Original Article

Clinical Experiences of Molecular Genetic Evaluation of Achondroplasia in Prenatal and Neonatal Cases

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Purpose: The purpose of this study was to assess the characteristics of achondroplasia (ACH) diagnosed in fetuses or neonates and to evaluate the usefulness of a molecular genetic testing to confirm ACH.

Materials and Methods: The medical and ultrasonographic records of 16 pregnant women, who had molecular genetic testing for ACH performed on their fetus or neonate at the Cheil General Hospital between February 1999 and April 2013, were retrospectively analyzed. Detection of G1138A and G1138C mutations of the fibroblast growth factor receptor 3 (*FGFR3*) gene was accomplished by polymerase chain reaction - restriction fragment length polymorphism analysis.

Results: Of the eight fetuses and two neonates who were suspected of having ACH during pregnancy, four fetuses and one neonate was confirmed to have ACH and they all carried the heterozygous G1138A mutation. Out of 6 cases which had a history of ACH in prior pregnancies, three had genetic information for the previous fetuses while the other three did not. All six fetuses had no mutations at G380R. However, the one fetus of pregnant woman with non-confirmed ACH showed shortened long bone on ultrasound thereafter and the fetus was identified as having oto-spondylo-megaepiphyseal dysplasia after birth.

Conclusion: Korean patients with achondroplasia have the heterozygous G1138A mutation that is most commonly defined in other countries. Molecular genetic evaluations of ACH are helpful not only for establishing diagnosis but for appropriate counseling with subsequent pregnancies.

Keywords: Achondroplasia, Prenatal diagnosis, Ultrasonography, Fibroblast growth factor receptor 3 gene

Introduction

Achondroplasia (ACH, OMIM 100800) is the most common form of short limbed dwarfism, occurring at a frequency about 1 in 15000.[1,2] ACH is characterized by short stature with disproportionately short arms and legs, a large head, characteristic facial features of frontal bossing and mid-face hypoplasia, exaggerated lumbar lordosis, genu varum and trident hands.[3] ACH is inherited as an autosomal dominant trait with 100% penetrance. More than 90% of affected individuals have de novo mutations associated with increased paternal age.[4,5] The gene responsible for achondroplasia, fibroblast growth factor receptor

3 (*FGFR3*), has been mapped to the short arm of chromosome 4 (4p16.3), and a predominant mutation, a G to A transition at nucleotide 1138, has been found in more than 95% of the affected individuals.[6,7] A second mutation described in about 2% of achondroplasia patients is a 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 protein.[6-8]

Since prenatal ultrasound has become common in routine obstetric care and often includes measurement of the long bones, unanticipated findings of shortened long bone are detected sometimes. This finding will often be secondary to ACH, but the diagnosis of ACH by ultrasound is known to be difficult. For this reason, molecular analysis has been used for a definite diagnosis in utero during the last few years.

The aim of this study was to assess the characteristics of achondroplasia which is diagnosed in pregnant Korean women and to evaluate the usefulness of molecular genetic studies to confirm the diagnosis of ACH.

Material and Methods

We retrospectively reviewed the medical records of fourteen pregnant women and two neonates who had molecular genetic studies performed for the verification of ACH at the Cheil General Hospital between February 1999 and April 2013. The study was approved by the Ethics Committee of the Institutional Review Board at Cheil General Hospital.

Genomic DNA was extracted from cultured amniotic fluid, umbilical cord blood and peripheral blood using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. PCR was amplified using the primers of Shiang et al., F: 5′-AGGAGCTGGTGGAGGCTGA-3′, R: 5′-GGAGATCTTGTGCACGGTGG-3′. PCR-restriction fragment length polymorphism analysis analysis using the MspI and SfcI restriction enzymes was performed for detection of G-to-A and G-to-C substitution at the position of the *FGFR3* cDNA sequence that causes ACH.[7]

Results

In a total of 16 cases, ten patients including eight fetuses and two neonates underwent genetic studies due to suspicion of ACH and six pregnant women were tested due to suspicion of or confirmed ACH history in a prior pregnancy. Characteristics of

the study population and the number of patients according to indication of the genetic studies are shown in Table 1.

1. Pregnant women who underwent genetic evaluation due to suspicion of achondroplasia on prenatal ultrasound (n=8)

Two cases who suspected of ACH on ultrasounds at about 20 weeks' gestation found no mutation on G380R. Four out of 6 cases between 27 and 32 weeks' gestation were confirmed to have mutation on G380R with the G to A transition at nucleotide 1138. One out of the four cases without a mutation at G380R showed a normal infantogram after birth. However, case 5 showed a low nasal bridge and proptosis as well as shortened long bone after birth and was ultimately referred to a tertiary pediatric center (Table 2).

2. Neonates who underwent genetic evaluation due to suspicion of achondroplasia after birth (n=2)

Case 9 was confirmed to have the G to A transition at nucleotide 1138. Case 10 with short neck, scoliosis, and cryptochidism as well as shortened long bones had no mutation at G380R. The mother of case 10 had an obstetric history of an antecedent fetus with hand deformity, scoliosis, and anorectal agenesis with rectovaginal fistula (Table 3).

3. Pregnant women who had genetic evaluation performed due to suspected or confirmed history of achondroplasia in prior pregnancies (n=6)

Among 6 cases with a history of ACH in prior pregnancies, three had molecular genetic information for the previous fetuses while the other three did not. All six cases showed no mutations

Table 1. Clinical Characteristics of the Study Population

Characteristic	Mean±SD or N (%)
Characteristics of study population	
Maternal age (years)	33.2±2.4
Paternal age (years)	35.0±2.8
Diagnosis of ACH on ultrasound (week)	27.5±4.9
Indication for genetic evaluation	
Suspected ACH on ultrasound	8 (50%)
Suspected ACH on appearance after birth	2 (12%)
Prior history of confirmed ACH	3 (19%)
Prior history of suspected ACH	3 (19%)

ACH, achondroplasia; SD, standard deviation

Table 2. Patients Suspected of Having Fetuses with Achondroplasia on Prenatal Ultrasound

Case no.	Maternal age	Paternal age	Diagnosis (wk)	Test (wk)	Material for genetic study	Karyotype	G380R mutation	Remark (outcome of this pregnancy)
1	32	33	30	30	CB	Normal	Positive (G1138A)	-
2	31	32	30	30	CB	Normal	Positive (G1138A)	-
3	34	35	27	27	CB	Normal	Positive (G1138A)	-
4	37	38	30	30	AF	N/M	Positive(G1138A)	-
5	31	30	27	27	AF	Normal	Negative	Low nasal bridge, proptosis
6	34	36	32	32	CB	Normal	Negative	Normal by infantogram
7	36	37	20	20	AF	Normal	Negative	Follow up loss
8	35	34	20	20	AF	Normal	Negative	Ongoing pregnancy

AF, amniotic fluid; CB, cord blood; N/M, not measured

Table 3. Neonates Suspected of Having Fetuses with Achondroplasia on Appearance after Birth

Case no.	Maternal age	Paternal age	Previous pregnancy	G380R mutation	Karyotype	Diagnosis week of short long bone on antenatal ultrasound	Remark (Outcome of this pregnancy)
9	36	36	Normal	Positive (G1138A)	Normal	35	-
10	34	37	Multiple anomaly*	Negative	Normal	24	Short neck, Scoliosis Cryptochidism

*hand deformity, scoliosis, and anorectal agenesis with rectovaginal fistula

Table 4. Patients with Antecedent Pregnancy History of Achondroplasia

Case no.	Maternal age	Paternal age	Material for genetic study	Previous ACH	G380R mutation	This fetus		
						Karyotype	Sonography	Remark (Outcome of this pregnancy)
11	31	34	AF	Confirmed	Negative	Normal	Normal	-
12	35	41	AF	Confirmed	Negative	Normal	Normal	-
13	34	35	AF	Confirmed	Negative	Normal	N/M	Follow up loss
14	30	37	AF	Suspected	Negative	Normal	Normal	Preterm delivery, VSD, PDA
15	30	32	AF	Suspected	Negative	Normal	Normal	Bilateral club foot
16	36	37	AF	Suspected	Negative	Normal	Abnormal	Oto-spondylo-megaepiphyseal dysplasia

ACH, achondroplasia; AF, amniotic fluid; VSD, ventricular septal defect; PDA, patent ductus arteriosus; N/M, not measured

at G380R and normal karyotype by amniocentesis between 16 and 18 weeks of gestation. Case 16 was recognized as having shortened long bones on mid-trimester ultrasound and showed micrognathia, bilateral club foot and flexion deformity in bilateral hip, knee and elbow joint as well as mildly disorganized epiphyseal growth zone. These findings are consistent with oto-spondylo-megaepiphyseal dysplasia (OSMED). Another fetus showed bilateral club foot but he did not have any other abnormal findings (Table 4).

Discussion

We examined 16 patients who had molecular genetic testing performed for the diagnosis of ACH. Among these cases, eight pregnant women were suspected to have ACH by abnormal ultrasound findings and underwent this study prenatally. Ultrasound is the primary imaging modality used for the diagnostic evaluation of a fetus with skeletal dysplasia. Lethal forms of skeletal dysplasia are frequently distinguishable by ultrasound even from the 18th week of gestation by femurs angulated like a telephone receiver, but heterozygous achondroplasia is typically only suspected during third trimester ultrasounds after detection of shortened long bones. Gaffney et al. reported four cases detected at 31-37 weeks,[9] while Doray et al. reported seven fetuses detected at 28-35 weeks.[10] However, there is a recent report in which rhizomelic shortening of the long bones less than 2 standard deviations was diagnosed at 17 weeks of gestation in a fetus with ACH.[11] According to the report by Modaff et al., misdiagnoses of heterozygous ACH can result in inappropriate pre- and perinatal decision-making regarding both interventions for fetal indications and termination of pregnancy. Therefore

they recommended that *FGFR3* mutation analysis (as well as cytogenetic assessment) should be offered in instances where a short limb disorder is detected ultrasonographically.[12] In the current study, two cases involved suspected ACH at around 20 week' gestation but they were found to have no mutation at G380R. The rest of the six cases were recognized from 27 to 32 weeks and four of them (66%) were then confirmed by genetic testing to have ACH. Furthermore, all fetuses showed normal karyotype except one that underwent only molecular genetic testing. Hatzaki et al. reported that all nine ACH fetuses in their study were recognized after 27 weeks and the fetuses with confirmed ACH had normal biometric parameters at around 20 weeks' gestation.[13] These findings support our data. Therefore, if shortened long bone is recognized on ultrasound at around 20 weeks' gestation, one must consider other forms of skeletal dysplasia. In addition, we have presented two neonate cases that had suspected of having ACH on appearance after birth and one of them was confirmed to have ACH by genetic testing, while the other was not. In these cases, the fetuses had already shown shortened long bone on the prenatal ultrasound, but genetic evaluations had not been carried during the pregnancies. In summary, five of ten fetuses suspected of having ACH were genetically confirmed to have ACH and the other five fetuses were not. Among the latter five, one fetus showed a normal infantogram. However, two of the non-ACH fetuses were suspected of having other forms of skeletal dysplasia and they were referred to a tertiary center (case 5 and 10).

It used to be assumed that the recurrence risk for siblings of children with an autosomal dominant condition of high pene-trance, whose parents were normal, was close to twice the mutation rate.[14] However, the occurrence of ACH in siblings of achondroplastic children with normal parents appears to be rare. According to the data from 11 Canadian genetic centers, recurrence risk was estimated to be 1/443.[14] Our data also showed that all three pregnant women, who had a history of a previous ACH fetus, had no mutation in this pregnancy. For these three cases, we were able to identify the results of previous molecular genetic studies and they also showed the G to A transition at nucleotide 1138. In the literature, more than 95% of patients with ACH from different ethnic groups carry the G380R mutation resulting a G to A transition at position 1138 in the *FGFR3* gene.[6,7] We identified the molecular genetic results of 8 ACH patients, including 5 fetuses and 3 siblings, and all carried the G to A transition at position 1138. According to our data and a review of the Korean literature, the homogeneity of the point mutation at 1138 is also authentic in the Korean population.[15] There were another three patients with

suspected ACH in previous pregnancies, but they terminated their pregnancies without genetic evaluations and they didn't carry out autopsies, so ACH could not be confirmed in these cases. For these three, their molecular genetic studies of ACH and karyotype also showed no abnormal findings in this pregnancy. However, one of fetuses showed shortened long bone in the mid-trimester ultrasound and the fetus had additional characteristics of OSMED. OSMED is an autosomal recessive dwarfism disorder characterized by limb shortening, multiple skeletal and radiological abnormalities, mid-face hypoplasia with a flat nasal bridge, small upturned nasal tip, and sensorineural hearing loss.[14]

Since the features of skeletal dysplasia on ultrasound are similar, accurate diagnosis of ACH is difficult without genetic evaluation. However, according to our data, diagnosis of achondroplasia on ultrasound during the third trimester is somewhat accurate. Therefore, if it is not overused, the molecular genetic test of ACH might be reasonable. Also, pregnant women, whose fetuses are confirmed to have ACH through genetic testing, must be offered counseling for subsequent pregnancies, such as recurrence risk. Furthermore, we must keep in mind that some kinds of skeletal dysplasias other than ACH occasionally recur.

In conclusion, Korean patients with ACH have the same mutation that has been most often defined in other countries. When definite shortened long bone is recognized in the third trimester, ACH might be the most likely cause in Korean pregnant women. However, if patients have a history of conceiving a fetus with a skeletal dysplasia other than ACH or if the findings of shortened long bones were shown at a mid-trimester ultrasound, we must consider the possibility of other forms of skeletal dysplasia.

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