Achondroplasia in Turkey is defined by recurrent G380R mutation of the FGFR3 gene

Sacide Pehlivan[1], Ferda Özkınay[2], Özlem Okutman[1], Özgür Çoğulu[2]
Ali Özcan[1], Tufan Çankaya[2], Ayfer Ülgenalp[3]

[1]Department of Molecular Biology, Faculty of Science and [2]Department of Pediatrics, Faculty of Medicine Ege University
and [3]Division of Genetics, Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey

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Achondroplasia, the most common form of skeletal dysplasia in man, has autosomal dominant inheritance and causes severe dwarfism. More than 90% of patients with achondroplasia have a G to A transversion or G to C transversion at position 1138 of the fibroblast growth factor receptor-3 (FGFR3) gene resulting in the substitution of an arginine for a glycine residue at position 380 (G380R) of the FGFR3 protein.

In this study, 12 unrelated Turkish patients with achondroplasia were evaluated for the G to A and G to C transversion at position 1138 of the FGFR3 gene. Eleven of 12 patients carried the G to A mutation heterozygously. None of the patients had the G to C mutation at the same position.

In conclusion, the vast majority of Turkish achondroplasia patients have the same mutation that has been most often defined in patients with achondroplasia from other countries. Our results give further support to the fact that the G380R mutation of FGFR-3 is the most common mutation causing achondroplasia in different populations.

Key words: achondroplasia, fibroblast growth factor receptor-3 gene, DNA, restriction endonuclease analysis.

Achondroplasia (ACH) is the most common form of skeletal dysplasia in humans. It is inherited in autosomal dominant fashion with a complete penetrance[1]. The combined frequency of the disease has been estimated to be 1 in 15,000 live births[2-4]. More than 80% of affected individuals have de novo mutations associated with increased paternal age[2-5]. Homozygous achondroplasia is usually lethal[4-6].

The gene responsible for ACH, fibroblast growth Factor receptor-3 (FGFR3), has been mapped to the short arm of chromosome 4 (4p16.3), and a predominant mutation, G to A transition at nucleotid 1138, has been found in more than 95% of the affected individuals[7-12]. A second mutation described in about 2% of ACH patients is G to C transition at the same position. Both mutations result in the substitution of an arginine for a glycine residue at position 380 of the FGFR3 gene[13,14].

In this study genomic DNA from 12 unrelated Turkish achondroplasia patients was evaluated for G to A and G to C mutations at nucleotide 1138.

Material and Methods

Blood samples from 12 unrelated patients with ACH aged between 2-23 years were obtained family history was not contributory. Parents of two patients were first-degree cousins but were normal[15].

To identify G to A and G to C mutations, 100 ng DNA isolated from lymphocytes was amplified with 5'AGGAGCTGGTGGAGGCTGA-3' and 5'-GGAGATCTTGTGCACGGTGG-3' primers according to polymerase chain reaction (PCR) conditions explained before[10,12]. SfcI (SfeI) and MspI digested the product of PCR, 164 bp. If an allele carries G to A mutation, then SfcI (SfeI)

digestion gives two-restriction fragments, 109 and 55 bp respectively. G to C mutation creates a restriction site for MspI and digestion results in 107 and 57 bp fragments. These enzymes do not cleave normal alleles. After checking PCR products on 2% agarose gel, 10 μl of each PCR product was incubated with 5 U SfcI and 10 U MspI separately at 37°C overnight. The products were electrophoresed on 8% polyacrylamide gel (500V, 2.5h)[10,12]. Silver stain was applied and
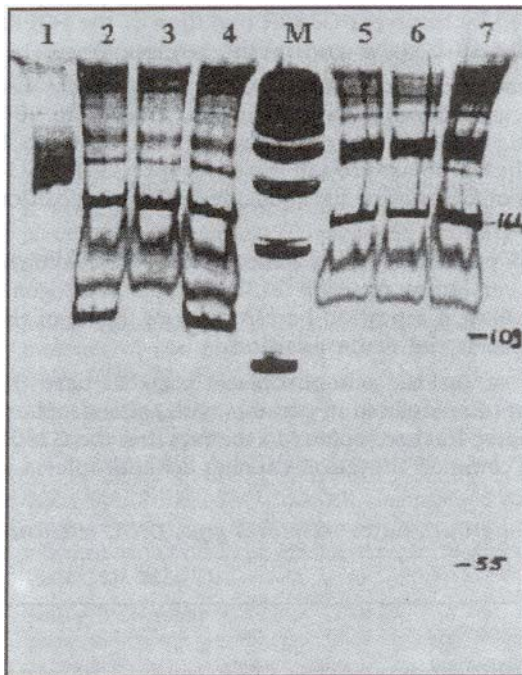
Fig. 1. SfcI (SfeI) and MspI digestion of PCR products. Nucleotide change G1138A creates SfcI (SfeI) digestion site whereas G1138C creates MspI digestion site.

Lane 1: Non-digested PCR product.
Lane 2: Heterozygous patient for G-A transition.
Lane 3: Patient who has no G-A transition.
Lane 4: Heterozygous positive control for G-A transition.
M: Marker (50 bp DNA stop ladder-promega, Cat# G4521).
Lane 5,6,7: Digestion of PCR product with MspI. None of the patients carry G-C transversion.

results were evaluated (Fig. 1).

In this study heterozygous positive control DNA was obtained from Aarhus University Hospital, Clinical Genetic Department, Denmak. Normal parents and sisters/brothers of patients were used as negative controls.

Results

Genomic DNA from 12 unrelated individuals (9 male, 3 female) with achondroplasia was studied for G to A and G to C mutations at nucleotide 1138 in the FGFR3 gene. Eleven patients of 12 were found as heterozygous for G to A transition. One patient had neither G to A transition nor G to C transversion. None of the patients carried G to C transversion. Neither mutations was detected in (–) control samples.

Both SfcI (SfeI) and MspI digestion of PCR products is shown in Figure 1.

Discussion

More than 90% of the patients with achondroplasia from different ethnic groups carry the G380R mutation resulting from G to A transition at position 1138 in FGFR3 gene[11,14,16,17]. Twelve Turkish achondroplasia patients studied here also showed this predominant mutation. Rarely, in 1% of the patients, it has been shown that G to C transversion at the same position caused G380R mutation[14]. None of our patients showed the second mutation. However, by increasing the number of patients, rare mutations may be found in the Turkish achondroplasia population. One patient in this report did not show either of the mutations investigated here. Recently a novel mutation of G to T transition at codon 375 has been reported, which resulted in substitution of a cysteine for a glycine[16]. This kind of mutation can be responsible for the clinical features in this patient and a more detailed investigation should be done.

Fibroblast growth factor receptors (FGFRs) have pleitropic effects on many different cell types at many stages of development. FGFR3 gene contains 19 exons and is one of the four FGFR genes, which show great homology but markedly different patterns of expression during development[18]. FGFR3 receptor has three domains: a big glycosylated extracellular region, a hydrophobic transmembrane region and an intracellular tyrosine kinase region. Two mutations involving nucleotide 1138 in achondroplasia patients are in the transmembrane domain of the FGFR3 gene[19]. FGFR3 is expressed in the articular chondrocyte. Experiments with transgenic mice showed that in ones with G to A mutation at nucleotide 1138 in the FGFR3 gene, chondrocyte proliferation and enchondral ossification were inhibited[20,21]. It has been established that FGFR3 mutations are responsible for many skeletal dysplasias

such as hypochondroplasia, thanatophoric dwarfism, and Crouzon syndrome. The site of the mutation, however, differs from that of ACH[19,22,23].

It is hard to amplify FGFR3 regions because these regions show a high rate of homology with other FGFR gene regions. To overcome this, heterozygous control DNA for G to A mutation was used in each PCR amplification and enzyme digestion. Because of the great homology at the molecular level, DNA analysis in ACH patients is an important tool to prove the clinical diagnosis and to plan care for the affected children. In the prenatal diagnosis of the fetus at risk of being homozygous for ACH, it is very important to identify the mutation because homozygous ACH is lethal.

In this report, a molecular defect was shown by DNA analysis in most of the individuals clinically diagnosed as ACH. More than 90% of Turkish ACH patients show the G1138A mutation as in other populations. ACH patients phenotypically show great similarities in all populations. Our results also support the argument that the more phenotypical similarities exist in a certain disease, the more the possibility of the same molecular defect.

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