A missense mutation in the neurofibromatosis 2 gene occurs in patients with mild and severe phenotypes

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**Article abstract—**We identified a missense mutation (T185 → C, Phe62 → Ser) in the neurofibromatosis 2 (NF2) gene in a family with mild and severe NF2 phenotypes. This mutation was previously reported in an unrelated family in which all affected individuals had mild phenotypes. These data demonstrate a lack of correlation between NF2 genotype and NF2 phenotype for this mutation.

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Neurofibromatosis 2 (NF2) predisposes to multiple benign tumors that primarily originate in the neural crest. NF2 occurs with an incidence of 1 in 33,000 to 40,000 individuals. Bilateral vestibular schwannomas (VSs) occur in approximately 90% of patients, but cranial and peripheral schwannomas, meningiomas, and ependymomas are also frequently observed.

The clinical characteristics of NF2 appear to conform to two subtypes, as reviewed by Mautner et al. The Wishart type is characterized by onset before age 20 years and multiple cranial and spinal tumors, in addition to bilateral VSs. The Gardner type has a more benign course, with late onset and tumors usually restricted to bilateral VSs. It has been suggested that the phenotypic subgroups may be caused by different mutations in the NF2 gene, and that missense mutations may be associated with mild phenotypes.

In this study, we identified a missense mutation in an NF2 family with individuals who had mild to severe phenotypes, and we compared the phenotypes of the affected members of the family. We also compared phenotypes between this family and an unrelated family that has the same mutation.

**Methods.** Genomic DNA was prepared from blood and subjected to polymerase chain reaction amplification of exon 2 of the NF2 gene using forward primer 5'-TTGCTCACAGTGTCCTTCCC-3', and reverse primer 5'-TCAGC-CCCACCAGTTTCA-3'. Cycling measurements were preceded by a 4-min initial denaturation at 94 °C, and included 38 cycles at 94 °C for 30 sec, 62 °C for 30 sec, and 72 °C for 1.5 min, followed by a 5-min final extension at 72 °C. Amplification was carried out in 20 μl in 50 mmol/liter KCl, 10 mmol/liter Tris-HCl, pH 9.0, 0.1% Triton X-100, and 0.25 mmol/liter each dNTP, with 10 pmol of each primer. SSCP and sequence analysis were conducted
as previously described, except that in amplifications conducted for single strand conformation polymorphism (SSCP) analysis, the dNTP concentrations were 37.5 μmol/liter each of dATP, dTTP, dGTP, and 18.8 μmol/liter dCTP, and included 2.5 μCi [α-32P]dCTP.

Results. The identification of the mutation resulted from SSCP analysis of 30 unrelated NF2 patients using intronic primers for each exon. SSCP analysis revealed an abnormal banding pattern for the proband, indicating the presence of a mutation in exon 2. Abnormal bands were not observed by SSCP analysis of other exons of the NF2 gene of the proband, or of exon 2 of other NF2 patients studied. DNA sequencing of this region demonstrated that the abnormal SSCP migration pattern was caused by a T → C transition at position 185, resulting in the replacement of phenylalanine by serine at codon 62, which was not observed in DNA from a normal individual (figure 1).

Three affected individuals of the family were clinically evaluated (family 1). The proband was a 65-year-old Caucasian man who presented with a skin schwannoma at age 25 years and had a mild clinical course. He was diagnosed with bilateral VSs (on the right, 3.5 cm; on the left, 1.8 × 1.3 cm) and had progressive hearing loss beginning at age 33 years in the right ear, and at age 39 years in the left ear. The right VS and a solitary meningioma were surgically removed at age 45 years. Slit-lamp examination revealed a left cortical spoke cataract. The proband's brother, 54 years of age, presented at age 26 years with gradual hearing loss in both ears. His tumors included bilateral VSs and multiple symptomatic cervical spinal tumors. He also had ambylophia and bilateral posterior lenticular opacities. A niece of the proband, 25 years of age, was severely affected and diagnosed with NF2 at the age of 14 years. Her symptoms included gradual hearing loss in the left ear and total loss of hearing in the right ear due to bilateral VSs. The right VS was surgically removed at age 18 years. She had reduced visual acuity due to ambylophia and bilateral posterior lenticular opacities.

Discussion. We analyzed 30 unrelated NF2 patients by SSCP analysis and found a missense mutation in one family with NF2 phenotypes of affected individuals ranging from mild to severe. Sequence analysis identified the mutation as T185 → C, Phe62 → Ser, in exon 2 of the NF2 gene. The same mutation was previously observed in an unrelated family with mild NF2 (family 2). In family 2, the proband and his sibling and father each had onset of symptoms between ages 40 and 43 years and bilateral VSs; the proband had a single meningioma. Family 1 had greater variability in age of onset of symptoms and number and types of tumors. The proband's niece was severely affected, although the proband had mild NF2. The presence of multiple spinal tumors suggests that the phenotype of the proband's

Figure 1. Direct sequencing of polymerase chain reaction–amplified NF2 exon 2 DNA demonstrated a T → C transition at position 185 (arrows) in both strands for the proband of family 1, but not in amplified DNA from a normal individual.

Figure 2. Positions of the eight germline (above) and five somatic (below) NF2 missense mutations in the NF2 gene. Also illustrated are the N-terminal (rectangle), α-helical (hatched rectangle), and C-terminal (lobed) domains of the NF2 protein. The numbers represent exons of the NF2 gene. Exon 16 is alternatively spliced.
brother is moderate. The ages of onset of symptoms in family 2 were considerably older than in family 1, and were nearly the same among the family members. All affected members of family 2 had mild NF2 and relatively slow growth of VSs since the diagnosis.

Missense mutations account for only about 9% of all NF2 mutations, and eight germline NF2 missense mutations are known: Phe62 → Ser, Glu106 → Gly, Val219 → Met, Asn220 → Tyr, Thr352 → Met, Leu360 → Pro, Leu535 → Pro, and Glu538 → Pro. Five others were identified in tumors and include Leu46 → Arg, Lys79 → Glu, Ile273 → Phe, Lys364 → Ile, and Arg418 → Cys. The NF2 missense mutations occur in various positions from exon 2 through exon 15 (figure 2) and probably affect important functional regions, or they may alter the protein on a larger scale by affecting conformation or degradation.

Knowledge of the relationship between mutations in the NF2 gene and phenotypes of affected patients aids in the understanding of the function of the NF2 protein. Because most NF2 mutations are predicted to result in truncated gene products, the impact of missense mutations on the function of the NF2 protein is not well understood. Both mild and severe phenotypes have been observed among germline NF2 mutations. The Leu360 → Pro missense mutation was observed in a family of three affected individuals, all of whom had mild phenotypes. However, phenotypic heterogeneity has also been seen within NF2 families, suggesting that complex mechanisms may modulate phenotype. In one such family, the identified mutation was a Glu538 → Pro missense. One affected individual had a mild NF2 phenotype, and the other had a moderately severe phenotype. In summary, our observations demonstrate that the same NF2 missense mutation can be associated with both mild and severe NF2 phenotypes. We found considerable intra- and interfamilial phenotypic variability for the identified mutation. Previous studies have also shown that NF2 patients and families with the same NF2 mutations may have variable phenotypes, including one study of affected monozygotic twins. These data suggest that the NF2 phenotype is modulated by additional factors that may be epigenetic, stochastic, or environmental in origin.

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References