—**Report on Experiments and Clinical Cases**—

Genetic Diagnosis of Werdnig-Hoffmann Disease: A Problem for Application to Prenatal Diagnosis

Makoto Migita[1,2], Yohko Uchikoba[1], Hideo Orimo[2], Takashi Shimada[1,2], Tae Matsumoto[1],
Jun Hayakawa[1], Osamu Fujino[1], Makiko Saitoh[3] and Yoshitaka Fukunaga[1]

[1]Department of Pediatrics, Nippon Medical School
[2]Department of Biochemistry and Molecular Biology, Nippon Medical School
[3]Department of Pediatrics, Faculty of Medicine, the University of Tokyo

Abstract

We report a floppy infant with Werdnig-Hoffmann disease (spinal muscular atrophy: SMA type 1) and Klinefelter syndrome. After genetic counseling with parents, a genetic diagnosis using DNA from the infant's peripheral blood mononuclear cells was performed. The parents' deletion of exons 7 and 8 of the survival motor neuron (smn) gene and exons 4 and 5 of the neuronal apoptosis inhibitory protein (naip) gene were noted in the infant, so he was confirmed to have SMA type 1. The parents wanted to receive a prenatal diagnosis on the next pregnancy. However this genetic test is achieved by confirming that a specific band can not be detected by PCR. Therefore, this method should be applied with great care to prenatal diagnosis using chorionic villi, which may be contaminated with maternal tissue.
(J Nippon Med Sch 2003; 70: 45–48)

Key words: Werdnig-Hoffmann disease (spinal muscular atrophy type 1; SMA type 1), survival motor neuron gene; SMN, neuronal apoptosis inhibitory protein gene; NAIP, prenatal diagnosis, polymerase chain reaction; PCR

Introduction

Spinal muscular atrophy (SMA) is a genetic disorder of the motor neurons that causes muscular weakness and muscular atrophy, predominantly in the truncal muscles and proximal limb muscles. This is a neurodegenerative, autosomal recessive disease that is characterized by the degeneration of spinal anterior horn cells. Clinically, the disease is classified into types 1 to 3 according to the time of onset and the severity of symptoms. Type 1;Werdnig-Hoffmann disease (MIM 253300) manifests immediately after birth or within 2 month of birth as a floppy infant with a weak voice, difficulty in swallowing, and dyspnea due to generalized muscle weakness. Type 2; An intermediate type (MIM 253550) occurs from the age of 2 months to 2 years, and Type 3; Kugelburg-Welander disease (MIM 253400) develops at the age of 2 years or older as weakness of the proximal limb muscles and muscular atrophy, especially affecting the lower limbs [1]. In 1990, the gene involved in SMA types 1 to 3 was found to be located in region 5q12-13 by linkage analysis[2,3]. In 1995, Lefebvre et al. and Roy et al. reported the smn gene 4 and the naip gene 5, respectively, as

those responsible genes for SMA. Recently, prenatal diagnose of SMA type 1 have been described in some reports[6,7]. In the present report, a case of SMA type 1 in addition to Klinefelter syndrome is reported and the application to the prenatal diagnosis of SMA type 1 is discussed.

Case Report

A 2,546 g male infant was born by normal spontaneous vaginal delivery, with Apgar scores of 7 at 1 min and 9 at 5 min. He was 10 days old on admission. After birth, the baby showed poor spontaneous movement and was admitted to our department as a suspected floppy infant. On admission, atrophy of the proximal muscles was obvious and the baby adopted the frog position in the supine position. Deep tendon reflexes were almost absent. The baby had longer arms and legs than normal. Hematology tests, biochemistry tests, blood gases, urinalysis, and cerebrospinal fluid findings were within the normal range. Muscle biopsy showed neurogenic changes. The karyotype of the baby was 47, XXY, showing the baby to be complicated with Klinefelter syndrome. Since it was difficult to explain the neuromuscular symptoms in this baby on the basis of this syndrome, complication by another disease was suspected. Just after admission, bulbar palsy occurred and the respiratory status of this baby became worse. One week after admission, he was put on a ventilator. At the age of 1 month, tracheotomy was performed. From around that time, deformity of the hands and fingers as well as fasciculation of the tongue were noted. Based on these findings, Werdnig-Hoffmann disease was strongly suspected. To make a definitive diagnosis, we obtained the consent of the parents for genetic testing. We examined the smn and naip genes responsible for SMA located in 5 q 13. PCR single-stranded conformational polymorphism analysis (SSCP) was performed on exons 7 and 8 to detect abnormalities of the smn gene as previously described 8. Deletion of a band which was present in the control and the parents was observed in the baby (**Fig. 1**). PCR-RFLP (restriction fragment length polymorphism) was performed on exon 8 using Dde I,
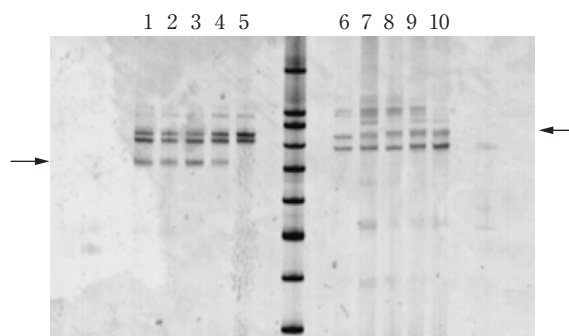

Fig. 1   SSCP analysis of SMN exon 7 and 8
The arrows mark the SMN bands.
Exon 7: Lane 1 and 2; healthy individuals, Lane 3; father, Lane 4; mother and Lane 5; affected baby
Exon 8: Lane 6; affected baby, Lane 7; mother, Lane 8 father, Lane 9 and 10 healthy individuals
Deletion of a band was observed in the affected baby, respectively.
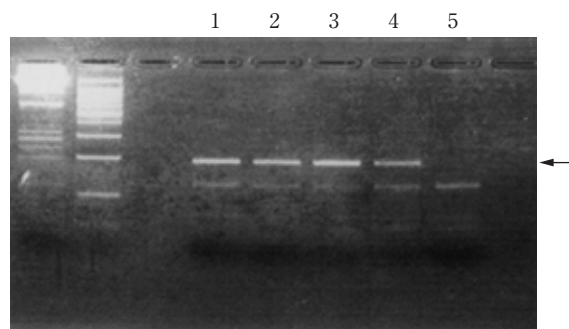

Fig. 2   PCR-RFLP of SMN exon 8 using Dde I
Lane 1 and 2; healthy individuals, lane 3; father, lane 4; mother and lane 5; affected baby. Dde I digests the smn pseudogenes amplified by PCR, but not the functional smn gene. No band of functional smn gene exon 8 was seen in the affected baby.

which digests the smn pseudogenes amplified by PCR, but not the functional smn gene. In the affected baby, the band indicating the presence of exon 8 was not observed, confirming the deletion of this exon (**Fig. 2**). Standard PCR was performed to detect abnormalities of the naip gene[6]. When exons 4 and 5 (plus exon 12 as a control) were amplified by PCR, the amplification of exon 4 and 5 was not observed in the baby (**Fig. 3**). Based on these findings, there was complete deletion of exons 7 and 8 in the smn gene as well as exons 4 and 5 in the naip gene. Finally the baby was confirmed to have SMA type 1 in addition to Klinefelter syndrome.
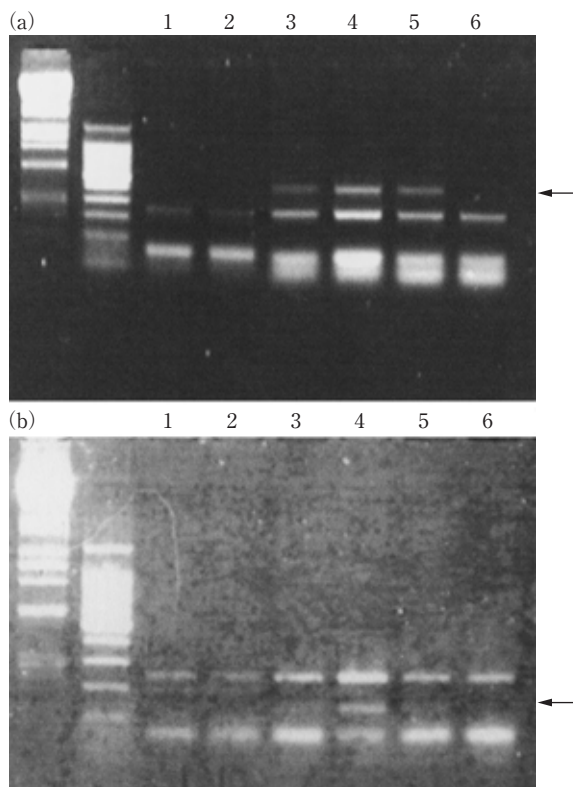
(a)

(b)

Fig. 3 PCR amplification of NAIP exon 4 and exon 5
　(a) exon 4 and exon 12 as a control
　(b) exon 5 and exon 12 as a control
　Lane 1 and 2; other patients diagnosed as
　SMN I, lane 3; healthy individuals, lane 4;
　father, lane 5; mother and lane 6; affected
　baby
　The affected baby had a deletion in both
　exon 4 and exon 5

Discussion

The most common mutation in SMA is deletion of exons 7 and 8 in the smn gene, and the deletion of exons 4 and 5 of naip, which is frequently observed in severe SMA type 1. Thus, it is considered that an abnormality of the smn gene induces the disease, and naip gene abnormalities determine the severity 7,8,9. Accordingly, genetic diagnosis in many cases of SMA, including assessment of the severity, can be achieved by investigating abnormalities in these 4 sites. In the present study, we extracted DNA from the peripheral blood of the parents and the baby by the method of Saitoh et al. ,and examined deletion of exons 7 and 8 in the SMN gene and exons 4 and 5 in NAIP. We found deletion at 4 sites in this baby.

SMN type 1 was diagnosed from these findings.

Recent technical progress in molecular genetics has allowed genetic diagnosis and even prenatal diagnosis of some hereditary diseases with serious symptoms. In genetic diagnosis, a sample containing DNA is collected directly from the blood cells, mucous membranes, or skin of an affected baby. However, in prenatal diagnosis, the DNA of a fetus is collected by amniocentesis or by sampling of chorionic villi. Employing chorionic cells has many advantages compared with amniotic cells. Firstly, the diagnosis can be performed as early as weeks 9 ～11 of gestation. Secondly, a large amount of cells and DNA can be obtained to carry out PCR and DNA analysis. In this study, SMA was diagnosed by the absence of particular bands using PCR, since the technique of chorionic villi sampling has the disadvantage of contamination with maternal cells, which may lead to false-positive prenatal diagnosis. To overcome this disadvantage, quantitative PCR may be one strategy to evaluate tcontamination by a few maternal cells[9]. Another option is the use of cultivated amniocytes. Because contamination with maternal cells occurs less commonly than with chorionic villi, prenatal diagnosis of SMA type 1 by DNA analysis using cultivated amniocytes was evaluated. However, the most obvious disadvantage is the absence or poor growth of amniocytes, which may be inadequate to perform the DNA analysis.

At this point, prenatal diagnosis using chorionic villi should be performed with great care, as they may be contaminated with maternal tissue.

References

1. ZerresK, Rudnik-Schonenborn S: Natural history in proximal spinal muscular atrophy. Clinical analysis of 445 patients and suggestions for a modification of existing classifications. Arch Neurol 1995; 52: 518–523.
2. Brzustowicz LM, Lehner T, Castilla LH, Penchaszadeh GK, Wilhelmsen KC, Daniels R, Davies KE, Leppert M, Ziter F, Wood D, Dubowitz V, Zerres K, Hausmanowa-Petrusewicz I, Ott J, Munsat TL, Gilliam TC: Genetic mapping of chronic childhood-onset spinal atrophy to chromosome 5 q 11. 2–13. 3. Nature 1990; 344: 540–541.
3. Hahnen E, Forkert R, Marke C, Rudnik-Schoneborn S, Zerres K, Wirth B: Molecular analysis of candidate genes on chromosome 5 q 13 in autosomal recessive

spinal muscular atrophy: evidence of homozygous deletions of SMN gene in unaffected individuals. Hum Mol Genet 1995; 4 : 1927–1933.

4. Lefebvre S, Burglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud M, Millasseau P, Zeviani M, LePalier D, Frezal J, Cohen D, Weissenbach J, Munnich A, Melki J: Identification and characterization of the spinal muscular atrophy determining gene. Cell 1995; 80: 155–165.

5. Roy N, Mahadevan MS, McLean M, Shutler G, Yaraghi Z, Farahani R, Baird S, Bersner-Johnston A, Lefebvre C, Kang X, Salih M, Aubry H, Tamai K, Guan X, Ioannou P, Crawford TO, de Jong PJ, Surh L, Ikeda J-E, Korneluk RG, MacKenzie A: The gene for neuronal Apotosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. Cell 1995; 80: 167–178.

6. Melki J, Abdelhak S, Burlet P: Prenatal prediction of Werdnig-Hoffmann disease using linked polymorphic DNA probes. J Med Genet 1992; 29: 171–174.

7. Stipoljev F, Sertiae J, Latin V, Rukavina-Stavljeniae A, Kurjak A: Prenatal diagnosis of spinal muscular atrophy type 1 (Werdnig-Hoffmann) by DNA deletion analysis of cultivated amniocytes. Croat Med J 1999; 40: 1427–1432.

8. Saitoh M, Sakakihara Y, Kobayashi S, Hayashi Y, Yanagisawa M: Correlation between deletion patterns of SMN and NAIP genes and the clinical features of spinal muscular atrophy in Japanese patients. Acta Paediatr Japon 1997; 39: 584 589.

9. Wirth B, Herz M, Wetter A, Moskau S, Hahnen E, Rudnik-Schoneborn, Wienker T, Zerres K: Quantitative analysis of survival motor neuron copies: Identification of subtle SMN 1 mutations in patients with spinal muscular atrophy, genotype phenotype correlation, and implications for genetic counseling. Am J um Genet 1999; 64: 1340–1356.