

## Report

### Elevated Risk for MPNST in *NF1* Microdeletion Patients

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**An *NF1* microdeletion is the single most commonly reported mutation in individuals with neurofibromatosis type 1 (NF1). Individuals with an *NF1* microdeletion have, as a group, more neurofibromas at a younger age than the group of all individuals with NF1. We report that *NF1* microdeletion individuals additionally have a substantially higher lifetime risk for the development of malignant peripheral nerve sheath tumors than individuals with NF1 who do not have an *NF1* microdeletion. This should be taken into account in the medical follow-up of individuals with an *NF1* microdeletion.**

Neurofibromatosis type 1 (NF1 [MIM 162200]) is an autosomal dominant disorder with a prevalence of 1/4,000 (Huson et al. 1989). *NF1* is a tumor-suppressor gene located on chromosome band 17q11.2. The main features of NF1 are benign neurofibromas, café-au-lait spots, axillary freckling, Lisch nodules, and learning disabilities. Most of the patients have a small truncating mutation in the *NF1* gene (point mutation, splice mutation, small deletion, insertion, or duplication). However, 5%–10% of individuals with NF1 have a microdeletion (Clementi et al. 1996; Cnossen et al. 1997; Rasmussen et al. 1998). Most of these have a 1.5-Mb deletion incorporating the entire *NF1* region (López-Correa et al. 2000; Dorschner et al. 2000). In rare cases, both bigger and smaller microdeletions have been observed (López-Correa et al. 1999; Dorschner et al. 2000; personal observation). Genome database analysis indicates that the common *NF1* microdeletion region contains at least 15 genes/transcripts, including *NF1* and the three embedded genes. Most of these genes have unknown functions.

The *NF1* microdeletion patients tend to have a different phenotype as compared with the general NF1 group. *NF1* microdeletion patients frequently have more neurofibro-

mas at an earlier age, a lower mean IQ, and nonfamilial facial features (Kayes et al. 1994; Wu et al. 1995; Leppig et al. 1997; Tonsgard et al. 1997; Wu et al. 1997). Although, as a group, the *NF1* microdeletion patients have distinct features, it remains difficult at the individual level to distinguish between deletion and nondeletion patients solely on the basis of clinical observations, and, at this time, there is a lack of genotype-phenotype correlation for individuals with NF1. Because of the enormous clinical variability in phenotypic expression, a medical surveillance program for all individuals with NF1 is necessary to detect a large range of possible complications. Each complication occurs in only a relatively small percentage of individuals with NF1 (Huson and Hughes 1994). The lifetime risk for malignant peripheral nerve sheath tumor (MPNST) in individuals with NF1 may be 8%–13%, as reported in a recent study (Evans et al. 2002), and it would be important to identify a subgroup of individuals with NF1 who have a higher risk of MPNST.

Elsewhere, we reported germline mutations in seven patients with NF1 and MPNSTs (Wu et al. 1999). Three of these had an *NF1* microdeletion, suggesting a possibly increased incidence of MPNSTs in *NF1* microdeletion patients. Dorschner et al. also reported two patients with MPNSTs in a group of 17 individuals with an *NF1* microdeletion and suggested a predisposition to malignancies in *NF1* microdeletion cases (Dorschner et al. 2000). We wanted to test the hypothesis that individuals with

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an *NF1* microdeletion represent a specific subgroup of individuals with NF1 and a higher risk of MPNST.

In a multicenter study (Wu et al. 1999; Birindelli et al. 2001; Leroy et al. 2001; unpublished cases), we tested the frequency of *NF1* microdeletions on a large number (38) of patients with NF1 and MPNST (all diagnosed as having NF1 according to the National Institutes of Health criteria [Stumpf et al. 1987]). This study on archival material was approved by the institutional review boards of the different institutes. On histology, all tumors corresponded to sarcomatous cellular proliferations, consisting of highly cellular, intersecting bundles of spindle cells, with unequivocal nuclear atypia, prominent typical and/or atypical mitotic figures, and variable areas of necrosis. Tumors were graded as low grade (I), intermediate grade (II), or high grade (III), on the basis of differentiation, mitotic rate, and amount of tumor necrosis (table 1). Of the 38 MPNST cases, 24 were high grade, 2 low grade, 9 intermediate grade, and 2 of unknown grades. Only two MPNSTs showed features enabling classification as either triton or epitheloid tumors.

The presence of microdeletions was assessed on nontumor tissue by different techniques, depending on the available material for analysis: a specific breakpoint PCR (López-Correa et al. 2001), FISH, semiquantitated PCR, and/or marker analysis (see table 1). The FISH experiments were performed with PAC clone RPC15-926B9 located within *NF1* (López-Correa et al. 1999). In several cases, only DNA was available, and FISH could not be performed. In these cases, a specific breakpoint PCR was performed to detect the common breakpoint junction fragment. If a specific deletion junction fragment was not detected, marker analysis was performed by assessing heterozygosity for five markers located in the *NF1* microdeletion region (D17S1800: 5'*NF1*-1, 3'*NF1*-1, 3'*NF1*-2, 3'*NF1*-3, and IVS/38-GT; see also López-Correa et al. [1999]). We excluded an *NF1* microdeletion if heterozygosity for markers located in the microdeletion region was observed in nontumor DNA. We realize that we might have missed cases with a smaller deletion confined to the *NF1* gene region. Only cases showing no heterozygosity for any of the five dinucleotide repeats were further analyzed with semiquantitative PCR for a potential microdeletion.

For the semiquantitated PCR analysis, exon 48 of *NF1* was amplified, together with exon 24 of *CFTR* as a control fragment (25 cycles). This semiquantitated PCR analysis was optimized using known *NF1* microdeletion and nonmicrodeletion individuals. The relative intensity of the *NF1* fragment was scored after electrophoresis on an ABI PRISM 310 Genetic Analyzer. All primer sequences and PCR conditions are available from the authors on request.

Nine of 38 patients with NF1 and MPNSTs (23.7%) showed an *NF1* microdeletion in the nontumor tissue

(table 1). This microdeletion frequency is significantly higher ( $P < .005$ ; binomial distribution) than expected in the general NF1 population (5%–10%) (Clementi et al. 1996; Cnossen et al. 1997; Rasmussen et al. 1998). To estimate the lifetime risk of MPNST in *NF1* microdeletion patients (Bayes's Theorem), we used conservative estimates of the different probabilities. We used 20% (this study 23.7%) for the frequency of an *NF1* microdeletion in individuals with NF1 and MPNST ( $P[\text{Del}/\text{MPNST}, \text{NF1}]$ ) and 10% (probably an overestimation) for the frequency that a patient with NF1 is a carrier of a microdeletion ( $P[\text{Del}/\text{NF1}]$ ).  $P(\text{MPNST}/\text{NF1})$  is an estimate of the lifetime risk for the development of MPNST in the general NF1 population.

$$P(\text{MPNST}/\text{Del}, \text{NF1}) = \frac{P(\text{Del}/\text{MPNST})}{P(\text{Del}/\text{NF1})} P(\text{MPNST}/\text{NF1}) ,$$

and

$$P(\text{MPNST}/\text{Del}, \text{NF1}) = \frac{0,20}{0,10} P(\text{MPNST}/\text{NF1}) = 2P(\text{MPNST}/\text{NF1}) .$$

Using these parameters, we estimate that the lifetime risk for MPNST in *NF1* microdeletion patients is twice as high as in the general NF1 population.

Previous cross-sectional studies showed that only 1%–2% of individuals with NF1 have MPNST (Huson et al. 1989). Specialist series found higher risks of MPNST in NF1, owing to a bias toward patients with important complications that require specialist treatment in a hospital setting (Greager et al. 1992). The lifetime risk of MPNST in patients with NF1 was recently estimated to be 8%–13%, in a population-based study with near-total ascertainment for NF1-related MPNST, using a dual approach for ascertainment (NF1 register and Cancer registry) (Evans et al. 2002). Evans et al. (2002) also found that the majority of patients with NF1 and MPNST clinically present with their malignancy in the 3rd or 4th decade of life (median 26 years). This is at a much younger age than individuals who have sporadic MPNST but are not afflicted with NF1 (median 62 years).

In the *NF1*-microdeletion patient group reported here, the age at diagnosis does not have a normal distribution curve because of an outlier (patient C12, 58 years). We observed a substantial difference in the median age at diagnosis between deletion and nondeletion cases: 22 years (microdeletion) versus 30 years (nonmicrodeletion). This difference of 8 years is not significant ( $P = .15$ ; Mann-Whitney U test). After exclusion of the outlier (C12), we observe a significant difference ( $P = .04$ ; Mann-Whitney U test) of 9 years in the median age of

**Table 1**  
**Overview of Patients with NF1 in This Report**

Sample	Del <sup>a</sup>	Age <sup>b</sup>	Grade <sup>c</sup>	Familial <sup>d</sup>	Test <sup>e</sup>	Reference
NF 00-4	+	15	III	–	F	Unpublished
NF 00-3	+	22	III	+	F	Unpublished
755	+	23	II	+	BP	Unpublished
14 L	+	29	II	–	BP	Leroy et al. 2001
NF 90-8	+	17	IIIe	U	F	Wu et al. 1999
NF 96-1	+	27	III	–	F	Wu et al. 1999
C12	+	58 <sup>f</sup>	U	–	F	Wu et al. 1999
2 L	+	18	I	–	BP	Leroy et al. 2001
10 L	+	20	III	–	SQ, M	Leroy et al. 2001
1 B	–	16	III	+	M	Birindelli et al. 2001
2 B	–	14	II	–	M	Birindelli et al. 2001
3 B	–	27	III	–	M	Birindelli et al. 2001
4 B	–	39	III	–	M	Birindelli et al. 2001
6 B	–	33	III	+	M	Birindelli et al. 2001
7 B	–	30	III	–	M	Birindelli et al. 2001
8 B	–	20	III	–	M	Birindelli et al. 2001
12 B	–	32	III	U	M	Birindelli et al. 2001
NF 89-3	–	15	U	U	F	Wu et al. 1999
NF 90-1	–	23	III	–	F	Wu et al. 1999
NF 88-3	–	33	III <sup>t</sup>	U	F	Wu et al. 1999
NF 88-18	–	60	II	–	F	Wu et al. 1999
1 L	–	20	II	+	M	Leroy et al. 2001
6 L	–	31	I	–	M	Leroy et al. 2001
NFR117	–	25	II	+	M	Unpublished
17 L	–	35	III	–	M	Leroy et al. 2001
11 L	–	18	III	–	M	Leroy et al. 2001
3 L	–	56	II	+	M	Leroy et al. 2001
9 L	–	49	III	–	M	Leroy et al. 2001
NFR525	–	31	III	+	M	Unpublished
560	–	22	II	+	M	Unpublished
632	–	30	II	+	M	Unpublished
790	–	19	III	+	M	Unpublished
5 L	–	55	III	+	M	Leroy et al. 2001
8 L	–	42	III	–	M	Leroy et al. 2001
12 L	–	18	III	+	M	Leroy et al. 2001
NF 93-2	–	28	III	+	M	Unpublished
NF 93-1	–	41	III	–	M	Unpublished
NF 96-4	–	?	U	U	M	Unpublished

<sup>a</sup> Presence (+) or absence (–) of deletion.

<sup>b</sup> Age in years at diagnosis of the MPNST.

<sup>c</sup> Grade of MPNST: e = epithelioid, t = triton.

<sup>d</sup> Familial (+) or sporadic (–) occurrence of NF1; U = unknown.

<sup>e</sup> The test performed to confirm the presence or absence of a deletion is indicated: M = marker analysis, BP = specific break point PCR, F = FISH analysis, SQ = semi-quantitated.

<sup>f</sup> Outlier in age at diagnosis distribution.

onset (21 years vs. 30 years). Therefore, it might be interesting to assess the significance of this difference in age at diagnosis in a larger study.

It is known that *NF1* microdeletion individuals as a group show a more severe phenotype than the general NF1 population. One might argue that mildly affected NF1 individuals with an MPNST have a lower chance of being diagnosed as NF1 and, thus, a higher chance of *not* being included in this study. Therefore, this study

could have a bias toward individuals more severely affected with NF1 with a higher likelihood of harboring an *NF1* microdeletion. We do not think that this bias plays a role in this study because the NF1 MPNST cases were diagnosed in centers with multidisciplinary outpatient clinics for neurofibromatosis. These centers have a high suspicion for the diagnosis of neurofibromatosis in cases presenting with MPNST. In addition, we believe that the potential ascertainment bias for diagnosing an

individual more severely affected with MPNST as having NF1 applies also for the referral of an individual with an NF1-compatible phenotype to a multidisciplinary clinic. The proportion of *NF1* microdeletion individuals in the total NF1 population is estimated on the basis of series from multidisciplinary clinics. These series might also show an ascertainment bias toward more severely affected cases, resulting in a potential overrepresentation of *NF1* microdeletion individuals. As a result, both ascertainment biases (NF1 in MPNST population and *NF1* deletion in NF1 multidisciplinary clinic population) will eliminate each other, to a great degree, in the equation.

Our study indicates that *NF1* microdeletion patients have a substantially higher risk for the development of MPNSTs compared with nonmicrodeletion NF1 individuals. If we assume that the lifetime risk of MPNST in NF1 individuals is 8%–13% (Evans et al. 2002), then *NF1* microdeletion patients have a lifetime risk for MPNST of 16%–26%. We therefore propose routine testing of individuals with NF1 for the presence of a microdeletion and follow-up of *NF1* microdeletion patients with a higher level of suspicion for MPNSTs. Complaints of pain and/or rapid growth of tumors should be actively investigated using a low clinical threshold.

Further molecular investigation of tumoral cells of neurofibromas and MPNSTs from both *NF1* microdeletion and nonmicrodeletion patients might shed some light on the molecular pathogenesis of the increased susceptibility for MPNST development in *NF1* microdeletion patients.

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## Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for NF1)

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