Clin Pediatr Endocrinol 2006; **15**(2), 61-64

Original Article

Prevalence of Mutations in the FGFR3 Gene in Individuals with Idiopathic Short Stature

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Abstract. FGFR3 (fibroblast growth factor receptor 3) is a gene responsible for the most common form of osteodysplasia, achondroplasia, which results in extreme short stature. An allelic disorder, hypochondroplasia, however, presents with a much milder phenotype and is sometimes indistinguishable from idiopathic short stature. In this study, in order to test the possibility of the mildest end of hypochondroplasia being labeled as idiopathic short stature and the possibility of polymorphism of FGFR3 acting as one of the stature genes of normal individuals, we examined the prevalence of sequence alterations of the FGFR3 gene among individuals diagnosed clinically with idiopathic short stature. Sequencing analysis of all exons of the FGFR3 gene on 54 individuals with idiopathic short stature did not reveal any sequence variations related to the stature of the individuals. These results suggest that hidden hypochondroplasia among idiopathic short stature individuals is not a common occurrence and the contribution of polymorphism of the FGFR3 gene as a determinant of stature in normal individuals is small if any.

Key words: fibroblast growth factor receptor 3 (FGFR3), idiopathic short stature

Introduction

Short stature is defined as a stature more than 2 S.D. below the age-adjusted mean and is one of the most common reasons for visits to pediatric endocrinology clinics. Diverse etiologies of short statue have been identified and include endocrinological short stature caused by deficient secretion of growth hormone and/or thyroid hormone, excessive secretion of cortisol due to pituitary/adrenocortical tumors, or premature closure of growth plates caused by excessive secretion of sex steroids. Other causes include syndromic short stature such as chromosomal anomalies or bony dysplasias, psychological short stature such as deprivation dwarfism, or constitutional delay of growth and puberty (CDGP), which is considered a normal variant. However, a number of patients with non-endocrinological short stature still remain undiagnosed and are therefore collectively labeled as having idiopathic short stature, which probably is a mixture of entities mostly influenced by multiple genetic factors.

The degree of genetic influences on the stature of normal individuals has been assessed

Received: November 17, 2005
Accepted: February 1, 2006
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Table 1 Profiles of the subjects

	Male	Female	Total
N	32	22	54
Age (year)	8.7 ± 2.1	8.3 ± 1.9	8.6 ± 1.8
Height SDS	–2.4 ± 0.3	–2.5 ± 0.4	–2.5 ± 0.3

mean ± SD.

by previous studies, and the consensus has been that stature is a multigenic trait and about 70–80% of stature is determined by genetic factors (1, 2). So far, however, only a few genes have been identified as causes of so-called idiopathic short stature.

Genetic defect of the fibroblast growth factor receptor 3 (FGFR3) is known as a cause of achondroplasia, which is one of the most common forms of bony dysplasia (3). Adults affected with this condition are extremely short: Japanese male adults measure ~130 cm in height and females are ~120 cm in height. A milder mutation of the FGFR3 gene, however, leads to a much milder phenotype, hypochodroplasia (3). The clinical phenotype of hypochodroplasia is variable, and a fraction of patients present with features that are almost indistinguishable from individuals with idiopathic short stature (4). In this study, in order to test the possibility of the mildest end of hypochodroplasia being labeled as idiopathic short stature and the possibility of polymorphisms of FGFR3 acting as one of the stature genes of normal individuals, we examined the prevalence of sequence alterations of the FGFR3 gene among individuals diagnosed clinically with idiopathic short stature.

Subjects and Methods

Study subjects

Fifty-four Japanese individuals clinically diagnosed with idiopathic short stature (32 male and 22 female) were analyzed (Table 1). Diagnosis of idiopathic short stature was made by the following criteria: height below the mean minus 2 standard deviations of the Japanese height standard, normal physical findings excluding syndromic short stature or extreme nutritional problems, normal laboratory screening results which included complete blood counts, urinalyses, blood chemicals/electrolytes, serum IGF-I, free thyroxin, and TSH. In order to exclude individuals with CDGP, individuals with delayed bone age and apparent family history of CDGP or obvious delay of puberty were excluded.

In addition, to test for the prevalence of the Arg65Gly polymorphism among the Japanese population, 171 male adult volunteers with normal (mean ± 2 standard deviations) or tall (over 2 standard deviations) stature were recruited for the study. The studies were conducted with subjects' informed consents and the study protocol was approved by the institutional review board of Kyoto University Hospital.

Genomic DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes with the QIAamp DNA mini kit (QIAGEN, Germany). All 15 exons were then amplified together with exon-intron boundaries in 25 μl reactions containing 50 ng of genomic DNA, 10 mM Tris-HCL (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 25 pmol of each primer and 1 U of AmpliTaq Gold polymerase (Perkin-Elmer, MA) with initial denaturation at 94°C for 10 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55–60°C for 30 sec, and extension at 72°C for 30

sec. The sequences of the primers are available from the authors. The amplified products were then purified using the Wizard PCR Preps DNA Purification System (Promega, WI) and directly sequenced with the BigDye Terminator Cycle Sequencing Kit (Roche, Switzerland) using one of the primers used for amplification. They were then analyzed by using the ABI Prism 3100 Automatic Sequencer (Applied Biosystems, CA).

Results

None of the subjects had previously known mutations for hypochodroplasia: Asn328Ile (5), Ile538Val (6), Asn540Lys (7, 8), Asn540Ser (9) Asn540Thr (10), and Lys650Asn (11). A previously known SNP, Arg65Gly in exon 3, was identified in 6 patients, including 5 in the heterozygous state and 1 in the homozygous state. In order to test the possibility that this SNP affected the stature of the subjects, 71 subjects with normal height (within ± 2 S.D.) and 100 subjects with tall stature (over + 2 S.D.) were tested for the presence of Arg65Gly. Six out of 71 individuals with normal stature and 8 out of 100 individuals with tall stature tested were positive for this SNP, suggesting that the SNP is not related to the height of the subjects. No other mutations were found in any of the exons of the FGFR3 gene.

Discussion

Although it is widely accepted that human stature is a multigenic trait, at present, very little is known about the genes that determine the stature of normal individuals. The number of stature genes and the exact nature of those genes are both largely unknown. Since there are a number of monogenic disorders that result in extremely short stature, one possibility is that milder polymorphisms of those disease genes affect the stature of normal individuals. The short stature homeobox containing gene (SHOX), which was originally identified as a gene responsible for short stature in Turner syndrome and Leri-Weill dyschondrosteosis, might be one of those genes, since mutations in the gene have been identified in approximately 2% of patients with idiopathic short stature (12). In this study, we tried to replicate the investigation using another gene, FGFR3, which is responsible for a common form of osteodysplasia, achondroplasia. The results of our study, however, indicate that hidden hypochondroplasia among idiopathic short stature subjects is not a common occurrence and the contribution of the polymorphisms of the FGFR3 gene as a determinant of stature in normal individuals is small if any (less than 2% since we did not find any pathological mutations in our 54 short individuals). One possibility is that there are a number of stature genes and the contribution of each gene is so small that it escapes the power of detection of a study with this sample size. In addition to the candidate gene approach as described in this study, a more systematic approach to determine the presence and the chromosomal locations of the major stature genes using a genome-wide high-density association study might be necessary to identify more influential stature genes.

Acknowledgment

This work was supported in part by a research grant from the Novo Nordisk Growth Award.

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