JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

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J. Clin. Endocrinol. Metab. 2004 89: 4840-4845, doi: 10.1210/jc.2004-0273

0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(10):4840–4845

doi: 10.1210/jc.2004-0273

Parathyroid Hormone Gene Polymorphism and Sporadic Idiopathic Hypoparathyroidism

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The pathogenetic mechanisms involved in the development of sporadic idiopathic hypoparathyroidism are currently under investigation. Although autoantibodies against the calcium-sensing receptor (CaSR) have been implicated to play a role, these could be demonstrated in only 49% of a group of 51 patients with sporadic idiopathic hypoparathyroidism that we previously studied. Therefore, we investigated 49 of these patients further, regardless of their antibody status, and looked for mutations in the section of the PTH gene sequence that coded for prepro-PTH as well as the 3′-untranslated region (3′-UTR) of the gene, which is believed to be involved in the stability of its mRNA. We also examined the relationship between the clinical manifestations of the disease and the occurrences of two commonly observed single nucleotide polymorphisms (SNPs) in the PTH gene. In 49 of the patients with idiopathic hypoparathyroidism and in 55 healthy controls, the SNPs were characterized by restriction analysis using *Dra*II and *Bst*BI enzymes. In a subset of these patients, exons 2 and 3 of the PTH gene (n = 37) and its 3′-UTR region (n = 40) were also sequenced. No mutations were observed in the segment of the PTH gene coding for the signal peptide, prohormone, or the 3′-UTR region. However, three well described SNPs were observed: 1) an A→G substitution in intron 1 in 35.1% of the patients; 2) a G→A substitution in intron 2, characterized by *Bst*BI, in one or both alleles in 27%; and 3) a C→A substitution at codon 52 (CGA) of exon 3, characterized by *Dra*II, in one or both alleles in 59.7% of the patients. There was no significant difference in the frequency of occurrence of these SNPs between the patient and the control groups. Furthermore, the mean age at onset of symptoms, body mass index, frequency of cataract, tetany, convulsion, basal ganglia calcification, serum calcium, inorganic phosphorus, and intact PTH were not significantly different between patients with and without the above-described SNPs. Thus, the data from this report demonstrate that in patients with sporadic idiopathic hypoparathyroidism, neither the clinical manifestations nor the biochemical indexes of the disease are related to the occurrence of mutations or SNPs in the PTH gene. Because neither patient nor control samples exhibited any variations in the sequence of their 3′-UTR regions, it is unlikely that mRNA instability is a factor in the pathogenesis of the disease. Additional studies are required to investigate the role of other genes and autoantigens that may be involved in the genesis of idiopathic hypoparathyroidism. (*J Clin Endocrinol Metab* 89: 4840–4845, 2004)

THE FUNDAMENTAL MECHANISMS involved in the development of idiopathic hypoparathyroidism still remain unidentified and are currently a subject of investigation. We recently demonstrated autoantibodies against the calcium-sensing receptor (CaSR) in 49% of a group of 51 patients with idiopathic hypoparathyroidism (1). The presence of CaSR autoantibodies in association with specific human leukocyte antigen DR haplotypes indicate organ-specific autoimmunity; therefore, this subset of patients could be classified as having autoimmune hypoparathyroidism. However, the remaining half of the patients in whom no CaSR autoantibodies could be detected would still have to be classified as having idiopathic hypoparathyroidism. Studies in patients with familial hypoparathyroidism have revealed point mutations in the signal peptide-coding region of PTH gene in some of them (2, 3). The human PTH gene has been cloned; it is located on chromosome 11 and consists of three exons. Exon 1 is untranslated, exon 2 codes for the 25-amino acid signal peptide and part of the prohormone, and exon 3 codes for the remaining part of the prohormone (six amino acids) and the whole PTH molecule (84 amino acids; Fig. 1). In humans, the terminal region of exon 3 consisting of 351 nucleotides remains untranslated [3′-untranslated region (3′-UTR)] (4, 5). In rats, the distal terminal portion of this UTR is related to the stability of PTH mRNA (6) and contains a 26-nucleotide-long *cis*-acting element (TATTGTTTATTCTTTTTAAAGTATGT) that binds a parathyroid cytosolic protein and is conserved among species, including humans. In the familial form of hypoparathyroidism, mutations have also been reported at a donor splice site in the intron 1-exon 2 junction (7). Suprasdngsin *et al.* (8) described a point mutation in the signal peptide-encoding region of the PTH gene in an isolated patient with the sporadic form of idiopathic hypoparathyroidism. A mutation occurring in the region coding for the signal peptide impairs the interaction of the nascent protein with the signal recognition particle and the translocation machinery (9). Three single nucleotide polymorphisms (SNPs) in the PTH gene sequence have been observed frequently in both Caucasian as well as Asian populations. These include an A→G substitution in intron 1, 10 nucleotides upstream of exon 2; a G→A substitution in intron 2, 54 nucleotides downstream of the 3′ end of exon 2; and a C→A substitution in codon 52 (CGA) of exon 3. The last

Abbreviations: CaSR, Calcium-sensing receptor; iPTH, intact PTH; SNP, single nucleotide polymorphism; 3′-UTR, 3′-untranslated region.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

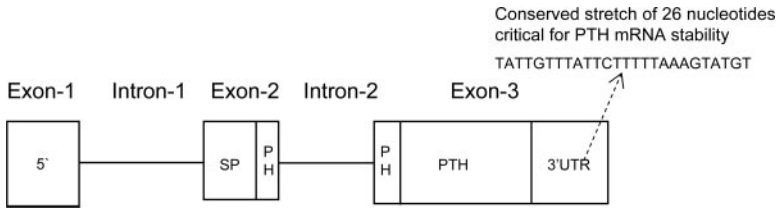

FIG. 1. A schematic representation of the arrangement of introns and exons in the PTH gene, which codes for the prepro-PTH molecule. SP, Signal peptide coding for 25 amino acids; PH, prohormone.

two SNPs have been analyzed in detail by restriction fragment length polymorphism studies using endonucleases *Dra*II and *Bst*BI, and some of the different genotypes detected in healthy populations have been found to be linked to the presence of osteoporosis (10, 11). In patients with primary hyperparathyroidism, Kanzawa *et al.* (12) reported a correlation between PTH gene polymorphism and the severity of the disease. Recently, Alvarez-Hernandez *et al.* (13) reported coinheritance of mutations in exon 7 of both the CaSR gene as well as the PTH gene SNPs in a family with autosomal dominant hypoparathyroidism.

In the present study we investigated 49 patients with idiopathic hypoparathyroidism regardless of their antibody status and looked for mutations in the section of the PTH gene sequence that coded for prepro-PTH. The 3′-UTR region was also sequenced, and the conserved stretch of 26 nucleotides concerned with the stability of PTH mRNA was specifically examined for alterations. We also studied the clinical manifestations of the disease as related to the occurrence of two commonly observed SNPs in the PTH gene.

Materials and Methods

The subjects in this study consisted of 49 patients (27 males and 22 females) with sporadic idiopathic hypoparathyroidism who attended the endocrine clinic at the All India Institute of Medical Sciences (New Delhi, India) from 1998–2003. The diagnosis of idiopathic hypoparathyroidism was based on the presence of hypocalcemia and hyperphosphatemia in association with subnormal or an inappropriately normal level of serum intact PTH (iPTH). Their mean age at presentation was 32.1 ± 13.8 yr (range, 10–59 yr), and the mean duration of their illness was 6.8 ± 7.7 yr (range, 1 d to 35 yr). Patients with postsurgical hypoparathyroidism were excluded. None of the patients had a family history of hypoparathyroidism or any features, such as mucocutaneous candidiasis or autoimmune adrenal insufficiency, that could be suggestive of hypoparathyroidism related to the polyglandular autoimmune type 1 syndrome/autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (14, 15). Autoimmune adrenal involvement was excluded by demonstrating normal ACTH and serum cortisol values at 0800 h coupled with no evidence of adrenal cortical autoantibodies on indirect immunofluorescence (16). Computerized tomography to determine basal ganglia calcification was carried out in all subjects, and cataract was diagnosed by examination with a slit lamp. The clinical features of the 49 patients included in this study are as follows. Both sexes showed a similar predilection for the disease, and the male/female ratio was 1.2:1. The most common features observed included basal ganglia calcification (76%), a history of generalized tonic clonic convulsions (63.2%), and cataract (36.7%). The absence of any clinical or biochemical evidence of adrenal insufficiency as well as mucocutaneus candidiasis in all patients suggests that the polyglandular autoimmune type 1 syndrome is rare among patients with idiopathic hypoparathyroidism, at least in India. From each patient, 10 ml blood were drawn in 3% EDTA, and genomic DNA was extracted from blood using the standard phenol-chloroform extraction technique (17). The institutional ethics committee of All India Institute of Medical Sciences approved the project, and written informed consent was obtained from all study subjects. Analysis of the two SNPs in the PTH gene using *Dra*II and *Bst*BI restriction endonucleases was performed using PCR-restriction fragment length polymorphism for all 49 patients with idiopathic hypoparathyroidism as well as 55 healthy controls. Sequencing of the PTH gene was performed in samples from 37 patients and five healthy volunteers.

Forward and reverse primers used to amplify exons 2 and 3 of the PTH gene were based on the report by Sunthornthepvarakul *et al.* (3). Besides amplifying exon 2 and the section of exon 3 that coded for PTH, these primers also amplified intron 2 and a part of intron 1 flanking the 5′ region of exon 1 (3). PAGE-purified oligonucleotide primers, 5′-gcttctcgtgaaacaacatggt-3′ (forward) and 5′-ccctacactgtctagagcag-3′ (reverse), were custom-synthesized (Microsynth GmbH, Balgach, Switzerland). The conditions for amplification by PCR were as follows: 100 ng genomic DNA, 100 pmol of each primer, 200 μmol/liter of each deoxy-NTP, 2.5 mmol/liter $MgCl_2$, 5 mmol/liter Tris-HCl (pH 8.0), 10 mmol/liter NaCl, 10 μmol/liter EDTA, 0.5 mmol/liter dithiothreitol, 5% glycerol, 0.1% Triton X-100, and 1.25 U *Taq* DNA polymerase. The initial denaturation was performed at 94 C for 5 min, followed by 35 cycles of 94 C for 1 min, 58 C for 2 min, and 72 C for 1 min and a final extension at 72 C for 7 min. In 40 patients and eight healthy controls, the 3′-UTR region of exon 3 of the PTH gene was amplified using primers designed based on the published sequence of the PTH gene (4). The forward primer sequence was 5′-cttggagaggcagacaaagc-3′, and the reverse primer sequence was 5′-catgtgggtatcagtgataacc-3′. The conditions for PCR for amplification of the 3′-UTR region were same as those described above, but the temperature for annealing was 55 C. Electrophoresis of the amplified DNA fragments was carried out on 1% agarose gel in Tris-borate/EDTA electrophoresis buffer and was visualized by ethidium bromide staining and a UV light illuminator. Serum calcium and inorganic phosphorus levels were measured using standard techniques (18). iPTH was measured using an immunoradiometric assay (DiaSorin, Inc., Stillwater, MN; normal range, 13–54 pg/ml; intraassay coefficient of variation, 4%).

Sequencing of PCR-amplified products

All PCR-amplified samples were purified using an ExoSAP-IT kit (Amersham Biosciences, Arlington Heights, IL) and were sequenced using both forward as well as reverse primers. Sequencing reactions were performed using the Big Dye Terminator Cycle Sequencing version 3.0 Ready Reaction Kit with AmpliTaq polymerase (Applied Biosystems, Foster City, CA). Each reaction consisted of 1 μl reaction mix, 1.5 μl dilution buffer, 1 μl (5 pmol) forward or reverse primer, 5 μl (150–200 ng) ExoSAP-purified DNA template, and 1.5 μl sterilized water in a final reaction volume of 10 μl. Sequencing PCR was carried out in an ABI thermocycler programmed for 25 cycles each at 96 C for 10 sec, 55 C for 5 sec, and 60 C for 2 min, followed by ethanol precipitation; air-dried for 5–10 min; and then dissolved in 10 μl Hi-Di formamide (Applied Biosystems). Sequencing was performed by running the samples in a fully automated 96-capillary-based ABI PRISM 3700 DNA sequencer for 4 h at 6000 V. A change in the base sequence was considered significant only when it was present in both the sense and antisense strands. BLAST (www.ncbi.nlm.nih.gov/blast) was used for identity search by comparing the sequence obtained with the published sequence of the PTH gene, including its 3′-UTR region.

Analysis of SNPs in the PTH gene

After amplification, a 40-μl aliquot of the PCR product of the PTH gene was incubated at 37 C with 10 U *Dra*II (Fermantas, Inc., Hannover, MD) for 3 h. In the case of *Bst*BI, 5 U enzyme were used for 20-μl PCR products. The digested products were electrophoresed on 1.5% agarose gel to visualize the fragments. The SNP leading to the C→A substitution at codon 52 (CGA) in exon 3 of the PTH gene results in loss of the restriction site for the endonuclease *Dra*II. The presence of normal allele (nucleotide C) at this position produces two fragments of 434 and 175

bp after restriction enzyme digestion (Fig. 2A). The individuals carrying this allele in homozygous form were designated DD in this study; those with all the three fragments (609, 434, and 175 bp) were heterozygotes and were referred to as Dd. The individuals carrying the mutant allele in the homozygous form as indicated by the presence of a single 609-bp fragment were designated dd. The SNP leading to the G→A substitution in intron 2 of the PTH gene resulted in loss of the restriction sites for the endonuclease *Bst*BI. Normal homozygotes (with 383- and 226-bp fragments), heterozygotes (609-, 383-, and 226-bp fragments) and mutant homozygotes (609-bp fragment) carrying the SNP for the *Bst*BI enzyme were referred to as BB, Bb, and bb, respectively (Fig. 2B).

Statistical analysis

Fischer's exact test was used to study the significance of the difference observed in the frequency of occurrence of different types of alleles between the patient and the control groups after *Dra*II and *Bst*BI digestion. The mean age of onset of disease; serum calcium, phosphorus, and iPTH levels; and frequency of various clinical manifestations were compared in different genotypes using *t* test and χ^2 analysis, respectively.

Results

The published sequence of the human PTH gene (4) was compared with the sequence data obtained from the analysis of samples from 37 patients and five controls in this study. The results revealed that there were no changes in the sequence of bases in the signal peptide, prohormone-encoding region of the PTH gene of any of the subjects. However, analysis of the sequence data of the PTH gene revealed that in 13 of the 37 patients (35.1%) and in two of the five controls (40%), there was an A→G substitution in intron 1. In five of the 37 patients (13.5%) and in one of the five controls (20%), there was a C→A substitution at codon 52 (CGA) in the exon 3 of the PTH-encoding region. However, codons CGA and AGA conserve the amino acid arginine at this position in the PTH molecule. Two of the patients also had a G→A substitution in the intron 2, 54 nucleotides downstream of 3′ end of exon 2 (Fig. 3). The possibility of the G→A substitution observed in the present study leading to the formation of a new mammalian branch point consensus sequence (and thus possibly altered splicing of PTH pre-mRNA) was calculated using the method described by Shapiro and Senapathy (19, 20) and the slice applet score chart (http://home.snafu.de/probins/Splice/shapiroSenapathy.html). The maximum score of any possible new combination of nucleotide sequence generated due to the G→A substitution (and thus a possible invariant A and a new branch point consensus sequence) was only 61% (Fig. 3).

All three single nucleotide changes described above are common and are located at well recognized sites for SNPs in the PTH gene (10, 21). When two of these three SNPs described above [C→A substitution at codon 52 (CGA) in exon

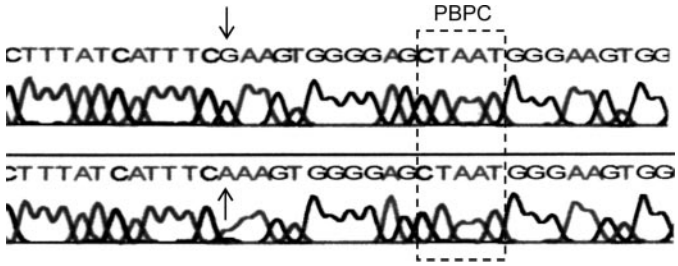

FIG. 3. A part of the sequence for intron 2 showing the G→A substitution at 54 nucleotides downstream of exon 2. The perfect branch point consensus nucleotide sequence (*rectangular box*) with a branch point score greater than 96% is already located 11 nucleotides downstream of the observed G→A substitution.

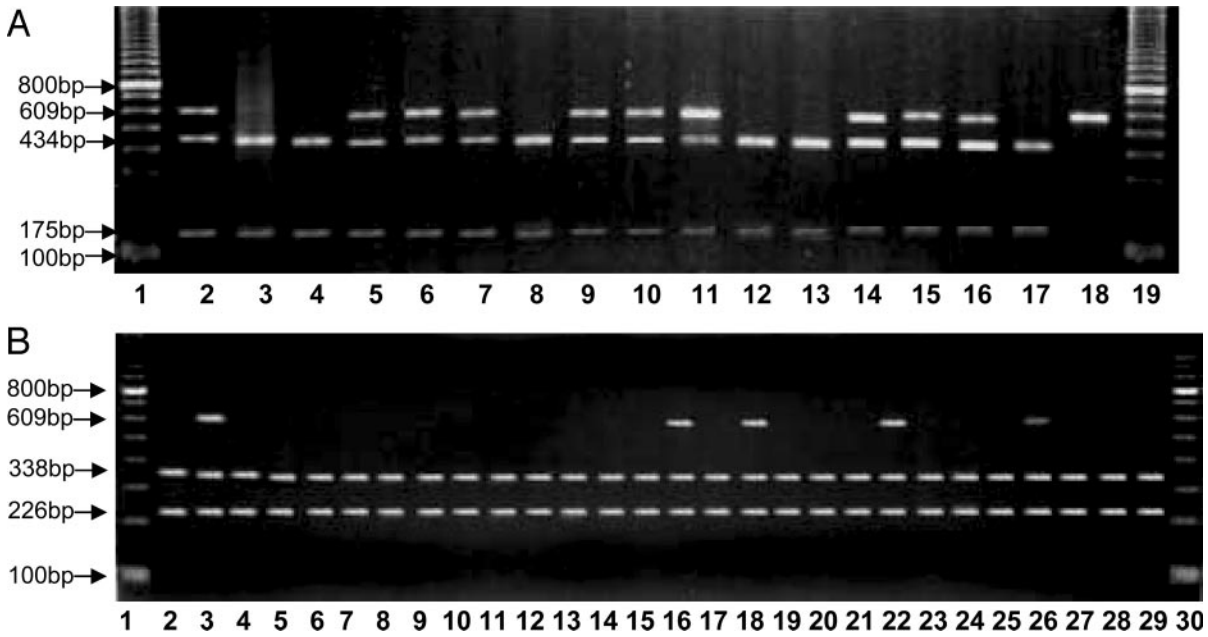

FIG. 2. A, A 1.5% agarose gel electrophoresis after *Dra*II restriction enzyme digestion showing 434- and 175-bp fragments of the PTH gene (lanes 2–18). Lanes 3, 4, 8, 12, 13, and 17 are samples from normal homozygotes (DD). Lanes 2, 5–7, 9–11, and 14–16 are from heterozygotes (Dd), and lane 18 is the homozygote with the SNP (dd). Lanes 1 and 19 contain a 100-bp DNA ladder. B, A 1.5% agarose gel electrophoresis after *Bst*BI restriction enzyme digestion of the PTH gene showing 383- and 226-bp fragments of the PTH gene (lanes 2–29). Lanes 3, 16, 18, 22, and 26 contain samples from heterozygotes with SNP designated Bb. Other lanes belong to the normal homozygotes with SNP (BB). A 100-bp DNA ladder was run in lanes 1 and 30.

3 and G→A substitution in intron 2] were further studied with restriction analysis using *Dra*II and *Bst*BI, respectively, there were no significant differences in the frequency of occurrence of various genotypes between the patient and control groups [patient *vs.* control groups: DD, 44.3% *vs.* 41.8% ($P = 0.48$); Dd, 46.1% *vs.* 49.1% ($P = 0.45$); dd, 9.6% *vs.* 9.1% ($P = 0.59$); BB, 73.0% *vs.* 74.4% ($P = 0.51$); Bb, 25.0% *vs.* 16.5% ($P = 0.28$); bb, 2.0% *vs.* 9.1% ($P = 0.12$%; Fig. 2].

To study the relationship between various PTH gene polymorphisms and clinical manifestations of the disease in idiopathic hypoparathyroidism, the patients were divided into groups based on the presence or absence of SNPs at the *Bst*BI and *Dra*II restriction sites. After restriction digestion with *Bst*BI, the number of patients bearing either the Bb allele or bb allele was few, and these subjects were therefore analyzed as a single group and compared with those having the BB genotype. For the same reasons, patients bearing the Dd and dd alleles were also grouped together and compared with those having the DD homozygote. Examination of the results showed that there was no significant difference between patients with and without the two PTH gene SNPs studied with regard to the mean age at onset of symptoms, body mass index, frequency of occurrence of cataract, tetany, convulsion, basal ganglia calcification, serum calcium, inorganic phosphorus, and levels of intact PTH (Table 1).

In the present study using the primers we designed to amplify 3′-UTR of the PTH gene, PCR yielded the expected product of 433 bp. Comparison of its sequence data with the published sequence (4) of the 3′-UTR of the human PTH gene did not reveal any variation in the nucleotide sequences of the 40 patients and eight controls studied.

Discussion

The pathogenesis of the sporadic form of idiopathic hypoparathyroidism is an issue presently under investigation. In a patient belonging to a family with autosomal dominant hypoparathyroidism, Arnold *et al.* (2) detected a T→C mutation in exon 2 of the PTH gene, which resulted in a cysteine to arginine substitution at position 18 of the 31-amino acid, prepro sequence of PTH. Suprasdngsin *et al.* (8) reported same mutation (T→C) in an isolated patient with sporadic idiopathic hypoparathyroidism. Parkinson and Thakker (7) studied three siblings with isolated hypoparathyroidism (two females and a male) whose parents were first cousins. They found that the affected individuals were homozygous for a mutation of the PTH gene that substituted C→G at the first nucleotide of intron 2 of the PTH gene. This mutation resulted in exon skipping and resultant loss of exon 2, which encoded the signal peptide, leading to PTH deficiency (7). In view of these reports of mutations in the PTH gene in the familial as well as sporadic forms of hypoparathyroidism (2, 3, 7, 8), we investigated a group of patients with sporadic idiopathic hypoparathyroidism and examined samples from them for the occurrence of similar mutations.

Although we did not find any mutations in the region coding for the signal peptide or the PTH gene in any of the patients, SNPs occurred frequently at three sites in both patients and controls. A similarly high frequency of occurrence of nucleotide substitution described above was reported earlier by Miric and Levine (21) in patients with familial isolated hypoparathyroidism as well as in a cohort of unrelated Caucasian individuals who had no known history of PTH deficiency.

Interestingly, in the present study 27% of the patients studied also had a G→A substitution in intron 2 at 54 nucleotides downstream of the 3′ end of exon 2 (or 50 nucleotides upstream of the 3′ splice acceptor site). In the process of splicing, a short conserved sequence, termed a branch point consensus nucleotide sequence, functions as the recognition signal for the spliceosome, which then excises the intron in the form of a lariat (19, 20). The branch point consensus nucleotide sequence is characterized by a highly conserved adenosine residue, which serves as an anchor for the formation of the spliceosome and is located between 10 and 50 nucleotides upstream of the 3′ splice site. The G→A substitution observed in the patients in this study can theoretically result in a new combination of nucleotide sequences, which may hypothetically produce another invariant adenosine residue and create a different branch point consensus nucleotide sequence. Shapiro, Senapathy and Harris (19, 22) studied the branch point nucleotide sequences of several genes and devised a scoring system for predicting the probability of a potential branch point existing in a specific nucleotide sequence. Using this method a sequence that scores higher than 96%, would indicate a branch point and corresponds to only four possible nucleotide sequences, *i.e.* CTGAC, CTAAC, CTGAT, and CTAAT. A recent report has shown the presence of a mutation in the branch point con-

TABLE 1. Clinical and biochemical indexes of patients with idiopathic hypoparathyroidism in relation to the occurrence of SNP in the PTH gene recognized by *Dra*II and *Bst*BI restriction enzymes

Parameters	Genotype characterized by *Dra*II enzyme		*P* value	Genotype characterized by *Bst*BI enzyme		*P* value
	DD (n = 21)	Dd or dd (n = 28)		BB (n = 36)	Bb or bb (n = 13)	
Males:females (no.)	10:11	17:11		19:17	8:5	
BMI (kg/m^2)	21.7 ± 4.1	20.9 ± 5.5	0.59	20.7 ± 4.6	22.6 ± 5.4	
Age of onset of symptoms (yr)	23.2 ± 14.1	26.8 ± 12.6	0.35	27.0 ± 12.8	20.4 ± 13.9	0.12
Convulsions [no. (%)]	14/21 (66.6)	17/28 (60.7)	0.89	21/36 (58.3)	10/13 (76.9)	0.73
Cataract [no. (%)]	7/20 (35.0)	11/28 (39.2)	0.76	14/35 (40.0)	4/13 (30.7)	0.25
Basal ganglia calcification [no. (%)]	15/20 (75.0)	21/27 (77.7)	0.82	26/34 (76.4)	10/13 (76.9)	0.81
Serum total calcium (mg/dl)	6.09 ± 0.77	5.68 ± 1.18	0.29	6.13 ± 0.98	5.60 ± 1.10	0.20
Serum inorganic phosphorus (mg/dl)	6.39 ± 1.14	6.27 ± 1.44	0.64	6.27 ± 1.35	6.57 ± 1.23	0.49
iPTH (pg/ml)	8.8 ± 5.3	11.7 ± 10.7	0.23	8.3 ± 4.0	11.2 ± 10.0	0.15

Results are expressed as mean ± SD. (Normal range for serum total calcium, 9.0–11.5 mg/dl; inorganic phosphorus, 2.5–4.5 mg/dl; and for iPTH, 13–54 pg/ml). SI conversion for serum total calcium is 0.2495 mmol/liter; for inorganic phosphorus 0.3229 mmol/liter.

sensus nucleotide sequence to be associated with familial hypercholesteremia (23). In view of this using the method described by Shapiro and Senapathy (20), we examined the data from the present study to determine whether the G→A substitution observed in intron 2 could have led to the formation of a new mammalian branch point consensus nucleotide sequence and consequent altered splicing of the PTH-pre-mRNA. The results of the analysis showed that this possibility was unlikely because a perfect branch point consensus sequence (CTAAT) with a score greater than 96% already existed at 30 nucleotides upstream of the 3′ acceptor site of exon 3 located in intron 2 (Fig. 3). Any other new combination of nucleotide sequences generated by the G→A substitution observed in this study had a maximum score of only 61% for possibly creating a new branch point consensus sequence and therefore would not affect the splicing of the pre-mRNA. In fact, in several Western and Japanese populations, the G→A substitution in intron 2 is a frequently reported polymorphism (10–11).

The C→A substitution at codon 52 (CGA) in exon 3 and the G→A substitution in intron 2 lead to disruption of restriction sites *BstB*I and *Dra*II. Using these restriction enzymes several studies recently reported a correlation between the presence of the above SNPs and the occurrence of osteoporosis. Recently, Kanzawa *et al.* (12) reported higher circulating levels of iPTH and alkaline phosphatase in patients with primary hyperparathyroidism belonging to the BBDD genotype. However, in the present study there was no significant association between the various clinical manifestations or biochemical indexes of the disease and the presence of these SNPs either in isolation or in various combinations in patients with sporadic idiopathic hypoparathyroidism. This further demonstrated that in the group of patients we studied, the SNPs observed in the PTH gene were silent polymorphisms and were not linked to hypoparathyroidism or any of its clinical manifestations, such as basal ganglia calcification, cataract, and convulsions in them.

The terminal nucleotides of the 3′-UTR of the PTH gene are related to the stability of the PTH mRNA transcript and protect the inherently unstable 3′-UTR section by binding to certain parathyroid-specific cytosolic proteins at a highly conserved 26-nucleotide sequence located in the distal terminal section of the 3′-UTR (6). In *in vitro* experiments carried out to assess the stability of PTH mRNA, Moallem *et al.* (24, 25) observed an increased stability of PTH mRNA probes without the 3′-UTR compared with that of probes with intact 3′-UTR. Prolonged hypocalcemia enhanced the stability, and hypophosphatemia led to decreased stability of PTH mRNA transcripts (24, 25). Because hypoparathyroidism is characterized by severe hypocalcemia with coexisting hyperphosphatemia, it could theoretically be associated with either an unchanged or an enhanced stability of the PTH mRNA transcript. Correspondingly in the present study we did not observe any change in the sequence of this 3′-UTR of the PTH gene, including the conserved stretch of 26 nucleotides. The presence of a normal 3′-UTR sequence in association with severe hypocalcemia and hyperphosphatemia should, in theory at least, lead to enhanced stability of the PTH mRNA transcript, rather than decreased stability; this rules out the role of mRNA instability in the pathogenesis of idiopathic hypoparathyroidism.

There are reports of patients with familial as well as sporadic forms of hypoparathyroidism and of mutations triggering constitutive activation of the CaSR (26, 27). Ding *et al.* (28) reported a patient with familial isolated hypoparathyroidism with a mutation in the gene coding for the recently discovered parathyroid cell-specific transcription factor GCMb, which belongs to the family of glial cell missing transcription factors. Transcription factors have also been reported to function as autoantigens in type 1 diabetes, systemic sclerosis, and lymphocytic hypophysitis, which are diseases well recognized to have an autoimmune basis (29–31). To understand the etiology of the various types of sporadic idiopathic hypoparathyroidism, additional investigations must be carried out to detect any modifications in the normal sequence of various candidate genes and to examine the concept of a parathyroid-specific transcription factor functioning as a potential autoantigen.

Hence, the findings of the present study suggest that the sporadic form of idiopathic hypoparathyroidism does not originate from mutations in either the PTH gene or in its 3′-UTR. The commonly described polymorphisms in the PTH gene did not segregate the patients with respect to the clinical manifestations of the disease. Although the present work did not contribute any new information regarding the mechanisms underlying the pathogenesis of idiopathic hypoparathyroidism, it adds positive information that mutations in the PTH gene or its SNPs are not an important cause of the sporadic form of idiopathic hypoparathyroidism. Additional studies are required to determine the involvement of other candidate genes and new autoantigens, other than the CaSR, in the pathogenesis of idiopathic hypoparathyroidism.

Acknowledgments

Received February 17, 2004. Accepted July 8, 2004.

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This work was supported by a Research Grant from All India Institute of Medical Sciences (to R.G. and N.G.).

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