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Japanese Cases of Type 1 Thanatophoric Dysplasia Exclusively Carry a C to T Transition at Nucleotide 742 of the Fibroblast Growth Factor Receptor 3 Gene

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Type I thanatophoric dysplasia (TD) is typically a lethal neonatal dwarfism, but a limited number of cases of type I TD cases survive more than one year, suggesting genetic heterogeneity. In this study, we analyzed the fibroblast growth factor receptor 3 (FGFR3) gene in 5 Japanese cases of type I TD with clinical symptoms ranging from lethal to long-survival. In every case, nucleotide sequence analysis of cDNA revealed a C to T transition at nucleotide 742 (C742T) in one allele of the FGFR3 gene, suggesting that type I TD is a rather homogeneous genetic condition, irrespective of clinical course. No association was found between C742T and C882T, although both nucleotides changes were from CpG dinucleotide in a near location.

Thanatophoric dysplasia (TD) is the most common form of sporadic lethal neonatal dwarfism, with an approximate incidence of 1 in 20 000 live births. TD is characterized by micromelic shortening of the limbs, relative macrocephaly with frontal bossing, reduced height of the vertebral bodies and shortened ribs resulting in a reduced thoracic cavity. From the pattern of bone deformity, TD has been divided into two types: type I TD patients have curved femurs with or without a cloverleaf skull, while type II TD patients always have a straight femur associated with a cloverleaf skull. Affected individuals generally die within minutes or days after birth, usually from respiratory failure. However, a limited number of cases of prolonged survival have been reported (1).

Fibroblast growth factor receptor 3 (FGFR3) is a cell surface protein consisting of extracellular, transmembrane and intracellular domains. It is particularly abundant in the cartilage growth plates (2). Mutations in different domains of the FGFR3 gene are responsible for a variety of chondrodysplasias ranging from very mild hypochondroplasia (3, 4) to lethal TD (5) and including chondrodysplasia (6, 7) and Crouzon syndrome (8).

Type II TD cases have been reported to have a single recurrent K650E change in the tyrosine kinase domain of FGFR3 (5). In contrast, type I cases have different mutations affecting either the extracellular or intracellular domains of FGFR3. However, mutations in the FGFR3 gene were identified in around only 60% of type I TD cases (5, 9, 10). These findings, and the range of symptoms observed suggest that type I TD is rather heterogeneous in genetic background.

We have followed a particularly interesting case of a Japanese type I TD for more than

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Abbreviations: TD, Thanatophoric dysplasia; FGFR3, Fibroblast growth factor receptor 3.

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9 years. This case may represent an extreme example of the clinically less severe course of type I TD that we considered might have been caused by a unique mutation. In order to identify the molecular abnormality in the index case and other four Japanese cases of type I TD, we analyzed the FGFR3 gene. Here, we report that all of the Japanese type I TD cases, including the index case, carry the same C742T mutation affecting the extracellular domain of FGFR3.

CASES AND METHODS

Cases and samples. Five Japanese type I TD cases were enrolled in this study. Though type I TD is usually lethal, one of our cases is still alive after 9 years. She (case K) was born to healthy parents after 36 weeks of gestation. Her elder brother is healthy. Her body weight at birth was 2904 gm and her height was 40 cm. She was intubated soon after birth because of respiratory failure and has been on the artificial ventilation ever since. She was diagnosed as type I TD on the basis of clinical findings and the presence of curved femurs, as revealed by radiology.

Another long-lived case is a $2\frac{1}{2}$ year old male who is also on an artificial ventilator. One type I TD case was stillborn and another two died within three weeks. All of these cases were diagnosed as type I TD because of their curved femurs and the absence of a cloverleaf skull. There was no apparent history of TD in any of their families. They were born in different parts of Japan spreading from Nagoya to Okinawa, separated by more than 1,000 km, and were unrelated.

Molecular analysis. Total RNA was extracted from four lymphoblastoid cell lines ($\sim 10^6$) and one autopsy sample (preserved at -80 C), and cDNA was synthesized as described before (11). DNA coding for the region between immunoglobulin like domains II and III (nt. 580 - 1018) in the extracellular domain of FGFR3 was amplified by the polymerase chain reaction (PCR) using the primer set described elsewhere (5). The resulting 439 bp amplified product was sequenced as described before (12).

The PCR amplified products from the type I TD cases and normal individuals were digested with *Bsi*HKAI restriction enzyme digestion as recommended by the supplier (New England Biolabs, Inc., Beverly, MA). The digested products were separated on a 4% agarose gel and stained with ethidium bromide.

RESULTS AND DISCUSSION

In order to identify the mutation in case K, we first focused our attention on the extracellular immunoglobulin-like domain of the FGFR3, since mutations affecting this domain have been detected in the majority of type I TD cases. A 439 bp-long region extending from nt 580 to

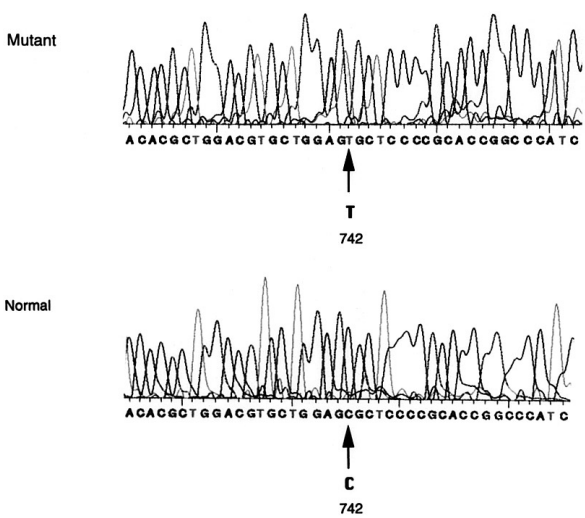

FIG. 1. Sequence of the DNA coding for the extracellular region between immunoglobulin-like domains II and III of FGFR3. The C at nt. 742 in the normal allele is mutated to T in the mutant allele. This mutation substitutes cysteine for arginine at 248th amino acid residue.

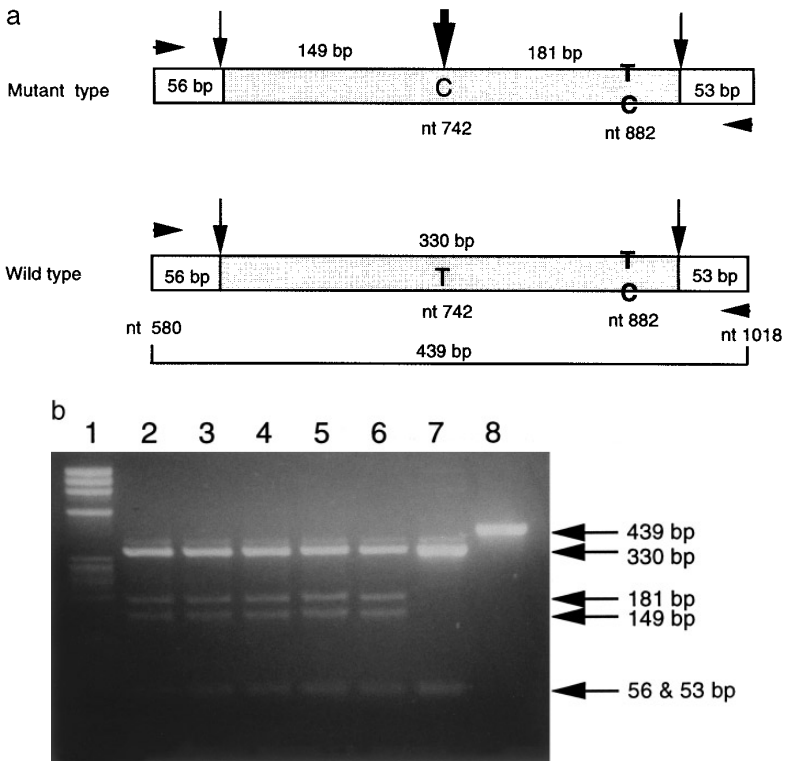

FIG. 2. BsiHKAI restriction digest analysis. (a) Schema of the recognition sites BsiHKAI in the DNA fragment. The wild sequence of the fragment (439bp) contains two sites for BsiHKAI, and digestion results in the appearance of three fragments of 330, 56 and 53 bp. The C742T mutation creates a third site for this enzyme, which therefore cuts the 330 bp fragment into fragments of 181 and 149 bp. A silent nucleotide change of T to C was identified at nt 882. The transverse arrowheads represent the position of the primers, vertical arrows represent BsiHKAI sites, thin= normal cutting sites, bold= novel cutting site due to the mutation. (b) Results of restriction enzyme analysis of amplified DNA from type 1 TD cases and normal individuals. Control cases produced two bands corresponding of 330 bp and the 56 and 53 bp fragments (lane 7). Four bands were observed after restriction of DNA amplified from type I TD individuals (lanes 2 to 6). Two were the same as the control (330 bp and the 56 and 53 bp) and the other two were the 181 and 149 bp products. Lane 1, size marker (*Hae*III-digested ϕx174 phage DNA). Lane 8 undigested product.

nt 1018 was amplified from cDNA prepared from a lymphoblastoid cell line. The size of amplified product matched that from the control (data not shown) indicating the absence of a major deletion or an insertion mutation.

When the amplified product was cloned and sequenced, two different clones were identified: one was the same as the wild-type and the other had a single nucleotide change, a C to T transition at nt 742 (C742T) (Fig. 1). C742T is located on the first nucleotide of codon 248 of FGFR3 and substitutes cysteine for arginine (R248C).

Surprisingly enough, C742T is the same mutation as that reported in nearly half of type I TD cases from the American and European continents (5, 9, 10). The C742T mutation created a novel restriction enzyme recognition site for the restriction enzyme *Bsi*HKAI (Fig. 2a), in addition to the two already present. Two bands (330 bp, and the mixture of 56 and 53 bp products) were visualized in all of 21 control samples after *Bsi*HKAI digestion (Fig. 2b, lane 7). However, when the amplified product from case K was digested with *Bsi*HKAI, five products were visualized as four bands (330, 181, 149 bp, and the 56 and 53 bp; Fig. 2b, lane

6), indicating that the case is heterozygous for the mutation. This is the first time that C742T in one allele of the FGFR3 gene has been shown to be responsible for Japanese type I TD.

Since the patient K was ranked as a very mild case of type I TD, amplified products from cDNA of the other four cases were also subjected to *Bsi*HKAI digestion. They all showed the same pattern of digestion as case K, i.e., heterozygous for C742T (Fig. 3, lanes 2,3, 4, and 5). This result suggests that type I TD is a rather homogenous condition, irrespective of race or clinical findings.

Similar to the universal occurrence of a G380R mutation affecting the transmembrane domain of FGFR3 in achondroplasia, the most common form of chondrodysplasia with a mild phenotype, some unknown mutagenic factors might make certain nucleotides in segments of genes coding for other functional regions of the protein particularly prone to mutation. The extracellular domain of FGFR3 is encoded by a C-G rich region of the gene. The CpG dinucleotide has been reported to be a mutation hot spot, because the C nucleotide is methylated and then deaminated to T (13). Since C at 742 is in CpG dinucleotide, this methylation reaction might be a predisposing factor for the C742T mutation.

Another nucleotide change was identified in the sequence of the amplified region of the same allele of case K: C to T at nt 882 (Fig. 2a). However, this nucleotide change did not induce a change at the protein level. C882T is also located in a CpG dinucleotide. Among all the amplified fragments sequenced, three of the 5 alleles harboring C742T also harbored C882T as did while 3 of the 5 normal alleles. From these data, it would appear that the mutations at C742 and C882, both of which could be due to methylation, did not occur at the same time even though they are located close to each other. Further analysis will be required to determine why the C742T mutation is common throughout the world.

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