Rapid prenatal diagnosis of spinocerebellar ataxia type 3 by using fluorescent PCR

Do-Jin Kim[1] · So-Yeon Park[1] · Mi-Jin Kim[1] · Moon-Hee Lee[1]
Sung-Han Shim[1] · Hyun-Mee Ryu[1,2]

[1]Laboratory of Medical Genetics, Medical Research Institute
Cheil General Hospital and Women's Healthcare Center, Seoul, Korea
[2]Department of Obstetrics and Gynecology, Kwandong University College of Medicine, Seoul, Korea.

Spinocerebellar Ataxia Type 3 (SCA3) is a rare autosomal dominative disorder in which one of the neurodegenerative disorders is caused by a CAG repeat expansion on chromosome 14q32.1. The age at onset of disease is related to the size of the expanded CAG repeat. We present the prenatal diagnosis of SCA3 in a woman whose husband was known to carry an unstable CAG repeat expansion in the MJD gene. The diagnosis was made using PCR with a fluorescent probe for an expanded MJD allele. The normal ranges of (CAG)n of SCA3 are 14~38 repeats. The husband, who had a family history of SCA3, has an expanded allele of 69 CAG repeats with a normal allele of 27 repeats. His wife had two normal alleles with 26 and 32 CAG repeats. The fetus had two normal alleles with 26 and 27 CAG repeats; consequently, the baby was healthy. We report a case of prenatal diagnosis of SCA3 using a fluorescent PCR which is rapid and accurate.

Key Words : Prenatal diagnosis, Spinocerebellar ataxia Type 3, Fluorescent probes

INTRODUCTION

Autosomal dominant cerebellar ataxias (ADCAs) are progressive neurodegenerative disorders[1)]. Mutations at different loci have been identified in ADCAs and some of the responsible genes have been cloned, including spinocerebellar ataxia type 1 (SCA1) on chromosome 6p[2)], SCA2 on chromosome 12q[3, 4)], SCA3/Machado-Joseph disease (MJD) on chromosome 14q[5)], SCA6 on chromosome 19p[6)], and SCA7 on chromosome 3p[7)]. To date, at least 13 different loci causing SCAs have been genetically mapped.

The clinical features of SCA3/MJD are variable: gait problems, speech difficulties, clumsiness, progressive ataxia, hyperreflexia, nystagmus, and often visible blurring and diplopia[8)]. The age of onset is also variable but is usually in the second to forth decade. SCA3 is associated with an unstable expansion of CAG trinucleotide repeats contained in the cording region of the MJD (Machado-Joseph disease) gene on chromosome 14q32.1[5)]. The (CAG)n repeat is highly polymorphic and varies in normal individuals from 13 to 44 repeats, whereas SCA3 patients have a CAG expansion ranging from 53 to 84 CAG repeats[5, 9)]. A strong inverse correlation has been observed between the repeat expansion size and the age of onset[10)]. Contrary to most other dynamic mutations, there is a clear range of (CAG)n that separates normal and affected patients; there are no

Corresponding author : Ryu Hyun-Mee, MD, Ph.D.
Department of Obstetrics and Gynecology, Cheil General Hospital & Women's Healthcare Center, 1-19 Mukjeong-dong, Jung-gu, Seoul 100-300, Korea
Tel : +82-2-2000-7683, Fax : +82-2-2278-4574
E-mail : hmryu@yahoo.com

alleles between 41 and 60. Identification of a CAG repeats mutation has enabled prenatal diagnosis of this genetic disorder.

Molecular diagnosis using a fluorescent PCR is a fast and effective method for detection of the short-tandem repeats expansion disorders. In this case report, this is the first case of prenatal diagnosis using a fluorescent PCR for SCA3 in Korea, as far as we know.

CASE REPORT

A 26-year-old female, whose husband had a family history of MJD, was referred to a genetic clinic in the early stages of pregnancy. Her husband, who had a family history of SCA3, was previously identified as carrying a 69 CAG repeat expansion in the MJD gene. Her husband didn't have any symptom for SCA3. His father clinically presented a gait problem, difficulty in tandem walking, clumsiness, and distal muscle atrophy, but he declined DNA testing. Three of his sisters also showed symptoms. Two were identified as carrying expanded alleles of 69 and 78 repeats, but the other denied a testing (Fig. 1).

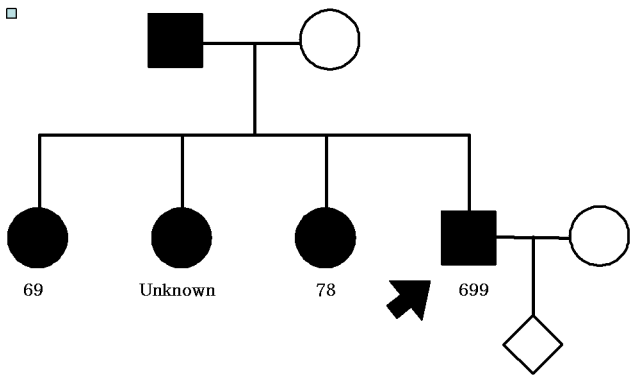

Fig. 1. Pedigree of the index family. Black/filled symbols represent the SCA3 patient and white/open symbols represent the unaffected family member. Number represents CAG repeats of the affected individual. The arrow indicates index case.

The couple decided the fetal diagnosis by amniocentesis after extensive genetic counseling. To investigate whether the fetus had an expanded allele, genomic DNA was isolated from amniotic fluid. Primers for amplification of a CAG repeat region of the MJD gene were obtained from Applied Biosystems, Netherlands. The forward primer for amplification, 5′ end-labeled with FAM (6-carboxyfluorescein), was 5′-CCAGTGACTACTTTGATTCG-3′ and the reverse primer was 5'-TGGCCTTTCACATGGATG TGAA-3′[5]. Fluorescent PCR production and size standard were analyzed by the ABI 3100-avant Genetic Analyzer (ABI PRISM Applied Biosystems, Netherlands) using POP-4™ as a polymer.

She was carrying normal alleles (26/32 repeats). Results of fluorescent-labeled PCR analysis of the mother, father, and fetus are shown in Fig 2. The father's expanded allele was confirmed (27/69 repeats). The fetal sample showed two normal signals of 26 and 27 CAG repeats.

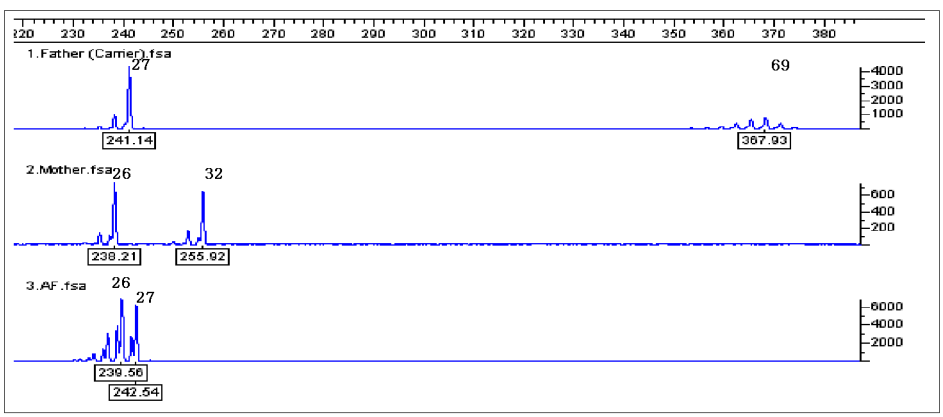

Fig. 2. Electrophenogram of the CAG trinucleotide repeat analysis for prenatal diagnosis. The length of the CAG repeats is represented on top of the electrophenogram in bp. The number of trinucleotide CAG repeats is shown next to the allelic CAG peaks.

DISCUSSION

CAG repeat expansion has been established in spinocerebellar ataxia (SCA) types 1, 2, 3, 6 and 7. In Korean patients, the most frequent SCA type was SCA2, followed by SCA3, SCA6, SCA1 and SCA7[11]. We obtained DNAs from amniotic fluid and blood for molecular genetic diagnosis.

To determine the exact number of CAG repeats, we used PCR with fluorescent probes. This method is fast and more safe and accurate than radioactive PCR analysis. We clearly genotyped the (CAG)n repeat of SCA3 after fluorescent PCR analysis and detection on the Genetic Analyzer ABI 3100-avant. The PCR product contained (CAG)n repeats and the size of the PCR product expected for (CAG)n repeats is 160 bp. Therefore, the size of repeats is (length of PCR product minus 160 bp)/3. We confirmed the father's expanded allele and the fetal sample showed two normal signals of 26 and 27 CAG repeats.

PCR amplification with fluorescent primers is more sensitive than non-radioactive PCR. The assay is also reliable when performed on minute amounts of DNA. Results can be obtained in 5-6 hours. These advantages make preimplantation genetic diagnosis (PGD) possible. There are several controversial ethical and legal issues in the prenatal diagnosis and termination of pregnancies of fetuses that carry mutations for late-onset genetic disorders. Ethical considerations of prenatal diagnoses of late onset diseases need to be discussed in Korea.

In conclusion, prenatal diagnosis of SCA3/MJD was successfully carried out from amniotic fluid using a fluorescent PCR, which is a rapid and accurate detection method for (CAG)n repeat expansions.

한글요약

척수소뇌성 실조증3는 신경세포의 손상으로 인해 생기는 질병으로 염색체14q32.1지역에 반복적인 CAG 삼염기 서열이 증가하면서 일어나는 것으로 알려져 있다. 본 증례는 척수소뇌성 실조증3으로 진단을 받은 부부에서 자연 임신한 태아를 산전진단한 경우로서 형광으로 포식된 표지자를 이용하여 CAG 지역을 증폭하여 빠르고 정확하게 반복수를 확인하는 방법을 이용하였다. 남편의 경우 CAG반복을 넘는 69개의 반복과 정상인 27개의 반복된 유전자를 갖고 있는 것으로 확인하였으며, 산모의 경우 정상인 26과 32개의 반복된 유전자를 갖고 있는 것으로 확인하였다. 태아는 부계의 27과 모계의 26개를 갖는 정상 유전자를 물려 받은 것으로 확인되어 건강한 아기를 분만하였다. 형광을 이용한 진단방법은 방사능을 사용하는 방법에 비해 안전하고 빠른 진단을 할 수 있으며 시료 채취 후 5-6시간 안에 정확하게 결과를 확인할 수 있는 방법이라 생각된다.

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