Achondroplasia In Diverse Jewish and Arab Populations in Israel: Clinical and Molecular Characterization

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Abstract

Background: Achondroplasia is the most frequent form of disproportionate short stature, characterized by rhizomelic shortening of the limbs. This disorder is inherited as an autosomal dominant trait, although most of the cases are sporadic, a result of a *de novo* mutation. A recurrent glycine to arginine mutation at codon 380 (G380R) in the transmembrane domain of the fibroblast growth factor receptor 3 gene was found to cause achondroplasia among different populations. This is most uncommon in other autosomal dominant genetic diseases.

Objectives: To determine whether this mutation is also common among Jewish patients from diverse ethnic groups and among the Arab population in Israel.

Methods: We examined the G380R mutation (G>A and G>C transition) and the mutation G375C (G>T transition at codon 375) in 31 sporadic patients and in one family diagnosed clinically to have achondroplasia.

Results: We found the G>A transition at codon 380 in 30 of our patients and the G>C transition in one patient. We were not able to detect any of the three mutations in two patients with an atypical form of achondroplasia.

Conclusions: Our results further support the unusual observation that nucleotide 1138 of the FGFR3 gene is the most mutable nucleotide discovered to date across different populations.

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Achondroplasia (MIM 100800), the most common form of chondrodysplasia in humans, was the first skeletal dysplasia shown to be caused by a mutated FGFR gene [1–3]. The main clinical features of achondroplasia are disproportionate short stature, a large skull with an abnormal cranial base, mid-face hypoplasia with depressed nasal bridge, a marked lordosis of the spine, small hands and short fingers, and limitation of elbow extension [4]. Although achondroplasia is transmitted as an autosomal dominant trait with complete penetrance, more than 90% of the patients carry *de novo* mutations that are thought to be of paternal origin. The gene FGFR3, responsible for achondroplasia, was mapped and cloned [5–7], and two recurring mutations (G>A and G>C) – both resulting in Gly380>Arg substitution in the transmembrane domain of the FGFR3 protein – were reported shortly thereafter in the vast majority of patients across the world [1–3, 8–11]. A different mutation, G375C, was reported once in a Croatian newborn [12]. These data suggest that the mutation rate at nucleotide 1138 is probably much higher than the average mutation rate at CpG dinucleotide island (only 10^{-9} to 10^{-10} per nucleotide per generation compared to 1.9X10 in achondroplasia) [4,5,13].

To further characterize the causative mutations of achondroplasia in the unique population of Israel, we studied the transmembrane domain of the FGRF3 gene for mutations in 31 sporadic patients with achondroplasia and in one familial case. The patients were of Jewish and Arab ethnicity.

Materials and Methods

Patients

The study group comprised 31 unrelated sporadic achondroplasia patients and one family with an affected father and son. The patients' ethnic background was analyzed. Blood samples were collected from each patient in EDTA tubes, and genomic DNA was extracted by standard techniques [14]. All samples were obtained with an informed consent.

Polymerase chain reaction-based restriction analysis

Detection of the G380R and G375C mutations was carried out by PCR amplification of 500–1,000 ng genomic DNA with the primers flanking the transmembrane domain.

FGFR3 = fibroblast growth factor receptor 3

PCR = polymerase chain reaction

The PCR amplification was performed in 50 mM KCL, 10 mM Tris HCL (pH 9.0), 1.5 mM MgCl2, 0.1% triton X-100, 200 μM dTTP, d CTP, dGTP, dATP, 10% DMSO, 0.2 mg/ml bovine serum albumin and 25 pmol of each primer and 1.25 units Taq Polymeraze (Appligen, France) in a 25 μl volume. We denatured the DNA for 5 minutes in 95^{0}C and continued with 30 cycles each consisting of denaturation at 94^{0}C for 45 seconds, annealing at 65^{0}C for 1 min and elongation at 72^{0}C for 1 min. Termination of the reaction was at final elongation in 72^{0}C for 5 min.

For the detection of the 1138G to A mutation, 8 μl of the PCR reaction was incubated at 25^{0}C overnight with 2 units of the restriction endonuclease Sfcl (New England Biolabs, USA), followed by an addition of 2 units of enzyme in the morning incubated for 1.5 hours at 37^{0}C. For the detection of G to C transversion the PCR product was digested with 20 units of MspI (New England Biolabs) at 37^{0}C overnight. For the detection of the G to T transition at codon 375, the PCR product was digested with 10 units of the restriction endonuclease NlaIII (New England Biolabs) at 37^{0}C overnight. The digested products were electrophoresed on a non-denaturing 6% acrylamide gel (Long Ranger FMC, USA).

Amplification of the genomic DNA with these primers generated a 164 base-pair band product. Digestion with Sfcl produced two fragments of 109 and 55 bp respectively. Digestion with MspI produced two fragments of 107 and 57 bp respectively. Digestion with NlaIII was expected to produce 122 and 42 bp bands respectively in the mutant allele [9,12].

Results

Patients

The patients were mostly Jewish (24 of the 32, 75%); 11 were of Sephardic origin (Morocco, Iran, Iraq, Tunisia, Yemen and Algeria), and 8 were of Ashkenazi background (Eastern Europe). Three patients were of mixed ethnicity – one parent being Ashkenazi and the other Sephardic, one Indian Jew and one Ethiopian Jew. The non-Jewish patients included one Druze, three Christian Arabs, two Moslem Arabs and three Bedouins.

PCR-based restriction analysis

The G to A transition at codon 380, recognized by the restriction enzyme SfcI, was documented in 29 sporadic patients with achondroplasia and in the familial case as well (father and son). All patients were heterozygous to this mutation. The G to C transition at codon 380 was documented in one patient.

DNA of two patients did not cleave with any of the three restriction enzymes – Sfcl, MspI and NlaIII. Efforts to identify the mutation causing achondroplasia in these patients involved direct sequencing of PCR products corresponding to the transmembrane domain of the FGFR-3 gene.

bp = base-pair

Discussion

We report here the molecular analysis of 33 achondroplasia patients in Israel. Of these, 31 (94%) were found to carry either G>A or G>C transition at nucleotide 1138. The three mutations described in the literature were not detected in the remaining two patients.

The patients described here mirror the ethnic diversity of the population in Israel, with each of the different ethnicities in Israel represented among the patient group although not in the same proportion as in the general population. The Jewish as well as the non-Jewish population in Israel have previously proven to carry unique genetic characteristics with regard to specific genetic diseases that are common in some of the ethnic groups [15]. These characteristic mutations are found in some common genetic diseases, for example cystic fibrosis [16,17], Gaucher's disease [18], and unique haplotypes such as reported for the fragile X syndrome [19]. These differences are attributed to the fact that both the Jewish and non-Jewish communities in Israel originated from closed populations. These differences also emphasize the need to study the local population for its unique genetics whenever a new disease gene is cloned and a mutation characterized.

This is the first report of achondroplasia patients in Israel. Despite the extreme ethnic diversity, 31 of the 33 patients were found to carry the G380R mutation, as was found in other ethnic groups [1,2,9,20].

The results of this study will enable us to provide prenatal diagnosis of achondroplasia for families who are interested, and to try to diagnose achondroplastic fetuses that are found on prenatal ultrasound examinations to have short extremities but no family history of bone dysplasia. If the characteristic mutation is found, we will be able to differentiate between achondroplasia and other possible types of dwarfism, some of which might be lethal such as thanatophoric dysplasia, and provide accurate genetic counseling to the families.

Our results further support the unexpected observation of an autosomal dominant disorder such as achondroplasia, which is frequently caused by a new mutation – the result of a substitution of the same nucleotide of the transmembrane domain of the FGFR-3 gene – across different populations. Although this mutation occurs in the context of a CpG dinucleotide, known to be a hot spot for transition mutations and especially a change of a G:C bp to A:T bp, it still represents an extraordinary high mutation rate. Furthermore, unlike other dominant genetic disorders such as neurofibromatosis type I and osteogenesis imperfecta, in which the vast majority of cases are sporadic and each patient has a different mutation, the mutation causing achondroplasia is identical in most cases.

Re-examination of the two patients in whom the mutation causing the disease was not found showed them to be somewhat atypical of classical achondroplasia. The first, an Arab-Christian girl, was the third child of healthy

unrelated parents, a 39-year-old father and a 33-year-old mother. Her two brothers were healthy. The family history was unremarkable and the patient had been delivered normally at term. Birth length was 46 cm (-2 SD), weight 2,755 g (10th centile), and head circumference 33 cm (10th centile). The face appeared unremarkable and short limbs were only evident at age 6 months. A skeletal survey disclosed radiological changes consistent with those in achondroplasia, including trident hands, narrow thorax, some modification of the vertebral bodies, hypoplastic ilia with trident spurs of acetabulum, short radiolucent proximal femora and metaphyseal changes of long bones [Figure 1]. However, overall, the skeletal abnormalities were milder than those in typical achondroplasia. Apart from a relatively large head, her craniofacial structure was considered normal. At 6 months, length was 56 cm (-4 SD), weight 5,500 g (3rd centile), head circumference 43 cm (75th centile), and arm span 54 cm. Short limbs were quite evident at this age. The clinical and radiological manifestations in this patient were consistent with achondroplasia, but to a milder degree, particularly the normal facial structure and milder metaphyseal modification.

The second patient, also a girl, was the third child of healthy unrelated Jewish parents from India, a 36-year-old father and a 26-year-old mother. Her two brothers were healthy. The family history was unremarkable. The patient was found to have short femora at 34 weeks of gestation on fetal ultrasound, but was delivered normally at term. Birth length was 48 cm (3rd centile), weight 3,600 g (50th centile), and head circumference 36.5 cm (97th centile). The facial appearance resembled those seen in achondroplasia, with frontal bossing and mid-face hypoplasia. Short limbs were noted at birth, as well as a large head and craniofacial structure as seen in classical achondroplasia. At 11 months her length was 66.5 cm (-3 SD), weight 8 kg (10th centile), head circumference 47 cm (90th centile), and arm span 64 cm. The clinical manifestations in this patient, particularly the abnormal facial structure and rhizomelia, were indeed quite characteristic of achondroplasia. A skeletal survey disclosed radiological changes somewhat consistent with those in achondroplasia and very similar to those described for the first patient [Figure 2]. Overall, the skeletal abnormalities were milder than those in typical achondroplasia, particularly with regard to spondylar deformity and metaphyseal cupping, and therefore provoked some confusion in the clinical diagnosis. Molecular studies in both cases failed to demonstrate known mutations of the FGFR-3 gene in achondroplasia [21].

Nevertheless, we would argue that these two patients had achondroplasia because of qualitative and quantitative similarities to "typical achondroplasia" in radiological findings and dissimilarities to other disorders in the "achondroplasia family" [22].

In general, achondroplastic individuals exhibit very little, if any, phenotypic variability, and "atypical achondroplasia" is extremely rare [23,24]. However, these two cases and the other few from the literature warrant that achondroplasia include, in part, unusual phenotypic variants, and that further investigation of phenotype-genotype correlation in achondroplasia is required to both elucidate these issues and clarify the diagnostic confusion exemplified in our two patients. Such analyses would enable us to

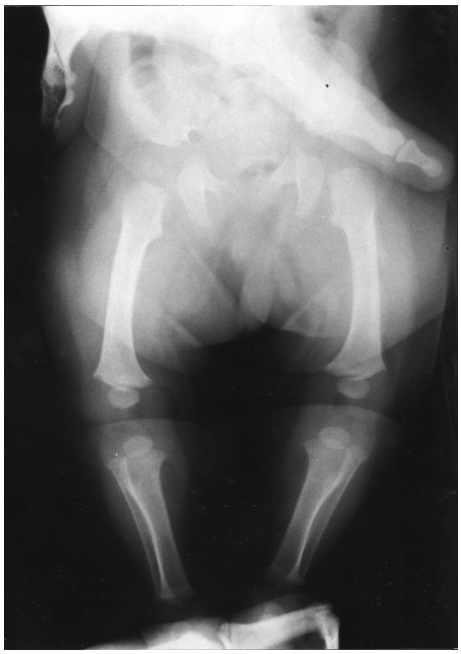

Figure 1. First patient as a neonate. Note hypoplastic/trident configuration to ilia, radiolucencies of proximal femora and metaphyseal changes of long bones, consistent with achondroplasia.

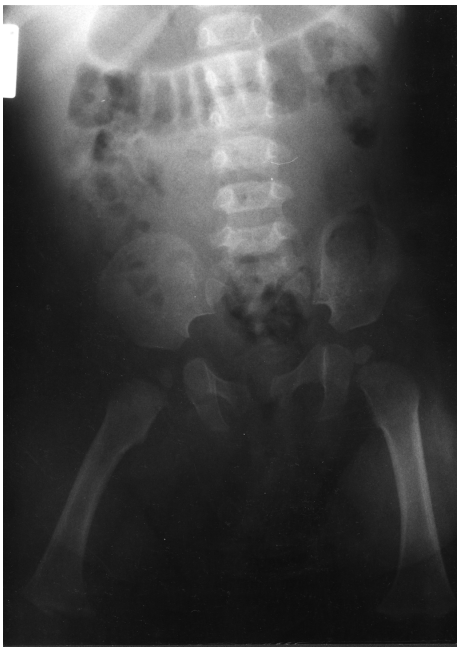

Figure 2. Second patient at 11 months. Note hypoplastic ilia with trident spurs of acetabulum, radiolucencies of proximal femora, and metaphyseal changes of long bones with mild metaphyseal cupping.

further understand the molecular basis and pathophysiology of this disorder and to characterize the local patient population. This can help physicians to reach conclusions that are of clinical significance, such as better genetic counseling and more accurate prenatal and postnatal diagnosis.

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Capsule

Polymerized bovine hemoglobin for autoimmune hemolytic anemia

Transfusions in patients with autoimmune hemolytic anemia are complicated by autoantibodies that react with erythrocytes from virtually all donors. In rare cases, transfusions cannot sustain the hematocrit at a level compatible with life because the autoantibodies rapidly destroy transfused red cells. The barrier presented by autoantibodies was circumvented in such a patient by the use of polymerized bovine hemoglobin for transfusion, and the patient eventually made a full recovery.

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