A Novel Deletion Mutation in the NF1 Gene in a Taiwanese Patient with Neurofibromatosis Type 1

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Neurofibromatosis type 1 (NF1) or von Recklinghausen neurofibromatosis is one of the most common autosomal dominant disorders in humans, primarily affecting cells of neural crest origin and resulting in developmental, pigmentary, and neoplastic abnormalities. NF1 affecting 1 in 3500 individuals and fully penetrant. Mutation detection is complex due to the large size of NF1 gene, the presence of pseudogenes and the great variety of lesions. Here we presented a 20-year-old female patient who had NF1 manifestation in school age and gene analysis revealed a novel deletion mutation (263delA).

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Key words: Neurofibromatosis 1 (NF1), von Recklinghausen neurofibromatosis, NF1 gene, Cafe-au-lait macules

第一型神經纖維瘤又稱為von Recklinghausen neurofibromatosis，是人類中最常見的體顯性遺傳疾病之一，最初影響神經嵴細胞，最終導致發展、色素及惡性變化等異常。NF1發生率約1/3500且一定會有臨床表徵。因為NF1基因很大，又有假基因及病灶有極大的變異性，使得突變基因的偵測有很高的困難度。我們在此報告一個20歲的女性患者，在求診時開始有NF1的表現，由基因突變分析發現一個新的單一核苷酸缺失突變(263delA)。(中華皮誌 24: 190-193, 2006)
INTRODUCTION

The neurofibromatosis (NF) is a heterogeneous set of genetic disorders having clinical manifestations that involve the skin, the nervous system or both. At least two distinct autosomal dominant disorders have been definitely recognized. The most common type of NF is neurofibromatosis type 1 (NF1; MIM# 162200) (85% patients), which should be distinguished from neurofibromatosis type 2 (NF2) (10% patients).

Neurofibromatosis type 1 (NF1), formerly known as Von Recklinghausen neurofibromatosis, is a common genetic disorder affecting approximately 1 in 3500 people. The condition appears to be fully penetrant but has a highly variable expression, even within families. Diagnosis is based on the clinical criteria recommended by an NIH Consensus Conference, which include multiple cafe-au-lait spots, cutaneous or subcutaneous neurofibromas, plexiform neurofibromas, axillary or inguinal freckling, optic gliomas, and iris Lisch nodules. Complications occur in some patients and include learning difficulties or mental retardation, focal neurological deficits, dysplastic skeletal lesions, hypertension, and, rarely, malignancy. The disease is fully penetrant and exhibits a mutation rate some 10-fold higher than that reported for most other disease genes. As a consequence, a high number of sporadic cases (up to 50%) are observed. Most disease features are present in more than 90% of patients at puberty.

The disease is caused by mutations in the NF1 gene, one of the largest human genes, composed of 60 exons and spanning more than 300 kb of genomic DNA. Due to the large number of coding exons and the considerable mutational heterogeneity, the determination of the NF1 mutational spectrum has been complex. In the present study we performed mutation analysis of NF1 gene in a Taiwanese family.

HISTORY

This 20-year-old female patient was quite well in the past. Multiple cafe-au-lait macules and neurofibromas, axillary freckles were developed during school age. Examination of the skin revealed areas with pigmented alterations consisting of numerous 1-5 mm sized light-brown macules in a speckled distribution, as well as several larger cafe-au-lait macules. In her family, only patient's mother has the similar skin lesions.

GENE ANALYSIS AND RESULT

The NF1 genemapes to chromosome 17q11.2. It spans a region of about 350kb of genomic DNA and contains 60 exons. Phenotype expression of individual patients in the same family can be uniquely different. Therefore gene mutation analysis was per-
formed with PCR amplification and autonomous sequencing.

Genomic DNA was extracted from peripheral blood of the patient and family members with informed consent. DNA samples were then subjected to mutation screening by amplification of segments of NF1 gene spanning all the gene using primers synthesized on the basis of intronic sequences (GenBank accession no. M82814.1 and NT010799.1). The first base (position +1) of the initiator methionine is taken as the start of the cDNA.

For PCR amplification, approximately 200 ng of genomic DNA, 12.8 pmol of each primer, 10 μM dNTP and 1.25 U of AmpliTaq Gold (Perkin Elmer, Roche Molecular Systems, Inc., Branchburg, New Jersey, USA) were used in a total volume of 50 μl. PCR and sequencing were performed over all exons. Only one deletion mutation was found over exon 3.

The NF1 primer sets used for exon 3 were 5'- CTG GGA GGT AAA ATG GAA GA and 5'- CTC TTG GTC CAC ATC TGT AC -3'. The amplification conditions were 94°C for 5 min, followed by 35 cycles of 94°C for 45 second, annealing temperature 55°C for 45 second and 72°C for 45 second, and extension at 72°C for 10 min. The PCR product (289 bp) was examined on 2% agarose gel. The PCR product was subjected to direct automated sequencing (377 ABI Advanced Biotechnologies, Columbia, MD).

Direct sequencing revealed 263delA in exon 3, which resulting frameshift and premature termination of codon (PTC + 15 aa) (Fig. 3).

**DISCUSSION**

NF1 is an autosomal dominant disease with variable clinical manifestations. Phenotype expression of individual patients in the same family can be uniquely different. The highly variable clinical features of NF1 are attributed to the face that NF1 mutations involve different functional domains of the protein and that other modifier genes could also affect the disease manifestation.

Mutation detection in NF1 has been made difficult by the large size of the gene, the existence of a number of homologous pseudogene sequences spread throughout the genome, and the lack of defined mutational hotspots. The most common transcript codes for a polypeptide of 2818 amino acids called neurofibromin. Analysis of the protein sequence of neurofibromin revealed striking similarity with the catalytic domain of proteins that function as negative regulators of Ras guanosine triphosphate (GTP)ase proteins. Loss of NF1 gene expression results in absent neurofibromin Ras GAP function, which leads to increased Ras activity and results in increased cell proliferation and tumor formation. To date, more than 709 NF1 gene mutation had been reported in the literature (http://archive.uwcm.ac.uk/uwcm/mg/search/120231.html). Frameshift and nonsense mutations that would be expected to truncate the reading frame constituted about 50% mutations detected. In this case, we detected a novel deletion mutation (263delA) in exon 3 which resulted in premature termination of codon (PTC+15aa). Mutation analysis in families with NF1 allows for genetic counseling and prenatal diagnosis.
REFERENCES