LETTER TO JMG

NF1 mutations and clinical spectrum in patients with spinal neurofibromas

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Neurofibromatosis 1 (NF1) is an autosomal dominant disorder associated with a variety of benign and malignant lesions such as café au lait spots, neurofibromas, phaeochromocytomas, pilocytic astrocytomas, and malignant peripheral nerve sheath tumours.[1–3] With an incidence of 1 in 4000, NF1 is caused by genetic alterations of the *NF1* gene located on 17q11.2.[4–6] Consisting of 60 exons, the *NF1* gene is a tumour suppressor gene which leads to tumorigenesis upon inactivation of both alleles.[7–9]

Spinal tumours cause neurological symptoms in about 2% of NF1 patients and can be detected in 40% of NF1 patients by magnetic resonance imaging (MRI).[10] Patients with multiple spinal tumours but very few or no other clinical symptoms of NF1, including three multigenerational families,[11–13] have been reported, suggesting a subgroup or a distinct genetic form of NF1, spinal neurofibromatosis (SNF).[14] In one of these multigenerational families, a truncating mutation in exon 46 of the *NF1* gene was found.[13] However, no systematic study of the clinical spectrum of patients with spinal tumours and of the *NF1* mutations associated with it has been carried out.

In this study, we performed a clinical and molecular examination of patients from 17 families with spinal tumours.

Key points

- Twenty patients from 17 families with spinal tumours were examined for clinical symptoms associated with neurofibromatosis type 1 (NF1) and for *NF1* mutations. Twelve patients from 11 families had typical NF1 symptoms. Typical *NF1* mutations were found in 10 out of the 11 index patients in this group, including eight truncating mutations, one missense mutation, and one deletion of the entire *NF1* gene.
- Eight patients from six families had no or only a few additional NF1 associated symptoms besides multiple spinal tumours, which were distributed symmetrically in all cases and affected all 38 nerve roots in six patients.
- Only mild *NF1* mutations were found in four out of the six index patients in the latter group, including one splicing mutation, two missense mutations, and one nonsense mutation in exon 47 at the 3′ end of the gene.
- Our data show that patients with spinal tumours can have various NF1 symptoms and *NF1* mutations. However, patients with no or only a few additional NF1 symptoms may be a subgroup or may have a distinct form of NF1, probably associated with milder *NF1* mutations or other genetic alterations.

PATIENTS AND METHODS

Neurofibromatosis type 1 (NF1) was diagnosed based on the NIH criteria.[15] The protocol was approved by the institutional review board and all participants provided informed consent. The 17 index patients and 11 family members were examined in our NF clinic in Hamburg. Detailed MRI results of the full spine were available from the 17 patients and three affected family members.[10]

DNA was extracted from blood using a QIAamp Blood Kit from Qiagen (Hilden, Germany). Mutation analysis was performed by direct sequencing of all *NF1* exons using a BigDye Sequencing kit as previously described.[16 17]

RESULTS

Clinical findings

The age at onset of symptoms caused by spinal tumours in the 17 index patients varied from 11 to 49 years (mean 32.8 years). Apart from two patients who had symptoms at ages 11 and 14, all the other 15 index patients had symptoms at adult ages (22 to 43 years). For the 17 index patients and three family members, we could count the number of affected nerve roots of the spine based on MRI: 10 subjects had tumours in 1 to 24 (an example of a single tumour is shown in fig 1A) while the other 10 had tumours in all 38 spinal nerve roots (an example is shown in fig 1B). Some nerve roots probably had more than one tumour but the exact number of tumours in each nerve root could not be determined. Nine patients had surgical interventions and one other patient had a biopsy. According to the pathology report, all removed or biopsied tumours were neurofibromas. However, some tumours were not solitary and not well circumscribed as shown by MRI and we thus could not exclude the possibility that some of them were plexiform neurofibromas.

Fifteen out of the 17 index patients met the NIH diagnostic criteria for NF1, that is, two or more of the following were found in each of them: six or more café au lait spots, two or more neurofibromas of any type, or one plexiform neurofibroma; axillary or inguinal freckling; optic glioma; two or more Lisch nodules; a distinct osseous lesion; a first degree relative (parent, sib, or offspring) with NF1 according to the above criteria for NF1.[15] Two other patients (Nos 142 and 42) had no additional signs of NF1 besides the multiple spinal tumours and thus did not meet the diagnostic criteria for NF1. The father of patient 42 had a phaeochromocytoma but no further sign of NF1. Although fulfilling the diagnostic criteria, patients 308 and 341 had only one and seven café au lait spots, respectively, besides spinal tumours. The diagnosis for patient 308 was made possible by the diagnosis of his daughter who had multiple spinal tumours and more than two Lisch nodules and thus met the minimum criteria for NF1. Three other NF1 patients from two families (Nos 824, 584, 584/child 1) had fewer than 10 neurofibromas and fewer than five café au lait spots. In total, eight patients from six families had no or only a few additional NF1 associated symptoms besides the multiple spinal tumours. In all these eight patients spinal tumours were distributed symmetrically and in six of them all 38 spinal nerve roots were affected.

One patient (No 406) had two children (aged 13 and 14 years) with identical *NF1* mutations but no spinal tumours

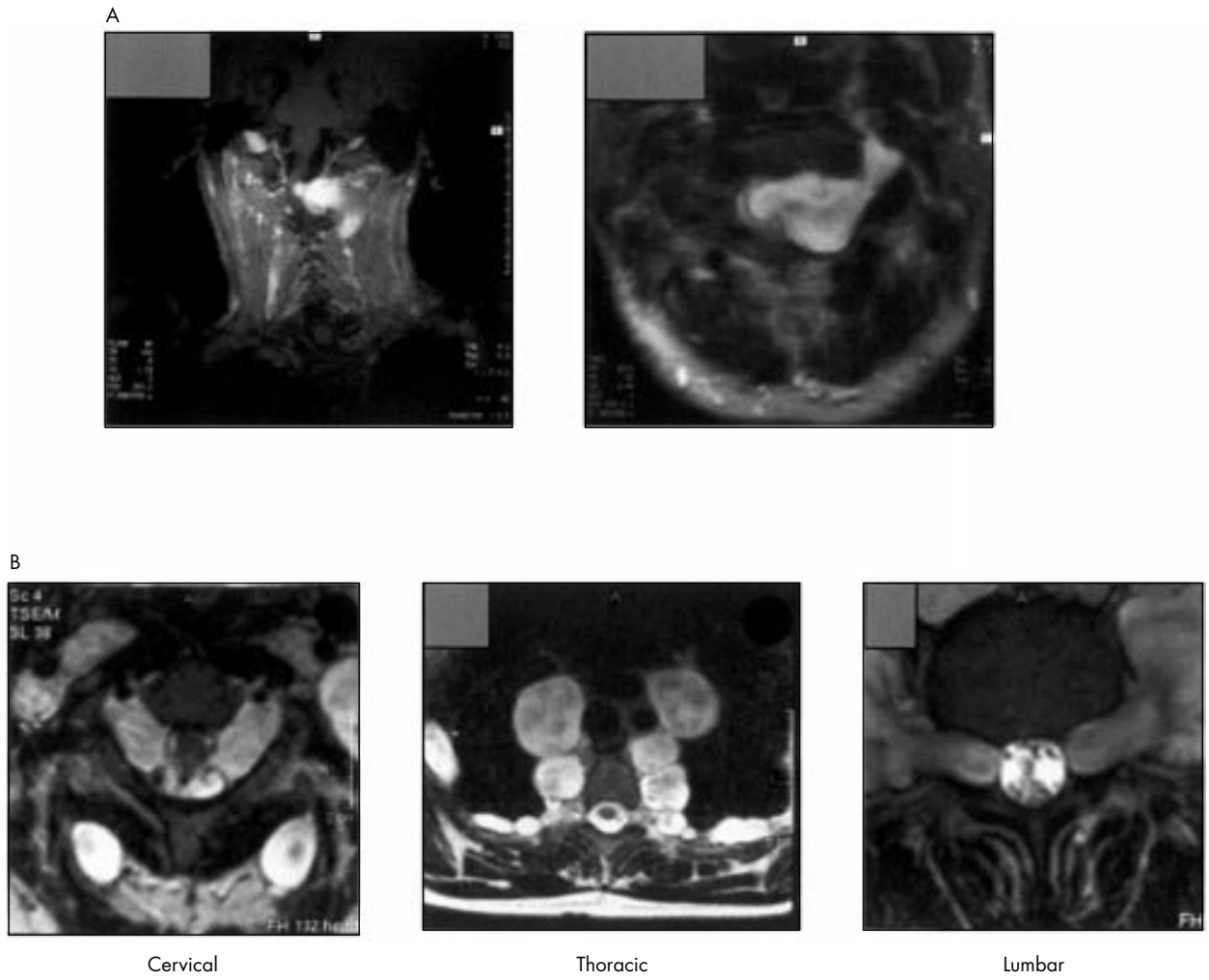

Figure 1 (A) A single tumour compressing the cervical segment of the spine in patient 406. (B) Symmetrical spinal tumours in patient 42.

were found on MRI. Patient 142 had one child with an identical missense *NF1* mutation; however, the child was not examined for spinal tumours owing to the young age (3 years).

Mutation analysis

DNA extracted from peripheral blood from the 17 index patients with spinal tumours was screened for *NF1* mutations by direct sequencing of all exons of the gene. As listed in table 1, *NF1* mutations including nine truncating (nonsense or frameshift), one splicing, and three missense mutations were found in 13 patients. In an additional patient, deletion of the *NF1* gene region was detected by FISH. Nine out of the 13 mutations were novel while four have been described previously (table 1)[15 16] (http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html).

Four mild *NF1* mutations were found in four out of the six families/patients with no or only a few additional NF1 symptoms besides the multiple spinal tumours: one splicing mutation in intron 5, two missense mutations (2759T>C, Leu>Pro and 6598C>G, Pro>Ala) in exons 16 and 35, and one nonsense mutation (8093C>G) in exon 47. No *NF1* mutations were found in the other two patients in this group.

DISCUSSION

The genetic disorder neurofibromatosis type 1 (NF1) has a wide clinical spectrum. Symptoms such as café au lait spots and neurofibromas are hallmarks of the disease and are found in more than 90% of patients.[1–3] Other symptoms such as plexiform neurofibromas, optical gliomas, or scoliosis are found only in subpopulations (10-30%) of the patients.[2 14 18] Spinal tumours are a part of the clinical spectrum of NF1, but are symptomatic in less than 2% of patients.[10] As shown in the present study, spinal tumours cause symptoms mainly in older patients (mean age 32.8 years). Also in the family described by Ars *et al*,[13] only the two older patients (aged 34 and 58 years) had symptoms while the other three younger family members (aged 12, 21, and 24 years) had only asymptomatic spinal tumours. In family 1 reported by Pulst *et al*,[11] no spinal tumours were found in the two youngest patients with the disease associated haplotype.

Our results showed that both typical and atypical clinical symptoms of NF1 can be found in patients with spinal tumours. Twelve patients from 11 families in our study had spinal tumours and typical NF1 symptoms, such as more than 20 neurofibromas, more than six café au lait spots, and multiple Lisch nodules. The number of affected spinal nerve roots varied from one to 38 in each of these patients and the tumours were usually distributed asymmetrically. Typical *NF1* mutations were found in these patients (table 1). It is reasonable to consider these patients as a subgroup of NF1 patients, comparable to those 30% and 5% of NF1 patients who develop plexiform neurofibromas[18] and pilocytic astrocytomas, respectively. No large families with spinal tumours and typical NF1 phenotypes have been observed.

Eight patients from six families in this study had spinal tumours but only a few or no additional NF1 symptoms,

Table 1 Clinical features and *NF1* mutations in patients with spinal tumours

Patient information				NF1 symptoms†							Spinal tumours‡				Molecular genetic findings§		
Patient No	Sex	Age at onset	Age at investigation	Family history	CNf	CLS	PNf	Lisch nodules	Axillary freckling	Scoliosis	Affected nerve roots	Symmetry	Symptom	OP	Exon/ intron	Mutation	Mutation type
Patients with spinal tumours but only a few or no other signs for NF1																	
308	M	41	54	No	0	1	No	1	0	No	38	Yes	Yes	Yes	**5**	**IVS5-2A>G**	**Sp**
Child 1	F	22	23	No	0	3	No	>2	0	No	38	Yes	Yes	No		Ditto	
Child	F		1	Yes	0	0	No	0	0	No	NE		No			Ditto	
Child 2	M	Died at 18		No	0	3	No	>2	0	No	Mult	Unk	Yes	No		NE	
142	M	25	35	No	0	2	No	0	0	No	12	Yes	Yes	No, Bs	**16**	**2759T>C**	**Ms (Leu>Pro)**
Child	M		4	Yes	0	0	No	0	0	No	NE					Ditto	
824	M	39	44	No	<10	5	Yes	>2	0	No	38	Yes	Yes	Yes	**35**	**6598 C>G**	**Ms (Pro>Ala)**
584	F	37	50	No	<10	4	Yes	>2	0	<10°	38	Yes	Yes	No	47	8093C>G	Ns[16]
Child 1	F	23	24	Yes	0	5	No	>2	0	No	38	Yes				NE	
Child 2	M	Died at 18		Yes				Unk			Mult	Unk				NE	
341	M	30	38	No	0	7	No	0	0	No	24	Yes	Yes	Yes		Not found	
42	F	29	38	No	0	2	No	0	0	No	38	Yes	Yes	Yes		Not found	
Father*	M	Died at 64		No	0	3	No	0	0	No	NE					NE	
Patients with spinal tumours and typical NF1 symptoms																	
158	F	40	50	No	>300	>6	Yes	>2	>2	No	2	No	Yes	No	7	910C>T	Ns
Child 1	F		16	Yes	Mult	>6	No	>2	>2	No	2	No	No			Ditto	
732	M	11	26	Yes	>150	>6	No	>2	0	<10°	20	No	Yes	No	**12a**	**1733T>G**	**Ms (Leu>Arg)**
Father	M			Unk	>30	>6	No	>2	>2	No	Mult	Unk	No			NE	
833	M	41	42	Yes	Milt	>6	Yes	>2	>2	No	7	No	Yes	No	12b	1935-1936delG	Fs[15]
406	M	39	41	No	>240	>6	No	>2	>2	<10°	1	No	Yes	Yes	**28**	**4905-4906ins11bp**	**Fs**
Child 1	F		14	Yes	Mult	>6	No	>2	>2	No	0		No			Ditto	
Child 2	F		19	Yes	Mult	>6	No	>2	>2	No	0		No			Ditto	
745	M	22	36	No	>20	>6	Yes	>2	>2	No	38	Yes	Yes	Yes	29	5242C>T	Ns
894	F	49	51	No	50-60	>6	Yes	>2	>2	No	1	No	Yes	Yes	**31**	**5788-5789delC**	**Fs**
548	F	40	41	No	>150	>6	No	>2	>2	No	1	No	Yes	No	**37**	**6757delG**	**Fs**
459	M	35	36	No	>200	>6	No	>2	0	No	7	No	Yes	No	**40**	**7257del 2bp**	**Fs**
280	F	43	53	Yes	>150	>6	No	>2	2	<10°	38	Yes	Yes	Yes	**41**	**7296C>A**	**Ns**
442	M	22	24	No	Mult	>6	No	>2	0	<10°	38	Yes	Yes	Yes		Large del	Large del
546	M	14	15	No	>150	>6	No	>2	>2	No	38	Yes	Yes	No		Not found	

*This parent had a phaeochromocytoma.
†: Unk = unkown; Mult = multiple; CNf = cutaneous neufibromas; PNf = plexiform neurofibromas; CLS = café au lait spots.
‡: OP, operation; Bs, biopsied; NE, not examined.
§: Ns, nonsense mutation; Ms, missense mutation; Sp, splicing mutation; Fs, frameshift mutation. Novel mutations are shown in bold.

meeting the description of spinal neurofibromatosis.[11–14 19] Spinal tumours in these patients are characterised by symmetry and multiplicity, often involving all 38 spinal nerve roots. SNF has been observed in large families.[11–13] Seven *NF1* mutations associated with SNF have been found (four in this study and three previously[13 19]): two splicing mutations, three missense mutations, and two nonsense mutations in exons 46 and 47, respectively, at the 5′ end of the *NF1* gene. These mutations differ from those found in unselected NF1 populations[16 20 21] and may be considered as milder mutations, which may explain the reduced clinical spectrum of NF1 in these patients. However, we do not understand why these mutations are associated with spinal tumours of high penetrance and it is unlikely that the *NF1* mutation alone can explain the given phenotype. For two patients with phenotypes of SNF in our study, no exonal *NF1* mutations were found. For these two cases, other genetic causes may be responsible for the spinal tumours. Indeed, three affected members with only spinal tumours and café au lait spots were not linked to the *NF1* or the *NF2* locus, suggesting involvement of another locus in a previously described family.[11] The involvement of NF2, which is characterised by schwannomas, is unlikely in the two patients in our study, since surgically removed spinal tumours from them were neurofibromas.

In summary, our data show that patients with spinal tumours may have various clinical features of NF1 and that, as in the case of plexiform neurofibromas, all types of *NF1* mutations can lead to spinal tumours in NF1 patients.[17] However, cases with spinal tumours but few or no other NF1 signs may be a subgroup of NF1, or a distinct disorder, and may have milder *NF1* mutations or other genetic causes.

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