A clinical study of patients with multiple isolated neurofibromas

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EDITOR—Neurofibromas are benign nerve sheath tumours of a heterogeneous nature consisting of Schwann cells, fibroblastic elements, and embedded axons. Neurofibromas may occur singly in genetically normal people at any point along the peripheral nervous system. Multiple neurofibromas are nearly pathognomonic for neurofibromatosis 1 (NF1). In patients with NF1, neurofibromas may be congenital and plexiform or, more commonly, may be smaller masses that begin to accumulate around the time of puberty. Cutaneous and subcutaneous neurofibromas may cause considerable cosmetic disfigurement, but rarely result in neurological dysfunction. Conversely, deep seated neurofibromas on peripheral nerves and spinal roots frequently lead to neurological disability. Inevitably, adult patients with NF1 have other stigmata of the disorder with the most common being café au lait spots, skin fold freckling, and Lisch nodules.^{1 2} NF1 is an autosomal dominant disorder with full penetrance and a defined genetic aetiology that shows no evidence of locus heterogeneity.³ Neurofibromatosis 2 patients are rarely found to have one or more neurofibromas.^{4 5}

Recently, we have become aware of a small number of patients with multiple pathologically proven neurofibromas, who have no other stigmata of NF1. Here we report the clinical characteristics and pathological findings of these patients, and propose the terminology “multiple isolated neurofibromas” to describe this rare condition.

Material and methods

The criteria for inclusion in the study were multiple, pathologically proven neurofibromas without other defining features of NF1. Careful family histories were obtained in order to

document other family members potentially affected, extending to all second degree relatives. Each participant underwent a clinical examination by one or more of the authors (PB, MM, MPS), which included a detailed neurological evaluation and inspection of the skin with a Wood's lamp. For patients who gave a positive family history, medical records from the potentially affected family member and tumour specimens were reviewed to confirm the diagnosis. Blood samples were collected and immortalised cell lines created for future studies.

All available histological and immunohistochemical materials from each patient were requested from treating institutions and reviewed centrally by a single neuropathologist (DNL). In all but two patients, two or more distinct tumours from separate procedures were obtained; in patient BNF9 only a single specimen was available and in patient BNF12 two specimens from the same procedure were reviewed. Specimens were also reviewed from the mother of patient BNF2 and the brother of patient BNF10.

Molecular genetic analysis of the *NF2* gene was limited to specimens from patient BNF11 and his affected son and was performed as previously described.⁶ No molecular analysis was performed of the *NF1* locus.

This study was approved by the institutional review board of Massachusetts General Hospital and informed consent was obtained from all subjects donating tissue.

Results

A total of 10 adult probands were identified who met our criteria of multiple neurofibromas without other stigmata of NF1 (table 1). Age of onset ranged from 6 to 53 years (mean 27.8 years). An

Table 1 Clinical characteristics of patients with multiple neurofibromas. Tumour distribution includes both surgically removed tumours and those still in place

Patient	Age (y)	Sex	Age of onset (y)	First symptom	Distribution of tumours	Findings associated with NF1	Tumour pathology (No of tumours)	Family history
BNF1	44	F	35	Painless mass	Brachial plexus and chest (bilateral)	Single CAL, migraine	Neurofibroma and plexiform neurofibroma (2)	Brother, osteosarcoma
BNF2	36	F	18	Abdominal pain	Scalp, spine and pelvis	Learning disability, migraine, single CAL	Neurofibroma (2)	Daughter, four subcutaneous masses Mother, multiple NF without CAL Maternal aunt and brother, multiple NF (not confirmed)
BNF3	39	F	6	Painless mass	Head and neck, spine, extremities, torso	Leg length discrepancy	Neurofibroma and plexiform neurofibroma (5)	None
BNF4	44	F	16	Facial palsy	Skull base, brachial plexus, spine, chest, pelvis, extremities	None	Neurofibroma (2)	None
BNF9	49	F	48	Chest pain	Brachial plexus (bilateral)	Migraine	Neurofibroma (1)	None
BNF10	47	M	33	Spastic gait	Spine	Skin tags	Neurofibroma (2)	Brother, multiple NF
BNF11	65	M	53	Painless mass	Shoulder, chest, arm (all cutaneous)	Three CAL	Neurofibroma (7)	Son, NF2
BNF12	62	F	20	Painless mass	Face, extremities (all cutaneous)	Three CAL	Neurofibroma (2)	Multiple relatives with “tumours”, none confirmed
BNF14	48	F	18	Facial pain	Head and neck (R greater than L)	Single GTC, hypertension	Neurofibroma (4)	None
BNF15	39	F	31	Hip pain	R hip and buttock	None	Neurofibroma with marked collagen deposition (2)	None

F = female, M = male, R = right, L = left, CAL = café au lait macules, GTC = generalised tonic clonic seizure, NF = neurofibromas.

Figure 1 Multiple masses in the right supraclavicular area in patient BNF1. A surgical scar from a debulking procedure is present.

initial diagnosis of neurofibromatosis was made in only two patients. All patients reported an increase in the size and number of tumours since diagnosis. Seven patients reported increased pain in association with the increase in tumour size. In five cases, the pain had been unresponsive to medical management and had necessitated a reduction or discontinuation of employment. Decreasing neurological function was reported by three patients. A total of 41 surgical procedures had been performed on these patients for pain, increasing tumour size, or cosmetic disfigurement. A single patient received radiation therapy because of aggressive growth of her tumours associated with bony erosion.

Four patients gave a history of relatives potentially affected by NF. In one case (patient BNF12), none of the potentially affected relatives nor their records were available for study. In a second case (patient BNF11), the proband's son had typical neurofibromatosis 2. Molecular genetic analysis of the son's tumour showed a typical truncating mutation of the *NF2* gene and loss of the paternal allele. Examination of genomic DNA extracted from peripheral blood lymphocytes from patient BNF11 showed that the *NF2* gene mutation seen in the son's tumour was not present. Two patients had first degree affected relatives with pathologically proven neurofibromas (BNF2 and 10). These 10 patients had a total of 15 children ranging in age from 9 to 40 years. Three of 15 were affected by the proband's report and one (child of BNF2) had been examined and found to have four subcutaneous tumours and no café au lait macules at age 9.5 years. The mean age at referral to our clinic was 42.7 years.

Figure 2 Right arm and hand of patient BNF3 showing nearly contiguous involvement of the forearm by tumour masses and abnormal nail beds.

Figure 3 Coronal MRI of the cervical spine in patient BNF10 shows multiple poorly enhancing tumours (*) with significant mass effect on the cord. Contrast enhanced T1 weighted image.

Examination of these patients showed that four had readily visible, firm, subcutaneous tumours and two had typical cutaneous tumours indistinguishable from those seen in NF1 (fig 1). Detailed skin inspection using a Wood's lamp showed that no patient had six or more café au lait macules (CAL) and no patient had skin fold freckling. Neurological examination was abnormal in four with weakness, sensory change, or Horner's syndrome that could be attributed to the tumours or to postoperative change. Slit lamp examination was performed in all patients and failed to show Lisch nodules, cataract, or retinal changes. A single patient had abnormalities of the fingernails (fig 2); no other dysmorphic features were seen.

MR imaging studies of the tumours showed T2 bright and T1 isointense masses which were often poorly or irregularly enhancing (fig 3). Radiographic progression of tumours was documented in seven patients. Cranial MRI scan was performed in seven patients and thin cuts through the skull base were performed in four patients. A single patient (BNF4) had enhancement along cranial nerves consistent with tumour formation (fig 4).

Pathological review was conducted on archival material from 23 of the 41 surgical procedures, including two specimens from potentially affected relatives. All specimens were neurofibromas (table 1). Two patients (BNF1 and BNF3) had neurofibromas that were classically plexiform (fig 5A), in addition to having typical non-plexiform neurofibromas. Two patients (BNF4, BNF15) and two affected relatives (the mother of BNF2 and the brother of BNF10) had diffusely infiltrative neurofibromas that were suggestive, but not diagnostic, of plexiform neurofibroma. All tumours were characterised by elongated, wavy nuclei, often in a prominent myxoid background (fig 5B). Both tumours from one patient (BNF15) had marked collagen

Figure 4 Imaging studies of patient BNF4. (A) Irregularly enhancing mass filling the right cavernous sinus (C) and extending forward into the orbit (arrowheads) causing proptosis. The right carotid artery is occluded at the level of the sinus. There is less prominent, irregular enhancement in the left cavernous sinus. T1 weighted, contrast enhanced image. (B) Multiple paraspinal masses at the level of L4 (arrowheads). T2 weighted image.

deposition. None of the tumours had defined capsules and many diffusely involved the affected nerves. Mitotic activity or necrosis was not noted in any specimen. None of the tumours had cytological or architectural features suggestive of schwannoma, such as Antoni A and B areas or Verocay bodies.

Discussion

In this report, we describe the clinical characteristics of 10 subjects with multiple, pathologically proven neurofibromas who do not have other diagnostic features of NF1 or NF2. These patients presented with a combination of pain and neurological disability that required frequent intervention and often prevented them from working. There was clear heterogeneity in tumour distribution, with five patients having anatomically localised tumours, and two patients having only cutaneous tumours. Detailed physical examination and imaging studies confirmed the lack of other features of the commonly recognised forms of NF. Pathological examination failed to show any distinguishing characteristics of their tumours; both plexiform and non-plexiform neurofibromas were seen. By the patients' reports, affected relatives were rare, and we were able to confirm a similarly affected relative in only two cases.

Careful classification of the neurofibromatoses has been essential to both natural history studies and the successful cloning and characterisation of the disease causing genes. Because of the pathology of their tumours, these patients were closely evaluated for the possibility of NF1. They met neither the original NIH diagnostic criteria⁷ nor the proposed revision.⁸ Especially remarkable is the lack of CAL spots (seen or reported in nearly 100% of adults with NF1), skin fold freckling (seen in 90%), and Lisch nodules (seen in 96%).⁹ Neurofibromas are only rarely associated with NF2, and none of our patients had features suggestive of NF2, such as vestibular schwannoma, meningioma, or cataract. A single patient had an NF2 affected child, but we were able to exclude the diagnosis of NF2 in the patient using molecular methods.

In addition to NF1 and NF2, a number of variant forms of neurofibromatosis have been identified. Patients with marked anatomical and

often dermatomal limitation of their tumours may represent segmental inactivation of the *NF1* tumour suppressor.¹⁰ Although we did not observe any patient with strict dermatomal limitation, our patients BNF1, BNF9, BNF14, and BNF15 did show a localisation that suggests mosaicism. Several reports have been made of persons with anatomically limited tumours, which are all cutaneous and appear late in life.^{11 12} Interestingly, two of our patients had only cutaneous tumours (BNF11 and BNF12), though neither had obvious anatomical limitation. The remainder of our patients had no cutaneous tumours, suggesting that cutaneous tumour formation may represent a distinct pathophysiological pathway. Finally, sporadic plexiform neurofibroma (in presumed genetically normal subjects) may adopt a complex anatomy which mimics multiple tumour masses. Without molecular analysis, we cannot completely exclude a single tumour in patients BNF1, BNF9, BNF14, and BNF15. However, we feel this is unlikely based on the radiographic appearance of the tumour burden.

There have been a number of recent studies suggesting that spinal neurofibromatosis is a separate entity. Closer evaluation of the families, however, suggests that many meet the diagnostic criteria for NF1. Pulst *et al.*¹³ reported two families with multiple spinal neurofibromas in the absence of cutaneous tumours, vestibular schwannomas, and Lisch nodules. In one of the families, however, all of the affected members had more than six CAL macules in association with their spinal neurofibromas. A similar family with spinal neurofibromas and CAL macules was reported by Poyhonen *et al.*¹⁴ The three generation family reported by Ars *et al.*¹⁵ also meets the criteria for a diagnosis of NF1 and indeed was found to have a typical truncating mutation of the *NF1* gene. All affected family members had spinal neurofibromas, CAL macules, and either Lisch nodules or cutaneous neurofibromas. Spinal neurofibroma may, in fact, not be unusual in NF1 patients with or without symptoms.¹⁶

Careful pathological review is especially important in the classification of the neurofibromatoses. This is perhaps best illustrated by the observation that NF1 patients rarely, if ever, develop benign schwannomas and NF2 patients rarely develop neurofibromas.^{4 5 17} In our review of the pathology of these patients and their affected family members, we found no patient with a schwannoma and no patient with malignant elements in their tumours. In clinical practice, pathological review of tumours in these patients was essential because their presentation was similar to those of patients with schwannomatosis (tumours often in an anatomically limited distribution and pain greater than neurological disability¹⁸). Interpretation of published reports of unusual NF phenotypes should be made with caution if detailed pathological descriptions are not given.

In summary, we present the clinical findings of 10 patients whose unifying feature is the presence of multiple neurofibromas without other stigmata of NF1. Those patients most severely affected have a unique, generalised

Figure 5 (A) Low power photomicrograph of plexiform neurofibroma from patient BNF3. The left half of the figure shows one ill defined nodule of neurofibroma, with a second, well defined fascicle in the right side of the figure. (B) High power photomicrograph of neurofibroma from patient BNF2. Note the wavy nuclei and myxoid background.

phenotype that at times was familial. Other patients may represent a mosaic form of NF1 with either localised manifestations or a generalised but attenuated form of the disease. Our current work is focused on identifying additional patients, clarifying the natural history of this phenotype, and studying patients' blood and tumour specimens in an effort to determine its molecular genetic aetiology.

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B cell immunodeficiency, distal limb abnormalities, and urogenital malformations in a three generation family: a novel autosomal dominant syndrome?

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We report on a three generation family with four affected members presenting with a combination of B cell immunodeficiency, distal limbs abnormalities, genitourinary malformations, and mild dysmorphic features. All affected patients had normal intelligence and growth. No chromosomal abnormalities were observed using both standard and high resolution banding methods on the patients' lymphocytes. The observation of affected subjects

of both sexes along with the occurrence of one male to male transmission suggests autosomal dominant inheritance of the trait with marked intrafamilial variable expression of the disease. While several multiple congenital anomalies (MCA) syndromes include both skeletal dysplasia and immune deficiency, the striking combination of congenital anomalies presented here, for which we propose the acronym BILU (B cell Immunodeficiency, Limb anomalies,