

Characterization of Mutations at Nucleotide 1138 of the Fibroblast Growth Factor Receptor 3 Gene in Filipino Patients with Achondroplasia

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ABSTRACT

Introduction. Achondroplasia is the most common form of short limbed dwarfism with a birth incidence between 1:7,500 and 1:70,000. In >97% of cases, this autosomal dominant disorder is associated with a G to A or a G to C mutation at nucleotide 1138 in exon 10 of the fibroblast growth factor receptor 3 (FGFR3) gene. Both mutations result in the substitution of a glycine (Gly) to arginine (Arg) residue at position 380 in the transmembrane domain of the FGFR3 protein.

Methods. To assess the presence of this mutation in 11 unrelated Filipino patients with achondroplasia, RFLP digestion of their PCR amplified genomic DNA was done. The PCR products were digested with the restriction enzymes *Sfcl* and *MspI* to determine the G1138A transition and the G1138C transversion, respectively.

Results. We report that ten of the 11 patients were heterozygous for the G to A mutation. Only one patient had the G to C mutation in the same position.

Conclusion. Majority of Filipino patients with achondroplasia have the same mutation most often defined in patients with achondroplasia from other countries. This further supports that the majority of patients with achondroplasia have a Gly to Arg substitution caused by a G to A change at nt 1138 of the FGFR3 gene.

Key Words: Achondroplasia, Filipino, FGFR3

Introduction

Achondroplasia (OMIM 100800) is the most common form of short limbed dwarfism in humans and is transmitted as an autosomal dominant trait with essentially complete penetrance.¹ It has an estimated birth incidence between 1:7,500 and 1:70,000.² The clinical features are distinctive at birth with diagnosis usually evident. The classic physical features include a characteristic facies with frontal bossing and midface hypoplasia, proximal shortening of the extremities, trident hand, genu varum, limitation of elbow extension, exaggerated lumbar lordosis and megalencephaly.^{3,4}

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Homozygous achondroplasia is usually lethal in the neonatal period and affects 25% of the offspring of matings between parents with achondroplasia.⁴ As is often the case among dominant traits, a high proportion of cases are new mutations (80-90% of all cases), but achondroplasia is unique in that the great majority are caused by either of two mutations at the same nucleotide in the transmembrane domain of the fibroblast growth factor 3 (FGFR3) gene. These mutations are the G1138A transition and G1138C transversion in exon 10 of the FGFR3 gene which result in a glycine (Gly) to arginine (Arg) substitution at codon 380 (G380R) in the transmembrane domain of the FGFR gene. It is among the most highly mutable single nucleotides known in the human genome and accounts for more than 97% of all reported cases of achondroplasia.^{1,5}

Achondroplasia is almost never detected on prenatal ultrasound before the third trimester. A retrospective study showed that 25% of achondroplasia patients were given an incorrect diagnosis of a lethal or very severe disorder, thus mutational analysis is a more effective method for prenatal diagnosis at the earliest gestational age possible since it allows better genetic counseling and avoids unnecessary terminations.⁷ The high degree of specificity of the FGFR3 G380R mutation for the achondroplasia phenotype thus has profound implications for patients, their families and their physicians.⁶

Molecular analysis of Filipino patients with achondroplasia has so far not been done. This study therefore aims to determine the presence of the G1138A transition and G1138C transversion in the FGFR3 gene of Filipino patients with achondroplasia using polymerase chain reaction-restriction length polymorphism (PCR-RFLP) analysis.

Methods

Subjects

Patients of Filipino descent with achondroplasia were identified and recruited with informed consent, from January 2004 to December 2007, through the Medical Genetics Clinics at the Philippine General Hospital and private physicians practicing in the Metro Manila area.

DNA amplification and mutation detection

DNA was isolated from whole blood using the

QIAamp®Blood DNA Midi Kit (QIAGEN, Hilden, Germany).

A 295-bp PCR product corresponding to exon 10 of the FGFR3 gene was obtained using 0.67 μ M of each of the primers flanking the exon (Forward: 5'-GCG CGT GCT GAG GTT CTG AG-3'; Reverse: 5'-GGA GAT CTT GTG CAG GGT GG-3' described by Wilkin et al 1998) and a 50 μ l reaction mixture containing 250 μ M dNTPs, 1x Titanium™ PCR Buffer (BD Biosciences, Palo Alto, California), 0.4x Titanium™ DNA Taq Polymerase (BD Biosciences, Palo Alto, California), and 200 ng genomic DNA. The reaction consisted of an initial denaturation at 95°C for 1 minute, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 70°C for 30 seconds, and extension at 72°C for 30 seconds, and a final extension at 72°C for 3 minutes. All PCR products were subsequently digested at 37°C for 4 hours with 5 units of *MspI* (New England BioLabs Inc., Beverly, Massachusetts) and 5 units of *SfcI* (New England BioLabs Inc., Beverly, Massachusetts) in separate reactions. The restriction fragments were separated by electrophoresis through a 2% agarose gel run in 0.5x Tris-borate buffer at 100 V, where the presence of the *SfcI* or *MspI* cutting sites produced 188-bp and 107-bp bands, indicating the presence of the G1138A transition or the G1138C transversion, respectively.¹⁰

Results

The 1138 G to A transition and G to C transversion created restriction sites (*SfcI* and *MspI*) in the FGFR3 transmembrane domain nucleotide sequence. These mutations were detected by PCR amplification of a 295-bp portion of FGFR3 (Figure 1) followed by digestion with either enzyme. Using this method, screening of genomic DNA from a collection of 11 Filipinos born to parents of average stature but presenting with clinical features associated with achondroplasia was done.

Confirmation of the G1138A transition was first analyzed. RFLP analyses of the 295-bp PCR products showed ten of the 11 patients (99%) to be heterozygous for the G1138A transition (Figure 2).

The PCR-RFLP technique based on *MspI* digestion of amplified DNA showed only one patient (1%) being heterozygous for the G1138C transversion (Figure 3).

Discussion

Achondroplasia is inherited as an autosomal dominant trait with essentially complete penetrance. Diagnosis of achondroplasia in the fetus is often made with certainty when one or both parents have this condition. The diagnosis is often first suspected late in gestation on the basis of long-bone foreshortening, incidentally discovered by ultrasonography. With the frequent use of ultrasonography, many cases of achondroplasia are first identified prenatally. However, disproportionately short limbs are observed in a heterogeneous group of conditions with prenatal diagnosis of many skeletal dysplasias difficult to make. Misdiagnosis

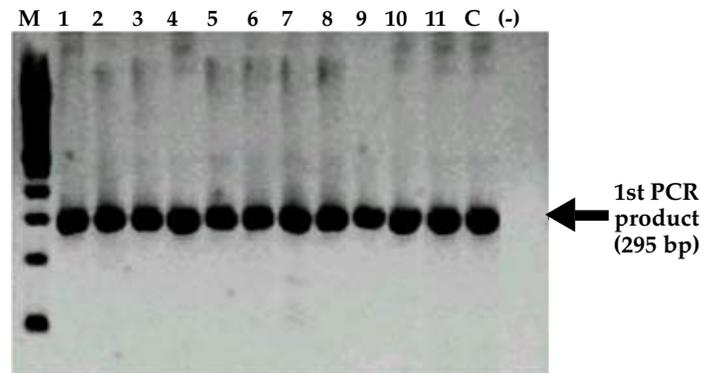


Figure 1. Undigested 295 bp PCR products corresponding to exon 10 of the FGFR3 gene of patients 1-11. M represents the 100 bp marker, lanes 1-11 represent patients 1-11, C represents the normal control.

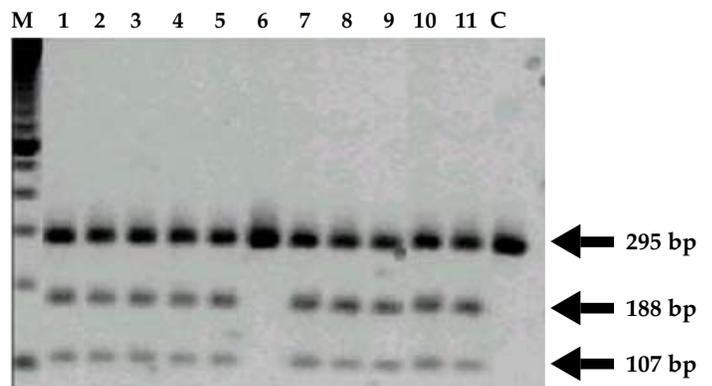


Figure 2. Confirmation of the G1138A mutation of FGFR3 in patients 1-11 by *SfcI* digestions of amplified genomic DNA. The undigested amplicon (lane 6) after *SfcI* digestion eliminates the possible G1138A mutation. Lanes 1-5 and 7-11 show the digested allele confirming the G1138A mutation (188 bp and 107 bp for *SfcI* digestion). M, 100 bp ladder; Lanes 1-11, patients 1-11 and C, normal control.

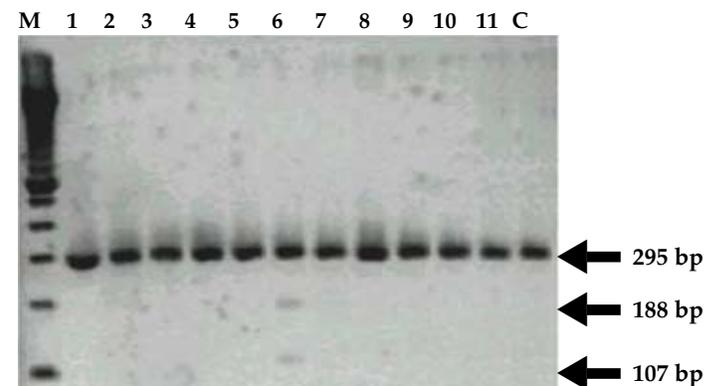


Figure 3. Confirmation of the G1138C mutation of FGFR3 in patients 1-11 by *MspI* digestions of amplified genomic DNA. The undigested amplicons (lanes 1-5, 7-11) after *MspI* digestion eliminates the possible G1138C mutation. Lane 6 shows the digested allele confirming the G1138C mutation (188 bp and 107 bp for *MspI* digestion). M, 100 bp ladder; Lanes 1-11, patients 1-11 and C, normal control.

and inaccurate prenatal counseling of families is then very possible.⁷

When both parents are of disproportionate short stature, the possibility of double heterozygosity or homozygosity for achondroplasia must be assessed. Some forms of double heterozygosity lead to life-threatening problems; infants with homozygous achondroplasia usually are stillborn or die shortly after birth.⁸

Achondroplasia is almost never detected on prenatal ultrasound before the third trimester. In the face of uncertainty, physicians sometimes elect to emphasize the most severe alternative diagnoses. Achondroplasia mutations are easily detectable by molecular means. By identifying mutations responsible for skeletal dysplasias, FGFR3 mutational analysis can be offered when a short-limb disorder is detected by ultrasound.⁹ If no such confirmation has yet been completed, caution should be exercised when counseling the family. Given the high degree of homogeneity of the achondroplasia mutation, prenatal diagnosis for the homozygous condition should be extremely reliable and readily available to all couples at risk. Molecular diagnosis is thus effective since this reduces the amount of incorrect and potentially harmful information provided to parents. The high degree of specificity of the FGFR3 G1138A mutation for the achondroplasia phenotype, therefore, has profound implications for persons with achondroplasia and their families.

Homozygous achondroplasia can be diagnosed prenatally with molecular testing of the fetus, by either chorionic villus sampling or amniocentesis. A pregnancy at risk of homozygosity should be followed with ultrasonographic measurements at 14, 16, 18, 22, and 32 weeks of gestation to distinguish homozygosity or heterozygosity from normal growth patterns in the fetus.⁹

One very positive outcome of the ability for molecular diagnosis is to provide couples at risk for children with homozygous achondroplasia with reliable prenatal diagnosis for the inevitably lethal condition. Additionally, as has been found with many genetic disorders in the past, understanding the physiology behind the achondroplasia family of disease, and other skeletal dysplasias, has the potential to help us understand the normal mechanisms of skeletal growth and development.⁶

Interestingly, there was some variation in the severity of the phenotypes observed despite the homogeneity of the mutations. The patients generally displayed the classical features associated with achondroplasia, including short stature, rhizomelic shortening of arms and legs, limitation of elbow extension, trident configuration of the hands, genu varum, thoracolumbar gibbus, exaggerated lumbar lordosis, large head with frontal bossing, midface hypoplasia and hypotonia. Some patients lacked as many as three of these features, while others presented with all of the features. Furthermore, no correlations could be drawn from the phenotypes of patients with the G1138A or G1138C mutations.

Conclusion

The most noteworthy implication of our data is that it extends and strengthens previous findings by Rousseau et al. (1994), Shiang et al. (1994), and Bellus et al. (1995), which imply that >97-99% of achondroplasia cases can be attributed to G380R mutations and that the 1138 G to C transversion accounts for >2%, despite inconsistencies in the severity of patients' phenotypes.

The common FGFR3 mutations causing achondroplasia both result in Gly380Arg amino acid substitutions. These results suggest that like other populations, the majority of, if not all, achondroplasia cases in Filipinos, can be traced to G380R amino acid substitutions in the FGFR3 gene.

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