Original Article

Detection of the G1138A Mutation in the *FGFR3* Gene for the Diagnosis of Achondroplasia by Allele-Specific Polymerase Chain Reaction

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Background: Achondroplasia is the most common inherited skeletal dysplasia caused by the G1138A (98%) mutation in the *FGFR3* gene. Detection of the mutation can be done by several methods such as DNA sequencing or restriction enzyme-based polymerase chain reaction (PCR).

Objective: We developed an allele-specific PCR (ASPCR) technique for the detection this mutation in the *FGFR3* gene that is more convenient, timesaving and cost effective than previous methods.

Materials and Methods: Genomic DNA from 7 patients with a clinical diagnosis of achondroplasia was extracted. The samples were analyzed using the new ASPCR technique and compared with restriction enzyme-based PCR and DNA sequencing.

Results: The G1138A mutation in the *FGFR3* gene was detected in all patients by the ASPCR technique, restriction enzyme-based PCR and DNA sequencing. However, the ASPCR technique had a shorter processing time and lower cost compared with the others.

Conclusion: ASPCR is a more rapid and convenient technique that is more cost-effective than either DNA sequencing or restriction enzyme-based PCR techniques for the diagnosis of achondroplasia.

Keywords: *FGFR3*, G1138A, Achondroplasia, Allele-specific PCR

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Achondroplasia is the most common inherited skeletal dysplasia that causes a disproportionate short stature in humans with prevalence of 1: 26,000 to 28,000 live births[1,2]. It is caused by missense mutations in the Fibroblast Growth Factor Receptor-3 (*FGFR3*) gene with 98% of cases caused by a G1138A mutation and the remaining 2% by a G1138C mutation[3]. These mutations cause a glycine to arginine substitution at codon 380 in the transmembrane domain of the FGFR3 protein that results in exaggerated physiological negative regulation of chondrocyte proliferation[4]. The classic physical features include disproportionate short stature, macrocephaly, frontal bossing, midfacial hypoplasia, rhizomelic shortening of the long bones, trident hand configuration and lumbar hyperlordosis[5]. Radiological findings include a small base of the skull, small cuboid vertebral bodies with short pedicles, narrowing of the lumbar interpedicular distance, thoracolumbar kyphosis with lumbar lordosis, small iliac wings, narrow greater sciatic notch, metaphyseal flare, short tubular bones and short femoral neck[6]. Although achondroplasia can be diagnosed by its clinical characteristic and radiological features, molecular analysis for the *FGFR3* gene mutations is still useful in patients who cannot be differentiated from hypochondroplasia. It is also necessary for confirming the diagnosis prenatally. Detection of a G1138A mutation in the *FGFR3* gene can be done by several methods, such as DNA sequencing, restriction enzyme-based polymerase chain reaction (PCR) or high-resolution melting analysis[3,7-10]. However, these procedures take several steps and are also expensive. Therefore, we aimed to develop an allele-specific PCR (ASPCR) technique for the detection of the G1138A mutation in the *FGFR3* gene that is more convenient than the previous methods, can save time and cost and yet produce a diagnosis of achondroplasia. This study was approved by the Ethics Committee in Human Research, Khon Kaen University, with the approval number of HE591329.

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Materials and Methods

This is a prospective study. Genomic DNA was

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collected from seven patients with a clinical diagnosis of achondroplasia. The genomic DNA was processed by 3 molecular techniques including restriction enzyme-based polymerase chain reaction, DNA sequencing and allele specific polymerase chain reaction for detecting of G1138A mutation in *FGFR3*.

DNA extraction

Genomic DNA collected from seven patients with a clinical diagnosis of achondroplasia was isolated from 3 mL of peripheral blood using isopropanol-fractionation with concentrated NaI and SDS technique as described in a previous study[12].

Restriction enzyme-based polymerase chain reaction

PCR was done using primers described by Shiang et al[7]. Primer A (5'-AGGAGCTGGTGGAGGCTGA-3') and primer B (5'-GGAGATCTTGTGCACGGTGG-3') as shown in Figure 1. The samples containing 100 ng of DNA were amplified in a 30 µl reaction mixture containing 10 mM Tris-HCl (pH 8.5), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 5 µM primer A, 5 µM of primer B and Taq DNA polymerase (GoTaq® colorless Master Mix, Promega®). PCR conditions were initial denaturation at 94°C for 5 min and then 30 cycles with denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, extension at 72°C for 30 seconds and 1 final extension cycle at 72°C for 5 min (nexus GSX1 Mastercycler®; Eppendorf®). The PCR product of 164 bp was subjected to restriction enzyme digestion. Ten µl of PCR product were digested with SfcI (New England Biolab) at 37°C for 1 hour. The fragments were separated by 2.5% agarose gel electrophoresis as shown in Figure 2.

DNA sequencing

PCR products were purified using GF-1 AmbiClean kit (Vivantis). All samples sequenced using an automated DNA sequencer, Applied Biosystems 3130/Genetic Analyzers to confirm the mutation. The sequencing reaction was performed as described in the kit's manual, BigDye Terminator v3.1 Cycle Sequencing kit.

Allele specific polymerase chain reaction (ASPCR)

The new ASPCR protocol for detecting the G1138A mutation of *FGFR3* was developed as follows. The *FGFR3* was amplified by using the set of oligonucleotide primers including primer A (5'-AGGAGCTGGTGGAGGC TGA-3'), primer B (5'-GGAGATCTTGTGCACGGTGG-3') as internal control and primer D that containing G→A mutation (5'-GCATCCTCAGCTACA-3') as shown in Figure 1. The samples containing 100 ng of DNA were amplified in a 20 µl reaction mixture containing 10 mM Tris-HCl (pH 8.5), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM primer A, 5 µM of primer B and primer D, and Taq DNA polymerase (GoTaq® colorless Master Mix, Promega®). PCR conditions were initial denaturation at 94°C for 5 min and then 30 cycles with denaturation at 94°C for 30 seconds, annealing at 63°C for 30 seconds, extension at 72°C for 30

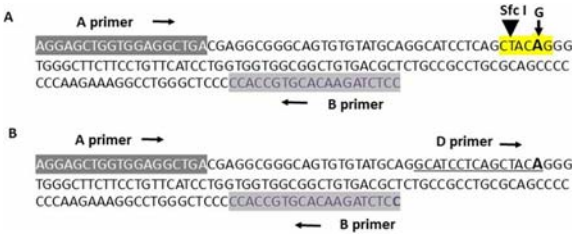

Figure 1. The diagram shows restriction enzyme-based PCR and allele-specific PCR. The conventional method of restriction enzyme-based PCR with A and B primers results in a 164 bp PCR product. If the G→A mutation is present, the PCR product digested by SfcI results in 109 bp and 55 bp fragments (A). The new allele-specific PCR (ASPCR) method with D primer that is specific for the G→A mutation at the 3' end results in 122 bp and 164 bp PCR products (B).

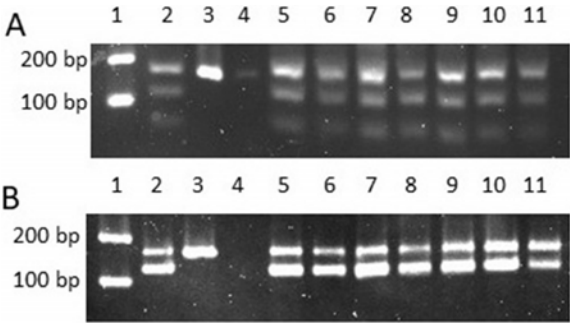

Figure 2. Agarose gel electrophoresis of amplification genomic DNA fragments surrounding the G1138A mutation in the FGFR3. A) restriction enzyme-based PCR technique. B) ASPCR technique. Lane 1, 100 bp ladder; Lane 2, positive control for heterozygous G1138A mutation; Lane 3, normal individual; Lane 4, distilled water; Lane 5 to 11, patients with achondroplasia (heterozygous for the G1138A substitution).

seconds and 1 final extension cycle at 72°C for 5 min (nexus GSX1 Mastercycler®; Eppendorf®). Ten µl of the PCR reaction mixture were analyzed on 2.5% agarose gel (AxyGEN®). After electrophoresis, the gel was stained with 0.5 µg/ml of ethidium bromide solution. The DNA in the gel was visualized under UV light and an image was taken with a CCD camera, ChemiDoc® MP Imaging System (BIO RAD). If the G→A mutationwas present, there were 2 PCR products with 122 and 164 bp fragments. The results of ASPCR were compared with those of the conventional method for detection of G1138A mutation[8].

Results

DNA samples collected from seven patients with a clinical diagnosis of achondroplasia were analyzed by ASPCR along with restriction enzyme-based PCR techniques. All samples were amplified by PCR with primer A and primer B to yield 164 bp products. For the detection with ASPCR, the primer D was additionally used to yield 122 bp products, while SfcI was used for the digestion of the 164 bp product using the restriction enzyme-based PCR technique to yield 109 bp and 55 bp products.

The overall findings of both techniques were compared as shown in Figure 2. ASPCR produced 164 and 122 bp fragments in individuals heterozygous for the G1138A substitution including samples in lane 2 (a positive control), 5, 6, 7, 8, 9, 10 and 11. A single band of 164 bp was detected in normal individuals including samples in lane 3 (a normal control). The results of ASPCR were compared with the method of restriction enzyme-based PCR. The restriction enzyme-based PCR produced 164, 109 and 55 bp fragments in individuals heterozygous for the G1138A substitution in lane 2 (a positive control), 5, 6, 7, 8, 9, 10 and 11. A single band of 164 bp fragment was detected in normal individuals including samples in lane 3 (a normal control). The DNA sequence for all patients and normal individual was analyzed and the results were confirmed as heterozygous for the G1138A substitution in all patients as shown in Figure 3.

Discussion

We successfully develop an ASPCR test as a rapid method to detect the *FGFR3* mutation G1138A in 7 achondroplasia patients in our hospital. This method was confirmed with the standard methods of restriction enzyme-based PCR and DNA sequencing. Basically, there are several techniques in regular practice to detect the G1138A mutation in the *FGFR3* gene, such as restriction enzyme-based PCR and DNA sequencing. The standard method that is used for detection in our laboratory is a restriction enzyme-based PCR technique. This technique requires 2 steps for analysis, DNA amplification followed by mutation detection. The mutation detection requires the digestion of an amplification product with the restriction endonuclease SfcI. This enzyme restricts the specific site of the mutant allele and produces 2 fragments of 109 and 55bp, but not for the wild type allele. Thus, an individual heterozygous for the G1138A mutation presents 3 fragments of 164, 109 and 55 bp, and the homozygous individual presents 2 fragments of 109 and 55 bp, but the normal individual presents only a fragment of 164 bp.

We developed a more rapid, convenient and timesaving technique by the ASPCR in our lab. This technique can be operated with a single DNA amplification, which easier than the previous more complex method. This technique can theoretically be used to confirm the diagnosis of a homozygous individual by presenting a single fragment of 122 bp, although this was not found in the patients presented in this study. This technique can save the time by eliminating the enzyme digestion step from the procedure. It also saves 665 Baht ($US 20.71) per assay by using this method compared with the restriction enzyme-based PCR and 1,805 Baht ($US 56.21) compared with DNA sequencing.

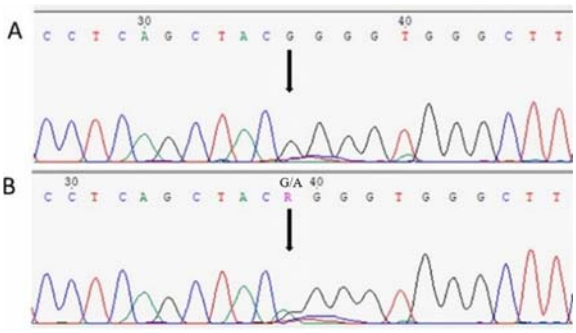

Figure 3. DNA sequencing data, the arrow in (A) shows no mutation was found at position 1138 in the FGFR3 gene in the normal control, the arrow in (B) shows a heterozygous G→A mutation at position 1138 in the FGFR3 in the patient.

Conclusion

ASPCR is a more rapid, convenient and cost-effective than either DNA sequencing or restriction enzyme-based PCR techniques for confirming the diagnosis of a G1138A mutation in the *FGFR3* gene that leads to achondroplasia.

What is already known on this topic?

Detection of the G1138A mutation in the *FGFR3* gene can be done by DNA sequencing and restriction enzyme-based polymerase chain reaction (PCR).

What this study adds?

The new ASPCR technique is more convenient, timesaving and cost-effective for the diagnosis of achondroplasia, especially in limited resource settings.

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Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Oberklaid F, Danks DM, Jensen F, Stace L, Rosshandler S. Achondroplasia and hypochondroplasia. Comments on frequency, mutation rate, and radiological features in

skull and spine. J Med Genet 1979;16:140-6.
2. Orioli IM, Castilla EE, Scarano G, Mastroiacovo P. Effect of paternal age in achondroplasia, thanatophoric dysplasia, and osteogenesis imperfecta. Am J Med Genet 1995;59:209-17.
3. Bellus GA, Hefferon TW, Ortiz de Luna RI, Hecht JT, Horton WA, Machado M, et al. Achondroplasia is defined by recurrent G380R mutations of *FGFR3*. Am J Hum Genet 1995;56:368-73.
4. Foldynova-Trantirkova S, Wilcox WR, Krejci P. Sixteen years and counting: the current understanding of fibroblast growth factor receptor 3 (*FGFR3*) signaling in skeletal dysplasias. Hum Mutat 2012;33:29-41.
5. Trotter TL, Hall JG. Health supervision for children with achondroplasia. Pediatrics 2005;116:771-83.
6. Langer LO Jr, Baumann PA, Gorlin RJ. Achondroplasia. Am J Roentgenol Radium Ther Nucl Med 1967;100:12-26.
7. Shiang R, Thompson LM, Zhu YZ, Church DM, Fielder TJ, Bocian M, et al. Mutations in the transmembrane domain of *FGFR3* cause the most common genetic form of dwarfism, achondroplasia. Cell 1994;78:335-42.
8. Lanning RW, Brown CA. An improved methodology for the detection of the common mutation in the *FGFR3* gene responsible for achondroplasia. Hum Mutat 1997;10:496-9.
9. Hung CC, Lee CN, Chang CH, Jong YJ, Chen CP, Hsieh WS, et al. Genotyping of the G1138A mutation of the *FGFR3* gene in patients with achondroplasia using high-resolution melting analysis. Clin Biochem 2008;41:162-6.
10. He X, Xie F, Ren ZR. Rapid detection of G1138A and G1138C mutations of the *FGFR3* gene in patients with achondroplasia using high-resolution melting analysis. Genet Test Mol Biomarkers 2012;16:297-301.
11. Liu J, Tang X, Cheng J, Wang L, Yang X, Wang Y. Analysis of the clinical and molecular characteristics of a child with achondroplasia: A case report. Exp Ther Med 2015;9:1763-7.
12. Wang L, Hirayasu K, Ishizawa M, Kobayashi Y. Purification of genomic DNA from human whole blood by isopropanol-fractionation with concentrated Nal and SDS. Nucleic Acids Res 1994;22:1774-5.