Nonsense mutations at Arg-1947 in two cases of familial neurofibromatosis type 1 in Japanese

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Abstract. We report two familial cases of NF1 presenting as C to T transitions changing an Arg-1947 codon to a stop codon. In one of the two families, cosegregation of the mutation with NF1 was demonstrated, indicating this mutation causes the disease in this family. As the same mutation at Arg-1947 has been reported previously in three cases of unrelated Caucasians (two are sporadic; the origin of the other is not reported), the codon at Arg-1947 (CGA) in the NF1 gene is considered to be a hotspot common among different ethnic groups and also among familial and sporadic cases.

Introduction

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen's neurofibromatosis, is an autosomal dominant disorder affecting about 1 in 4000 individuals in all ethnic groups. So far, few cases of NF1 have been reported presenting with abnormalities in the NF1 gene (reviewed by Gutmann and Collins 1993). In the present study, we describe nonsense mutations at Arg-1947 (numbering based on Marchuk et al. 1991) in two Japanese families shown to be unrelated by using a new polymorphic restriction fragment length polymorphism (RFLP) marker within the NF1 gene. We also show that the NF1 messages from the mutant allele and from the normal allele are transcribed in an Epstein-Barr (EB) virus-transformed lymphoblastoid cell line from one of the patients.

Materials and methods

NF1 patients

Twenty-five unrelated Japanese NF1 patients were studied; 15 had a family history of the disease and 10 were sporadic.

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Fig. 1. Top Pedigree of the family of case 1.
a Direct genomic sequencing of exon 31;
b EcoRI RFLP detected by probe GE2
(Marchuk et al. 1991); c direct sequencing of
the RT-PCR product of exon 31 from a lympho-
blastoid cell line of one patient. The location of
the mutation in a and c is denoted by arrows

by Reyniers et al. (1993). The frequency of the two-allele
polymorphism with bands at 7.3 kb (A1) and 4.2 kb (A2)
were 0.48 and 0.52, respectively, when estimated from 80
unrelated Japanese individuals.

In order to find more subtle abnormalities in the NF1
gene, we performed PCR-SSCP analysis of exon 31. Two
out of 25 patients (cases 1 and 17) showed bands of altered
mobility. The nucleotide sequence of patient DNA
revealed the transition of the codon CGA for Arg-1947 to
the stop codon TGA in both cases. Case 1 was a 40-year-
old male and case 17 was a 25-year-old female, both of
whom had a family history of the disease and presented
with multiple neurofibromas and café-au-lait spots. Other
abnormalities, such as malignant tumors, were not found
in either of the two cases or members of their families. All
other family members of case 1, including both parents
and three siblings, were studied for the C to T transition at
ARG-1947 to confirm that this mutation is responsible for
their NF1 (Fig. 1a). The perfect coinheritance of the non-
sense mutation at Arg-1947 and NF1 in case 1 family indi-
cates the importance of the mutation for the pathogene-
sis of NF1. The affected siblings of case 1 showed homo-
zygosity for A2, whereas the father was a heterozygote
for A1 and A2 (Fig. 1b). This indicates that the mutation
is on the A2 allele. On the other hand, case 17 is homozy-
gous for A1 (data not shown), indicating that the mutation
is on the A1 allele and that the two families are unrelated.

Although the same mutation has been reported indepen-
dently in three cases of NF1 (Cawthon et al. 1990; Estivill
et al. 1991; Ainworth et al. 1993), our cases are the first
described in an ethnic group other than Caucasians. In ad-
inclusion, the present study is the first report describing fa-
milial NF1 cases presenting with the nonsense mutation,
because two out of the previously reported cases with the
same mutation were sporadic and the origin of the other
case was not reported. The C at the codon for Arg-1947
(CGTA) is the only hotspot so far detected in the NF1 gene.
The frequency of this mutation in our Japanese NF1 pa-
tients is 8.0% (2 in 25 patients), whereas that in Cau-
casians is 1.1%, estimated from the data from the previous
three reports (3 in a total of 158 patients) and that of
Upadhyaya et al. (1992) (none in 110 patients). Further
study should clarify whether the frequency of this muta-
tion at Arg-1947 is different among different ethnic
groups.

Finally, we showed that the NF1 gene on the normal
and mutant allele are both transcribed in a lymphoblastoid
cell line derived from case 1 (Fig. 1c). If no other muta-
tions reside in the coding region of the mutant allele, the
mutant message encodes abnormal neurofibromin that
lacks its carboxy one third (Arg-1947 to Val-2818), but
that still preserves the GTPase-activating protein-related
domain (GRD). It will be of great interest to study the bio-
chemical and biological properties of this mutant neurofi-
bromin in order to understand the structure-function rela-
tionships of native neurofibromin.

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References

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