CLINICAL AND MOLECULAR CHARACTERISTICS OF THAI PATIENTS WITH ACHONDROPLASIA

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Abstract. Achondroplasia is an autosomal dominant disorder characterized by disproportionately short stature, frontal bossing, rhizomelia, and trident hands. Most patients appear sporadically resulting from a *de novo* mutation associated with advanced paternal age. A glycine to arginine mutation at codon 380 (G380R) of the fibroblast growth factor receptor 3 gene (*FGFR3*) was found to be the most common cause of achondroplasia in various populations. We identified and clinically characterized 3 Thai patients with achondroplasia. In all of them, we also successfully identified the G380R mutation supporting the observation that this is the most common mutation in achondroplasia across different ethnic groups including Thai.

INTRODUCTION

Patients with short stature display an extremely long list of differential diagnoses. Achondroplasia is one of them. Clinical manifestations and molecular defects of patients with achondroplasia have been described in various ethnic groups. Here we report three Thai patients with achondroplasia whose molecular abnormalities were successfully identified, providing a specific method for molecular diagnosis of patients and for prenatal diagnosis in families at risk.

MATERIALS AND METHODS

Case reports: Three patients coming to the Genetics Clinic at King Chulalongkorn Memorial Hospital were diagnosed with achondroplasia. Patient 1 was born at term to a 37 year-old G3P2 Thai mother and a 43 year-old unrelated Thai father. Neither the parents nor the two elder sisters of patient 1 were affected. Pregnancy and delivery were uncomplicated. His birth weight was 3,590 g (+1 SD), length 47 cm (-2 SD), and head circumference 38.5 cm (+3 SD). In addition to short stature, physical examination revealed increased upper to lower trunk ratio (2.2:1) (normal 1.7:1), frontal bossing, rhizomelia, trident hands, left hydrocele, and lordosis (Fig 1A). Achondroplasia was diagnosed soon after birth. At 8 months of age, his head circumference was 49 cm (+4 SD). Due to the rapid increase of his head size, a CT scan of the brain was performed revealing hydrocephalus. A ventriculoperitoneal shunt was placed. Developmental assessment by the Gesell Developmental schedule showed a developmental quotient of 73 at the chronological age of 1 year and 8 months. The left hydrocele was surgically repaired at 1 year and 9 months. Polysomnography performed at 2 years and 6 months was normal. At 4 years and 6 months, growth hormone provocative tests by insulin and clonidine showed maximum growth hormone levels of 1.9 and 6.4 ng/ml, respectively, indicating growth hormone deficiency. The IQ test by WISC III revealed verbal IQ, performance IQ and full IQ of 84, 103, 93 respectively at 8 years of age. Radiography of the lumbar spine showed caudal narrowing

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of the spinal canal with short pedicles (Fig 2A). At his last follow-up at 8 years and 1 month, his height was 100.2 cm (-4 SD), weight 19.6 kg (-1 SD), and head circumference 56 cm (+2.5 SD).

Patient 2 was born at term to a 27-year-old G1P0 Thai mother and a 27-year-old unrelated Thai father. The parents were unaffected. Pregnancy, labor and delivery were unremarkable. His birth weight was 3,500 g and his length 47 cm. Physical examination at 4 months of age revealed macrocephaly with a head circumference of 43 cm (+2 SD), increased upper to lower trunk ratio (40:19.5 = 2.05:1), large anterior fontanel, frontal bossing, depressed nasal bridge, trident hands, and rhizomelia (Fig 1B). Radiography revealed decreased interpeduncular distances of his lumbar vertebrae. A diagnosis of achondroplasia was made. CT scan of the brain at 10 months revealed hydrocephalus requiring ventriculoperitoneal shunt. Developmental assessment by the Gesell Developmental schedule showed a mental age of 39 weeks at the chronological age of 79 weeks. The IQ test according to Stanford Binet revealed an IQ of 82 at 5 years of age. Echocardiogram performed at 2 years and an eye examination at 3 years were unremarkable. Noisy breathing was developed at the age of 5 years. Obstructive sleep apnea was found by polysomnography and his hypertrophied tonsils and adenoids were removed at the age of 5 years and 10 months. The following tests were normal: blood cell counts, blood sugar, BUN, Cr, electrolytes, prothrombin time, and partial thromboplastin time. At his last visit at the age of 6 years and 10 months his height was 99.3 cm (-2.5 SD), weight 31.4 kg (+2.5 SD), and head circumference 54 cm (+1.5 SD).

Patient 3 was born at term after uncom-

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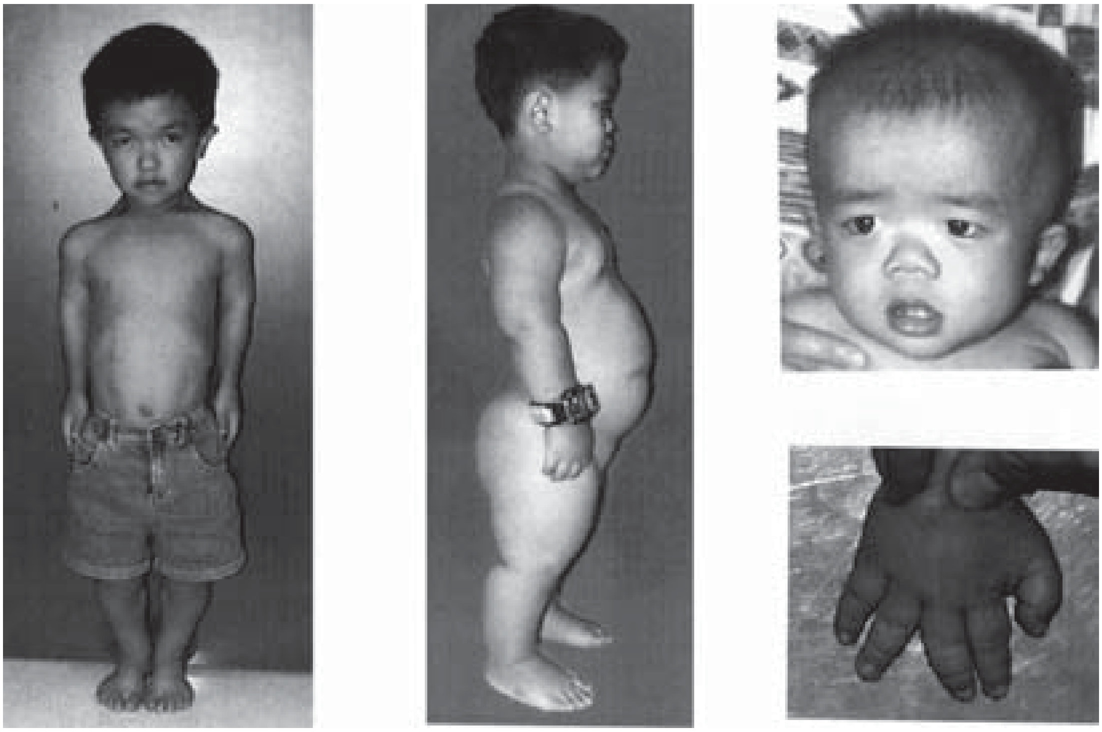

Fig 1–Clinical features. A. Patient 1 at 7 years of age showing disproportionate short stature with rhizomelia. B. Patient 2 at 6 years old revealing frontal bossing, overweight, and lumbar lordosis. C. Patient 3 at 11 months old showing maxillary hypoplasia (upper panel) and a trident hand (lower panel).

plicated pregnancy and delivery to a 36-year-old G3P2 Thai mother and a 39-year-old unrelated Thai father. Parents and the two elder siblings of patient 3 were unaffected. His birth weight was 3,400 g. Physical examination at the age of 11 months showed his weight at 6,900 g (-2.5 SD), length 62 cm (-5 SD), head circumference 47.5 cm (+2 SD), and arm span 58 cm. He had frontal bossing, midface hypoplasia, trident hands, kyphosis, and rhizomelia (Fig 1C). Developmentally, at 1 year of age, he could not sit unsupported but was able to do pincer grasp and talked a few words. Radiography of the spine revealed dextroscoliosis and narrowing of the interpeduncular distance of the lumbar vertebrae. Echocardiogram performed at 1 year of age was normal. CT scan of the brain at 1 year of age showed communicating hydrocephalus requiring lumboperitoneal shunt placement (Fig 2B).

Mutation analysis

After informed consent was obtained in accordance with the standards set by local institutional review boards, 6 ml of peripheral blood were obtained for DNA isolation by a standard method. *FGFR3* exon 10 was PCR amplified using the following two primers: 5′ CTC TGG GCC AGG GGA ATC CAT 3′ and 5′ GGCTGC AGA GAG GGC TCA CAC 3′. The PCR conditions were 30 seconds at 94ºC and 90 seconds at 68ºC for 35 cycles. The PCR products were digested with *SfcI* according to the manufacturer's specifications and electrophoresed on a 2% agarose gel (Promega) stained with ethidium bromide on preparation.

RESULTS

The PCR amplification was used to generate a 372 bp fragment. The 1138G→A transition of the *FGFR3* gene creates an *SfcI* restriction site. Hence, in the mutant allele, the 372 bp product is cleaved by *SfcI* into 234, 131 and 7 bp fragments. After digestion with *SfcI*, the PCR products of all three patients yielded 3 bands of 372, 234 and 131 bp. The expected

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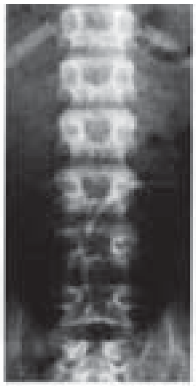

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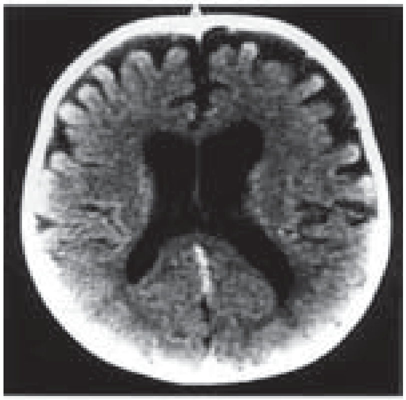

Fig 2–Imaging. A. Radiograph of lumbar spine of patient 1 at 7 years of age revealing caudal narrowing of the spinal canal and a shadow of a ventriculoperitoneal shunt. B. CT scan of the brain of patient 3 at 11 months old revealing hydrocephalus.

7 bp band could not be seen due to its small size. These results indicated that all of them were heterozygous for the 1138G→A transition.

DISCUSSION

Achondroplasia (MIM 100800), is the most common form of short-limbed dwarfism in humans. Its prevalence is estimated to be 1 in 20,000 (Stoll *et al*, 1989). The physical features evident at birth include frontal bossing, midface hypoplasia, rhizomelia, trident hands, genu varum, limitation of elbow extension, and exaggerated lumbar lordosis (Hall, 1992). The characteristic radiological features include caudal narrowing of the interpedicular distance (Oberklaid *et al*, 1979). We found

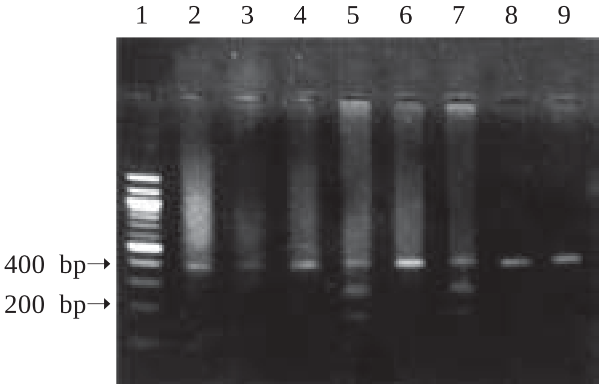

Fig 3–Restriction enzyme detection of the G380R mutation in achondroplasia. Lane 1 represents a 100 bp marker with the bands at 200 and 400 bp indicated with arrows. Lanes 2 and 3 were of the mother of patient 1; lanes 4 and 5 patient 1; lanes 6 and 7 patient 3; lanes 8 and 9 the mother of patient 3. Lanes 2, 4, 6 and 8 were PCR products without adding restriction enzymes and only the undigested 372 bp bands were presented. Lanes 3, 5, 7, and 9 were PCR products mixed with *SfcI*. The new bands of 231 bp and 134 bp in lanes 5 and 7 demonstrate that these individuals are heterozygous for the 1138G→A mutation. The products of their mothers in lane 3 and 9 were not cleaved by *SfcI*, which serves as negative controls.

3 patients with features typical for achondroplasia. In addition, they all have hydrocephalus requiring shunt placement to decrease the intracranial pressure. Ventriculomegaly in achondroplastic children was shown to accompany hydrocephalus, which is likely secondary to increased intracranial venous pressure due to hemodynamically significant stenosis of the jugular foramen and jugular venous obstruction at the level of the thoracic inlet (Steinbok *et al*, 1989). Patient 2 also had obesity. Obesity has been shown to be a significant health problem in achondroplasia (Hecht *et al*, 1988). Weight should be closely monitored and dietary intervention instituted whenever patients are overweight (American Academy of Pediatrics Committee on Genetics, 1995). All of our patients displayed noisy breathing, which is one of the known complications in achondroplasia (Stokes *et al*, 1983). Although delayed in early motor development, all of our patients showed intelligence within the normal range, consistent with most achondroplastia patients (Brinkmann *et al*, 1993).

Genetically, achondroplasia is inherited in an autosomal dominant fashion with complete penetrance (Tanaka, 1997). Eighty to 90% of cases are sporadic and associated with advanced paternal age (Stoll *et al*, 1989). After the gene had been cloned, molecular work has confirmed that mutations of the *FGFR3* gene in sporadic cases of achondroplasia occur exclusively on the paternally derived chromosome (Wilkin *et al*, 1998). All of our three achondroplastia patients are sporadic cases. The paternal ages of patients 1 and 3 were advanced (43 and 39 years).

Molecularly, the gene responsible for achondroplasia has been mapped to chromosome 4p16.3 (Velinov *et al*, 1994; Le Merrer *et al*, 1994; Francomano *et al*, 1994). Shortly after the gene had been mapped, the mutation of the fibroblast growth factor receptor-3 (*FGFR3*) gene was identified (Shiang *et al*, 1994; Rousseau *et al*, 1994). More than 99% of achondroplasia is caused by an *FGFR3* G380R mutation. Bellus *et al* (1995) found that 150 out of 154 unrelated patients showed the 1138G→A transition and 3 the 1138G→C transversion. Achondroplasia patients of other ethnic groups including Swedes, Chinese, Japanese, Jews and Arabs also have the most common mutations resulting in the G380R (Alderborn *et al*, 1996; Niu *et al*, 1996; Tanaka, 1997; Passos-Bueno *et al*, 1999; Katsumata *et al*, 2000; Falik-Zaccai *et al*, 2000). This study revealed that Thai achondroplasts also had the 1138G→A transition resulting in G380R as the most common mutation. Even though the patients are all sporadic reducing the recurrence risk to far below 50% in younger siblings of the patients, the risk is not negligible. Owing to advanced molecular techniques, a powerful method to perform prenatal diagnosis is now available to the parents.

In summary, we have identified three unrelated Thai patients with achondroplasia. They all display the 1138G→A mutation of the *FGFR3* gene supporting the observation that this is the most common mutation responsible for the phenotype across different populations.

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