.
10. Marx, S. J., Attie, M. F., Spiegel, A. M., Levine, M. A., Lasker, R. D., and Fok, M. (1982). *N. Engl. J. Med.* **306,** 257–264.
11. Heath, H. III., Jackson, C. E., Otterud, B., and Leppert, M. F. (1993). *Am. J. Hum. Genet.* **53,** 193–200.
12. Lloyd, S. E., Pannett, A. A. J., Dixon, P. H., Whyte, M. P., and Thakker, R. V. (1999). *Am. J. Hum. Genet.* **64,** 189–195.
13. Trump, D., Whyte, M. P., Wooding, C., Pang, J. T., Pearce, S. H., Kocher, D. B., and Thakker, R. V. (1995). *Hum. Genet.* **96,** 183–187.
14. Okazaki, R., Chikatsu, N., Nakatsu, M., Takeuchi, Y., Ajima, M., Miki, J., Fujita, T., Arai, M., Totsuka, Y., Tanaka, K., and Fukumoto, S. (1999). *J. Clin. Endocrinol. Metab.* **84,** 363–366.
15. Baron, J., Winer, K. K., Yanovski, J. A., Cunningham, A. W., Laue, L., Zimmerman, D., and Cutler, G. B. Jr. (1996). *Hum. Mol. Genet.* **5,** 601–606.
16. Pearce, S. H., Williamson, C., Kifor, O., Bai, M., Coulthard, M. G., Davies, M., Lewis-Barned, N., McCredie, D., Powell, H., Kendall-Taylor, P., Brown, E. M., and Thakker, R. V. (1996). *N. Engl. J. Med.* **335,** 1115–1122.

17. Finegold, D. N., Armitage, M. M., Galiani, M., Matise, T. C., Pandian, M. R., Perry, Y. M., Deka, R., and Ferrell, R. E. (1994). *Pediatr. Res.* **36,** 414–417.
18. Mancilla, E. E., De Luca, F., and Baron, J. (1998). *Mol. Genet. Metab.* **64,** 198–204.
19. Stock, J. L., Brown, R. S., Baron, J., Coderre, J. A., Mancilla, E., De Luca, F., Ray, K., and Mericq, M. V. (1999). *J. Clin. Endocrinol. Metab.* **84,** 3036–3040.
20. De Luca, F., Ray, K., Mancilla, E. E., Fan, G. F., Winer, K. K., Gore, P., Spiegel, A. M., and Baron, J. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2710–2715.
21. Watanabe, T., Bai, M., Lane, C. R., Matsumoto, S., Minamitani, K., Minagawa, M., Niimi, H., Brown, E. M., and Yasuda, T. (1998). *J. Clin. Endocrinol. Metab.* **83,** 2497–2502.
22. Lienhardt, A., Garabedian, M., Bai, M., Sinding, C., Zhang, Z., Lagarde, J. P., Boulesteix, J., Rigaud, M., Brown, E. M., and Kottler, M. L. (2000). *J. Clin. Endocrinol. Metab.* **85,** 1695–1702.
23. Chikatsu, N., Fukumoto, S., Suzawa, M., Tanaka, Y., Takeuchi, Y., Takeda, S., Tamura, Y., Matsumoto, T., and Fujita, T. (1999). *Clin. Endocrinol. (Oxf.).* **50,** 537–543.
24. Aida, K., Koishi, S., Inoue, M., Nakazato, M., Tawata, M., and Onaya, T. (1995). *J. Clin. Endocrinol. Metab.* **80,** 2594–2598.
25. Heath, H. III, Odelberg, S., Jackson, C. E., Teh, B. T., Hayward, N., Larsson, C., Buist, N. R., Krapcho, K. J., Hung, B. C., Capuano, I. V., Garrett, J. E., and Leppert, M. F. (1996). *J. Clin. Endocrinol. Metab.* **81,** 1312–1317.
26. Pearce, S. H., Trump, D., Wooding, C., Besser, G. M., Chew, S. L., Grant, D. B., Heath, D. A., Hughes, I. A., Paterson, C. R., Whyte, M. P., et al. (1995). *J. Clin. Invest.* **96,** 2683–2692.
27. Chou, Y. H., Pollak, M. R., Brandi, M. L., Toss, G., Arnqvist, H., Atkinson, A. B., Papapoulos, S. E., Marx, S., Brown, E. M., Seidman, J. G., et al. (1995). *Am. J. Hum. Genet.* **56,** 1075–1079.
28. Ward, B. K., Stuckey, B. G., Gutteridge, D. H., Laing, N. G., Pullan, P. T., and Ratajczak, T. (1997). *Hum. Mutat.* **10,** 233–235.
29. Kobayashi, M., Tanaka, H., Tsuzuki, K., Tsuyuki, M., Igaki, H., Ichinose, Y., Aya, K., Nishioka, N., and Seino, Y. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2716–2719.
30. Schwarz, P., Larsen, N. E., Lonborg Friis, I. M., Lillquist, K., Brown, E. M.,and Gammeltoft, S. (2000). *Scand. J. Clin. Lab. Invest.* **60,** 221–227.
31. Janicic, N., Pausova, Z., Cole, D. E., and Hendy, G. N. (1995). *Am. J. Hum. Genet.* **56,** 880–886.
32. Carling, T., Szabo, E., Bai, M., Ridefelt, P., Westin, G., Gustavsson, P., Trivedi, S., Hellman, P., Brown, E. M., Dahl, N., and Rastad, J. (2000). *J. Clin. Endocrinol. Metab.* **85,** 2042–2047.
33. Kanazawa, H., Tanaka, H., Kodama, S., Moriwake, T., Kobayashi, M., and Seino, Y. (2000). *Endocr. J.* **47,** 29–35.
34. Nakayama, T., Soma, M., Rahmutula, D., Izumi, Y., and Kanmatsuse, K. (1997). *Lancet* **349,** 1887,1888.
35. Nakayama, T., Soma, M., Takahashi, Y., Rehemudula, D., Kanmatsuse, K., and Furuya, K. (2000). *Circ. Res.* **86,** 841–845.