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CASE REPORT

A novel mutation in the GATA3 gene of a Japanese patient with PTH-deficient hypoparathyroidism

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Abstract Hypoparathyroidism is a disease characterized by hypocalcemia and hyperphosphatemia derived from deficient actions of parathyroid hormone (PTH). We report the case of 43-year-old Japanese man with PTH-deficient hypoparathyroidism introduced to an endocrinologist in our hospital. As he had complained of hearing disturbance since the age of 20, we decided to investigate the *GATA3* gene. Direct sequencing of PCR products identified a novel heterozygous mutation, 432insG, in the *GATA3* gene. The mutation introduces a premature stop codon at exon 4 (K302X), which results in a loss of both zinc finger domains of the GATA3 protein. However, because the mutation in the *GATA3* gene found in this patient is highly likely to impair GATA3 function, we speculate that it is extremely unlikely that this patient has mutations in other genes that cause PTH-deficient hypoparathyroidism, in addition to the *GATA3* mutation described here.

Keywords GATA3 · HDR syndrome · Hypoparathyroidism

Introduction

Hypoparathyroidism is a disease characterized by hypocalcemia and hyperphosphatemia derived from deficient actions of parathyroid hormone (PTH). The impaired secretion of PTH causes PTH-deficient hypoparathyroidism, and the resistance to PTH underlies pseudohypoparathyroidism [1, 2]. Most patients with PTH-deficient hypoparathyroidism other than clear secondary causes, such as parathyroidectomy, have been diagnosed with idiopathic hypoparathyroidism. Recent genetic analyses identified several genes for PTH-deficient hypoparathyroidism. Therefore, diseases caused by mutations in these genes have been shown to be distinct from idiopathic hypoparathyroidism. For examples, *calcium-sensing receptor (CASR)*, *glial cells missing 2 (GCM2)* and *PTH* genes have been identified to be responsible for familial isolated hypoparathyroidism (OMIM #146200) [3–7]. In addition, *GATA-binding protein 3 (GATA3)* and *tubulin-specific chaperone E (TBCE)* genes were shown to cause HDR (hypoparathyroidism, sensorineural deafness and renal disease) syndrome and HRD (hypoparathyroidism–retardation–dysmorphism) syndrome, respectively [8, 9].

GATA3 is a DNA-binding protein with two zinc finger domains. There are six GATA proteins (GATA1 to 6) which bind to the consensus 5′-(A/T)GATA(A/G)-3′ sequence [10–13]. Two zinc finger domains are shown to be necessary for the binding and the stabilization of the binding of GATA proteins to DNA [14, 15]. The *GATA3* gene is located on chromosome 10p15 and consists of six exons. Exon 1 is a non-coding exon and the transcription start site exists in exon 2 [8]. Homozygotes of *GATA3* knockout mice are embryonic lethal indicating that GATA3 is essential for fetal development at least in mice [16, 17]. GATA3 is highly expressed in T-cells and has been shown to be involved in the regulation of several genes in T-cells. However, haploinsufficiency of GATA3 has been shown to result in HDR syndrome [8]. Here we show a novel mutation in the *GATA3* gene in a Japanese

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patient with PTH-deficient hypoparathyroidism and discuss the diversity of phenotypes caused by mutations in *GATA3*.

Case report

A 43-year-old Japanese man was introduced to an endocrinologist in our hospital. He was born as an full-term baby without asphyxia from a healthy mother. He experienced sudden loss of consciousness at the age of 12. Since then, he sometimes suffered from generalized tonic–clonic seizure. He was diagnosed with epilepsy and had been under treatment for this disease. At the age of 24, he was admitted to our hospital suffering from lung tuberculosis. Laboratory examinations revealed hypocalcemia (corrected Ca by albumin 6.1 mg/dl) and normophosphatemia (3.3 mg/dl). His renal function was normal, and PTH measured by C-terminal assay was below 0.3 ng/ml. He showed normal phosphaturic response and increase of urinary cyclic AMP excretion in Ellsworth–Howard test using 100 U of human PTH(1–34) [18]. From these results, he was diagnosed with idiopathic hypoparathyroidism and has been under treatment with active vitamin D_3. He also complained hearing disturbance since the age of 20. Otological examination revealed sensorineural deafness. Audiogram conducted at the age of 32 showed the following hearing acuity: 60 dB at 500 Hz, 65 dB at 1,000 Hz and 65 dB at 2,000 Hz in the left ear and 85 dB at 500 Hz, 90 dB at 1,000 Hz and 90 dB at 2,000 Hz in the right ear. He has no family history of hearing disturbance or disorders of calcium metabolism.

Physical examination showed no abnormality except for hearing disturbance. He could communicate without any problem wearing a hearing aid in his left ear. Laboratory examinations revealed mild hypocalcemia (corrected Ca 7.5–8.2 mg/dl), normophosphatemia (3.4–4.1 mg/dl) and normal renal function (creatinine 0.86–0.95 mg/dl). Intact PTH was 9 pg/ml when serum corrected Ca was 8.2 mg/dl. Urinalysis was normal. Computed tomography of abdomen vvshowed no abnormality in the size and contour of kidneys.

Methods

After written informed consent was obtained, genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit (QIAamp DNA Blood Mini Kit, QIAGEN, Tokyo, Japan). The entire coding sequence and the exon–intron junctions of the *GATA3* gene were amplified using polymerase chain reaction (PCR). The primers used are 5′-CACCGAAAGCAAATCATTCAAC-3′ (forward) and 5′-TTTTTTGTAAATGAACCAGGAACG-3′ (reverse) for exon 2, 5′-CCTTCATTCTGCTACATTTGATGG-3′ (forward) and 5′-TTGTTTGTCTTTTTTCCTATCCCAG-3′ (reverse) for exon 3, 5′-CTCAACTTTGGAGCATCTTGGA-3′ (forward) and 5′-ACACGATTGGAGGCTATCCTGT-3′ (reverse) for exon 4, 5′-TTTCAAGCCTGTCTTCATAGTGATG-3′ (forward) and 5′-ATTATTTGGAACCTGTCATCTGCC-3′ (reverse) for exon 5, and 5′-CATTTCAGAGGCAGCAAAAAAGT-3′ (forward) and 5′-TTGCTTTCTGCCTTCAAAAACATA-3′ (reverse) for exon 6. PCR was carried out using 86 ng DNA (2 µl), 2.5 µl 10× buffer, 2 µl 2.5 mmol/L dNTPs, 1 µl each primer (forward and reverse), 16 µl distilled water and 0.5 µl Taq polymerase (Takara, Otsu, Japan). The mixture was amplified under the following conditions: initial denaturation for 3 min (94°C), followed by 30 cycles of denaturation for 60 s (94°C), annealing for 30 s (60°C), elongation for 60 s (72°C), with a final step of elongation for 10 min (72°C). The PCR products were separated by electrophoresis with 1% agarose gel, purified using Wizard SV Gel and PCR Clean-Up system (Promega, Madison USA) and directly sequenced with the same primers used for PCR.

Results

When he came to our attention, we found that he has been suffering from hearing disturbance, which lead us to investigate the *GATA3* gene in this patient. Direct sequencing of PCR products identified a novel heterozygous mutation, 432insG, in the *GATA3* gene. Figure 1 shows the position of this mutation. The mutation is considered to introduce a premature stop codon at exon 4 (K302X), which results in a loss of both zinc finger domains of the GATA3 protein.

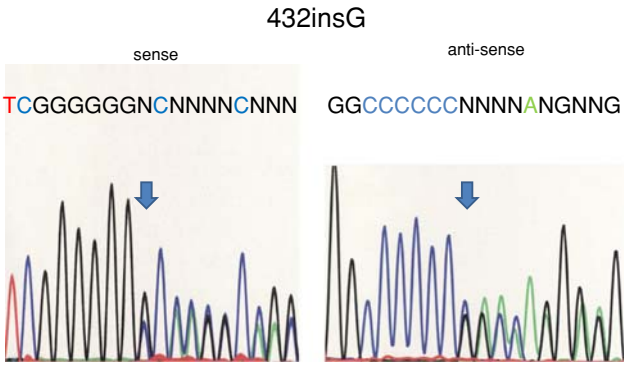

Fig. 1 Direct sequencing of PCR products for the *GATA3* gene revealed the novel insertion mutation in this patient. Both sense and anti-sense strands are shown. The *arrows* indicate the positions of the starting nucleotides that show heterozygosity in each sequencing

Discussion

More than 40 kinds of abnormalities in the *GATA3* gene have been reported in patients with HDR syndrome so far. These include missense and nonsense mutations, frame shift mutations and deletions, including at least some part of the *GATA3* gene [8, 19]. The insertion of guanine in codon 144 found in our patient is considered to change amino acids of both zinc finger domains which are coded by codons after 260 and result in early termination at codon 302 in this allele. Because several frame shift mutations around nucleotide 432 have been already reported in patients with HDR syndrome [19], the insertion of guanine in codon 144 seems to be a disease-causing mutation. We have not examined other genes that were reported to cause PTH-deficient hypoparathyroidism in this patient. However, because the mutation in the *GATA3* gene found in this patient is highly likely to impair GATA3 function, we speculate that it is extremely unlikely that this patient has mutations in other genes that cause PTH-deficient hypoparathyroidism, in addition to the *GATA3* mutation described here.

Clinical features in patients with HDR syndrome are reported to be quite variable even within the same family [14, 20]. In addition, the genotype–phenotype correlation has not been demonstrated in patients with HDR syndrome. This variability and lack of correlation between genotype and phenotype may be explained by compensatory or redundant functions of GATA proteins. The medical record of our patient indicated that he complained of hearing disturbance since he was 20 years old. However, it is uncertain whether his hearing acuity was not impaired before that time. Considering the congenital nature of HDR syndrome, it is reasonable to think that he had been suffering from hearing disturbance since infancy. Nonetheless, because he can communicate without difficulty, it is unlikely that this mutation caused a severe hearing disturbance in this patient.

GATA3 mutations have not been reported in patients with isolated hypoparathyroidism [19]. Actually, our patient showed a hearing disturbance in addition to hypoparathyroidism. Therefore, it would be impractical to screen mutations in the *GATA3* gene in patients with hypoparathyroidism who do not have hearing disturbance and/or renal abnormalities. On the contrary, we propose that it is necessary to assess the hearing acuity in patients with PTH-deficient hypoparathyroidism, because *GATA3* mutation may cause mild impairment of hearing as shown in our patient. In conclusion, we have described a novel mutation in the *GATA3* gene in a patient with PTH-deficient hypoparathyroidism.

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