Presymptomatic diagnosis for children of sporadic neurofibromatosis 2 patients: A method based on tumor analysis

Lan Kluwe, PhD[1], Reinhard E. Friedrich, MD[2], Marcos Tatagiba, MD[3], and Victor F. Mautner, MD[4]

Purpose: To provide presymptomatic diagnosis for children of sporadic neurofibromatosis 2 patients in whom no *NF2*-mutations were found by screening their blood-DNA. **Methods:** Tumors of four patients were analyzed for *NF2* allele losses and mutations. **Results:** Nonsense *NF2* mutations and *NF2* allele losses were found in all tumors. None of these alterations was found in any of eight children examined, suggesting that these children did not inherit the disease. **Conclusions:** Finding two genetic alterations of a tumor suppressor gene in associated tumors is useful for presymptomatic diagnosis. Identification of the lost allele in tumors alone also enables exclusion of disease transmission in 50% of cases. ***Genet Med* 2002:4(1):27–30.**

Key Words: *NF1, loss of heterozygosity, mosaicism, presymptomatic diagnosis*

Neurofibromatosis 2 (NF2) is a genetic disorder with an incidence of 1:40,000. Although bilateral vestibular schwannomas are the hallmark of this autosomal dominant disorder,[1–3] other cerebral tumors (e.g., meningiomas), cutaneous tumors,[4] and ophthalmologic abnormalities[5] are also common. NF2 is caused by mutations in the *NF2* tumor suppressor gene on the long arm of chromosome 22.[6,7] More than half of NF2 patients are sporadic and, thus, have new mutations.[8] Recent findings suggest that 20% to 30% of sporadic NF2 patients may be mosaic.[9,10] In some mosaic cases, the leukocytes of the patients may not bear the mutations and, thus, the mutations cannot be found by screening their blood-DNA. Our previous study demonstrated that, in such cases, analysis of tumors can provide an additional opportunity to find constitutional mutations.[10] In the present study, we used tumor analysis for presymptomatic diagnosis for eight children of four sporadic NF2 patients in whom no *NF2* mutations were found by screening their blood-DNA.

PATIENTS AND METHODS

Neurofibromatosis 2 patients were ascertained through our outpatient NF clinic at Hamburg, in cooperation with the Department of Neurosurgery, Hospital Nordstadt, and Hannover Medical School, Hannover, Germany. The diagnosis of NF2 was based on the updated NIH criteria.[11] Single vestibular schwannomas were removed from Patients 72 and 148. Three cerebral meningiomas were removed from Patient 74, and one skin schwannoma was removed from Patient 358. For all tumors, a part was frozen at the operation. The protocol was approved by the institutional review board, and all participants provided informed consent.

DNA extraction from blood and tumors, and exon-scanning using temperature gradient gel electrophoresis (TGGE) are as described in our previous study.[10] Haplotype analysis of the patients, their children, and unaffected spouses as well as allelic loss analysis of the *NF2* gene in tumors were performed using five microsatellite markers flanking or within the *NF2* gene: *CRYB2, D22S275, NF2CA3, D22S268,* and *D22S430*.[12,13]

RESULTS

As listed in Table 1, the clinical features of the four sporadic NF2 patients fulfill the updated NIH diagnostic criteria for NF2.[11] The phenotypes in these patients are rather mild, however, especially with respect to the age at diagnosis. In one patient (Patient 148), a unilateral vestibular schwannoma, a single cerebral tumor, and a cataract were all found on the left side. Mutation-screening using blood-DNA and exon-scanning TGGE did not reveal any alteration in the *NF2* gene in any of the four patients.

Single tumors were available from Patients 72 (vestibular schwannoma), 148 (vestibular schwannoma), and 358 (skin schwannoma), whereas three meningiomas were available from Patient 74. These tumors were subjected to loss of heterozygosity (LOH) analysis, using five flanking or intragenic microsatellite markers for the *NF2* gene, as well as to *NF2* mutation analysis, using exon-scanning TGGE (an example in Fig. 1). In all tumors, loss of one *NF2* allele was found as revealed by LOH of informative markers. Mutations in the *NF2* gene were also found in these tumors. All were nonsense mutations lead-

From the [1]Laboratory for Brain Tumor Biology, Department of Neurosurgery; [2]Department of Oral and Maxillofacial Surgery, University Hospital Eppendorf, Hamburg; [3]Department of Neurosurgery, Medical College Hannover; and [4]Department of Neurology, Klinikum Nord Ochsenzoll, Hamburg, Germany.

Lan Kluwe, PhD, Laboratory for Brain Tumor Biology, Department of Neurosurgery, University Hospital Eppendorf, Martinistr. 52, 20246 Hamburg, Germany.

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Table 1
Clinical and genetic features of the patients[a]

Patient	Sex	First symptom	Age (years) at first symptoms/diagnosis	Number of lesions			
				Vestibular schwannoma	Cerebral tumor	Spinal tumor	Cataract
72	M	Vestibular schwannoma	21/38	2	0	0	2
74	F	Meningioma	44/44	2	3	Multiple	2
148	F	Vestibular schwannoma	19/45	1 (left)	1 (left)	Multiple	1 (left)
358	M	Epiglotic schwannoma	29/38	2	1	0	0

[a]Patients 72 and 148 have been described previously.[12] M, male; F, female.

ing to truncation of the *NF2* gene product (Table 2). An identical mutation was found in all three different tumors from Patient 74, suggesting that it is constitutional because it is unlikely that the same somatic mutations occurred in three different tumors. For the other three patients, the mutations found are also likely to be constitutional, although we cannot exclude the possibility that the allelic loss of the *NF2* gene represents constitutional microdeletion of the *NF2* region. Lack of the mutations in blood-DNA of the four patients can be explained by mosaicism.

Patient 148 had one, patients 72 and 358 had two each, and Patient 74 had three children. Haplotypes of the *NF2* gene region was determined for the patients, the children, and unaffected spouses of the patients. Blood-DNA of the children was also examined for the *NF2* mutations that were found in tumors of their affected parents. Figure 1 shows an example of analysis of blood and tumor DNA from Patient 358 and blood-DNA from his two children. In total, five children (Children 72.1, 72.2, 74.3, 148.1 and 358.2) inherited the *NF2* alleles that were lost in the tumors of their affected parents (designated as allele-2 in Table 2) and, thus, did not inherit the mutation-bearing alleles. Indeed, the mutations found in the tumors of the patients were not detected in the blood of these five children. The other three children inherited the *NF2* alleles that remained and were mutated in the tumors of their affected parents (designated as allele-1 in Table 2). However, the mutations were not found in the blood of these children. This finding may be explained by the possibility that some or all germline cells of their affected parents were spared from the mutations due to mosaicism. Therefore, we were able to exclude inheritance of NF2 for all eight children of four affected parents.

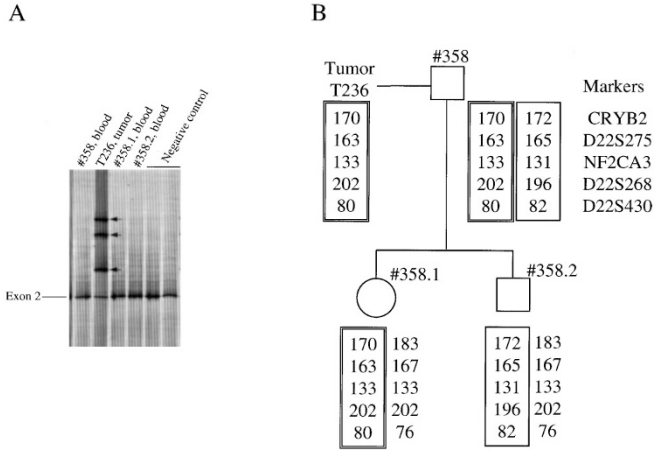

Fig. 1 Analysis of blood and tumor DNA from Patient 358 and blood-DNA from his two children. A: TGGE analysis. Additional bands (arrays) due to a mutation of 193C→T in exon 2 were detected in the tumor (T236) but not in the blood-DNA of Patient 358 or in the two Children 358.1 and 358.2. B: Haplotyping analysis. The allele boxed with single lines was lost in the tumor and inherited by Child 358.2. The allele boxed with double lines remained in the tumor and bore the mutation. This allele was inherited by Child 358.1

DISCUSSION

By screening exons using blood-DNA, *NF2* mutations have been found in between 30% and 60% of screened patients.[14–18] There are several explanations for the missing mutations: mosaicism in sporadic NF2 patients,[9,10] exon or multiexon deletions, insertion between exons, mutations in introns that disturb splicing, and alterations in the gene-regulatory regions. For sporadic cases, presymptomatic/prenatal diagnosis has not been possible without identified constitutional *NF2* mutations. Although linkage analysis can be applied for families with at least two affected members, its practical application is limited because more than half of NF2 cases are sporadic, and, in familial cases, it is often not possible to obtain blood from more than one affected member. In the present study, we demonstrated that analysis of tumors of sporadic NF2 patients enables presymptomatic/prenatal diagnosis in the absence of identified *NF2* mutations after screening blood-DNA. This is because, if we can find two genetic alterations (e.g., one mutation and one allele loss of the *NF2* gene, or two mutations) in tumor(s) of a patient, we know that one of them is constitutional. We can, thus, examine children/fetuses for either genetic alteration and determine whether or not they have inherited the disease.

Three children in this study inherited the alleles that carried the mutations in the tumors of their affected parents. However, the mutations were not found in the blood of these children. This finding can be explained by the possibility that some or all germline cells of the patients do not have the mutations due to gonadal mosaicism. This result is in concordance with the expectation and previous finding[9] that the risk of disease-transmission from founders to the next generation is reduced to

Table 2
Status of mutations and allelic loss of the *NF2* gene in the tumors of the patients and in their children

Patient	In the tumor		Child	Allele inherited from the patient	Mutation
	Allele-1 (mutation[a])	Allele-2			
72	Exon 2: 169C → T	Lost	72.1	Allele-2	No
			72.2	Allele-2	No
74	Exon 6: 586C → T	Lost	74.1	Allele-1	No
			74.2	Allele-1	No
			74.3	Allele-2	No
148	Exon 13: 1396C → T	Lost	148.1	Allele-2	No
358	Exon 2: 193C → T	Lost	358.1	Allele-1	No
			358.2	Allele-2	No

[a]All mutations are nonsense mutations.

below 50%, which is the expected transmission rate in a dominant genetic disorder.

Our study further demonstrated that identification of the lost *NF2* alleles in the tumors of the patients alone also enables presymptomatic/prenatal diagnosis in some cases. This is because statistically 50% of children/fetuses inherit the alleles that were lost in the tumors of their affected parents (e.g., Children 72.1 and 72.2 in this study, Table 2). According to the "two-hit" model, the allele lost in the tumor is the normal allele, and the allele retained may then be inferred to be that carrying the constitutional mutation. These 50% of children/fetuses, thus, have not inherited the mutation-bearing alleles. Although in a mosaic case, it is possible that the allele-loss represents a constitutional microdeletion of the *NF2* region, disease transmission to these 50% of children/fetuses can still be excluded because they inherited the allele and thus did not inherit the microdeletion. Furthermore, it is often possible to examine whether the apparent allelic loss is constitutional, because most somatic allelic loss involves large chromosomal regions or the whole chromosome. For the other 50% of children/fetuses who inherited the alleles that remained in the tumors of their affected parents, high risk for inheriting the disease is expected. However, due to mosaicism in 20% to 30% of sporadic NF2 cases, a small portion of children or fetuses in this group may not inherit the genetic defect (e.g., Children 74.1 and 74.2 in this study, Table 2). Further studies are needed to determine the extent to which the disease transmission risk is reduced in sporadic NF2 cases. For application of this LOH analysis–based presymptomatic/prenatal diagnosis, careful clinical diagnosis of the NF2 patients is extremely important. Non-NF2 patients who present with clinical symptoms and genetic alterations similar to NF2, e.g., schwannomatosis,[19,20] should be excluded. For clinically ascertained NF2 cases, the LOH analysis–based presymptomatic/prenatal diagnosis has several advantages: (1) it is unnecessary to find a mutation and can, therefore, be applied in cases in which no mutations have been found; (2) it is applicable for familial and sporadic cases, whereas linkage analysis can only be used in familial cases; (3) it includes only simple LOH analysis using four to six microsatellite markers and, thus, is inexpensive, fast, and requires only very small amounts of DNA. The possibility of obtaining data within 2 days is especially advantageous for prenatal diagnosis. Major limitations are the availability of tumors and that it can only by applied for cases with LOH in at least one tumor. Therefore, it is important to obtain as many tumors as possible to increase the probability of finding LOH. Skin tumors, which are present in approximately half of NF2 patients,[4] may serve as a tumor source for such testing because they can be removed easily.[21]

Presymptomatic/prenatal diagnosis based on tumor analysis can also be applied for other tumor suppressor gene syndromes, such as retinoblastomas,[22] tuberous sclerosis,[23] von Hippel-Lindau disease,[24] and neurofibromatosis 1 (NF1). NF1 patients often have a large number of neurofibromas,[25] which can be easily removed. This condition ensures LOH identification in one or more of the tumors, which would enable testing to exclude NF1 transmission in 50% of children/fetuses of affected parents. Because mosaicism is frequent in NF2 and in some cases is the reason for the failure to find *NF2* mutations in blood-DNA of the patients,[9,10] it would be generally desirable for a genetic analysis of a sporadic NF2 patient to start with screening blood-DNA; if no mutation can be found, then screening tumor specimens whenever possible is the next step.

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