Chapter 3
The Neurofibromatoses: Differential Diagnosis and Rare Subtypes

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Introduction

Accurate diagnosis of the type of neurofibromatosis is important for patient management and genetic counseling. In the majority of people with neurofibromatosis type one (NF1) and type two (NF2), the diagnosis is straightforward. In a specialist neurofibromatosis clinic, approximately 2% of new NF1 referrals will have an alternate non-NF diagnosis and 5% will have a specific NF subtype. This chapter reviews the differential diagnosis of and conditions related to NF1 and NF2.

The critical features used to differentiate the different types of NF are:

- The presence/absence of café au lait (CAL) spots with/without skin fold freckling
- The presence/distribution/histology of benign nerve tumors
- Eye features (often asymptomatic)

These disease features develop at different ages[1,2] (Table 3.1) – in NF1 the main feature in early childhood are the CAL spots which are nearly always obvious by age two. Conversely, in an adult the dermal neurofibromas become the key feature and CAL spots are known to become less obvious/decrease in number with age. With modern neuroimaging NF1 and

R.E. Ferner et al., *Neurofibromatoses in Clinical Practice*,
DOI: 10.1007/978-0-85729-629-0_3,

Table 3.1. Distinguishing the major forms of neurofibromatosis: key features and age they develop.

NF Type	Gene/chromosome	Pigmentary changes	Peripheral nerve tumors	Ophthalmic features
NF1	*NF1*/17	*CAL spots*: most patients have ≥ 6 or more spots by their second birthday. Numbers increase to early teens but CAL fade and disappear in some adults *Skin-fold freckling*: tends to develop from around third birthday, usually obvious by early teens except the submammary freckles in women which develop as breasts enlarge	*Dermal* (*cutaneous*) *neurofibromas*: develop mainly from teens onward and are main cutaneous feature in adults. Present in majority of NF1 adults, who can have several hundred *Nodular (subcutaneous) neurofibromas*: subcutaneous lesions on peripheral nerves. Develop mainly from teens; present in only a proportion of patients (5–10%) *Plexiform neurofibromas*: externally visible plexiform neurofibromas present in 25–30% patients, usually obvious by age 10 and major disfiguring lesions on face by 3 years, elsewhere by 5 years	*Lisch nodules*: develop during childhood and present in ~95% adult patients

Legius syndrome	*SPRED1*/15	Exactly the same as in NF1	NONE	NONE
NF2	NF2(MERLIN)/22	*CAL spots*: present in 43% of cases in one large series BUT only 1% had six; 24% had one spot, 11% had two spots, and 7% had 3 or 4	*Overall*: Peripheral Schwannomas present in 68% of patients BUT only 10% have >10 and in one large study maximum number was 27	Lens opacities/ cataracts
		Skin-fold freckling: not seen in NF2	*NF2 plaques*: present in 48% of patients and develop from early childhood	Amblyopia (no obvious cause)
			Peripheral nerve schwannomas: present in 43%	Astrocytic hamartomas
			Dermal (cutaneous) schwannomas: look exactly like dermal neurofibromas in NF1, present in 27% but in much lower numbers than in NF1	Combined retinal and retinal pigment epithelium hamartomas
Schwannomatosis	INI1/SMARCB1/22	NONE	*Peripheral nerve and spinal nerve root schwannomas*: usually develop from teens onward	NONE

NF2 are rarely confused in adults but NF1 is often the first diagnosis considered in children presenting with NF2-related skin changes. Very occasionally a major NF1 complication may present before the CAL spots are obvious – I have met parents who have been accused of nonaccidental injury prior to the correct diagnosis of tibial pseudarthrosis been made.

Although molecular genetic testing is now available for NF1 and NF2, for neither condition is mutation detection 100% and the presence of genetic mosaicism in sporadic cases complicates the situation further.[3-6] Prior to any molecular testing full clinical evaluation, with radiological and histological review if indicated is essential. In patients where things do not fit together, review in a specialist NF clinic may be of benefit prior to any molecular genetic testing.

Case History: The Importance of Clinical Diagnosis Prior to Genetic Testing

A 14-year-old girl was referred for genetic assessment with possible NF1. The previous year she had several tongue lesions removed which were reported as neurofibromas and the question of NF1 raised. Around the same time she had had some eye problems and thickened corneal nerves noted coincidentally. She was referred for pediatric assessment. She had one CAL spot. NF1 gene testing was requested and no mutation identified. She was then referred for genetic assessment.

There was no significant family history. The preoperative pictures of her tongue lesions showed multiple small papillomatous lesions. On examination the only skin feature was the CAL spot but she was thin with hyperextensible joints and prominent lips. She had an asymmetrically enlarged thyroid gland. A clinical

diagnosis of MEN 2B was made and this was confirmed by the finding of the M918T mutation in the *RET* proto-oncogene. Investigations and subsequent surgery diagnosed metastatic medullary thyroid carcinoma.

Key Points

- The tongue is not a common site for small neurofibromas in NF1; plexiform lesions of the area show diffuse enlargement of the tongue.
- One CAL spot is within normal limits.
- Thickened corneal nerves are classically associated with multiple endocrine neoplasia type 2B not NF1.

Clinical Assessment

The points I highlight in this section are those that assist in establishing the type of NF. Some patients will have clinical symptoms/signs that take clinical priority – for example a patient with NF1, NF2, or schwannomatosis may present with symptoms/signs of cord compression which requires urgent assessment. Even then it is usually possible to diagnose the type of NF during the same assessment and this allows perioperative planning, for example, in sporadic cases of NF2 and schwannomatosis collection of fresh frozen tissue for DNA analysis is helpful. Figure 3.1 shows a flow chart to aid in assessing a child with multiple CAL spots.

Past Medical History

Regardless of the age of the patient, I find it useful to take a full medical and social history from birth onward. This builds up a picture of the age of appearance of key disease features, any major medical problems, and whether learning and behavior problems have been an issue.

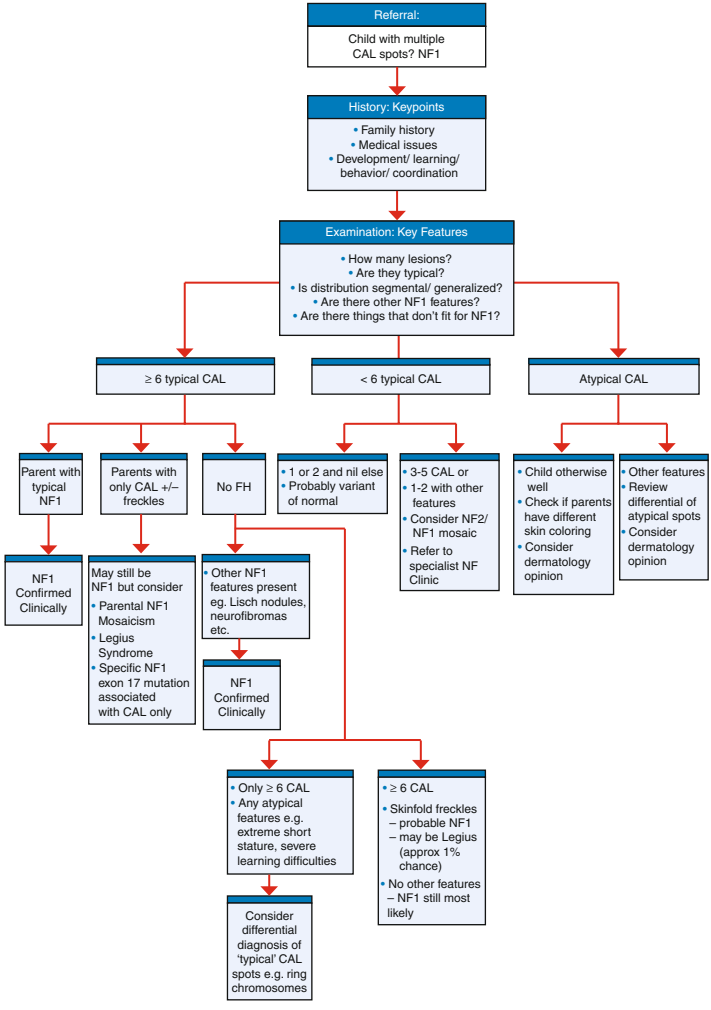

FIGURE 3.1. Diagnostic approach to a child with multiple CAL spots.

Family History

In both NF1 and NF2 approximately 50% of patients are the first in their families and the diseases are fully penetrant (so they do not "skip" generations in the pedigree[6]). Recording the family tree, going back to the grandparents of the index case, allows you to:

- Build up a picture of the family's experience of the disease.
- Identify which family members need assessing.
- Identify any unusual features – for example, affected siblings/cousins with NF1 and unaffected parents are very unusual. Prior to the recognition of the constitutional mismatch repair deficiency phenotype (CMMR-D, which is discussed later in the chapter[7]), several of the families had been presumed to have NF1 because of multiple CAL spots in one or more sibs/cousins. In retrospect the clues to the alternate diagnosis were the family history of cancers seen in hereditary nonpolyposis colon cancer and consanguinity.

Examination

The main systems that assist in the differential diagnosis are examination of the skin, eyes, and nervous system; in possible NF1 this is complemented by checking for disease complications that may be present in a patient of that age. In assessing NF-related skin pigmentation, an ultraviolet light is rarely necessary other than in very pale skinned people. Eye examination needs to be done by an Ophthalmologist familiar with NF – a slit lamp is needed to distinguish Lisch nodules from the iris nevus and the lens opacities in NF2 are often only seen by slit lamp.[8]

Radiology/Histology Review

In atypical cases the value of having radiology and histology reviewed by colleagues familiar with NF cannot be understated. Radiologists familiar with neurofibromatosis may

pick up subtle features on review which point to the subtype. Neurofibromas and schwannomas can often not be differentiated clinically or radiologically and then histology is vital.

NF Pigmentary Changes: Key Clinical Features

Café au Lait Spots

CAL spots or patchy pigmentation of other kinds[9] are listed as features of a large number of genetic syndromes, of which NF1 is by far the most common. What is important clinically is to ensure that what are being labeled as CAL spots are typical for NF1. Figure 3.2 shows different kinds of CAL pigmentation. The key features of CAL in NF1 are[6,9]:

- Macular (flat), any lesion with any thickening is not a CAL spot.
- They usually have an oval shape with a smooth edge and the pigmentation is uniform within the spot.
- In pale skinned individuals they are coffee colored, whereas in dark skinned individuals they may be dark brown or black.
- They may be present at birth but more usually develop in the first few months of life and are nearly always obvious by the second birthday.
- They grow with the child, becoming larger with age.
- They can occur anywhere on the body but are mainly seen on the trunk and limbs; they are rarely found on the face, scalp, palms, and soles.
- Very small, nonsignificant patches are not counted; the NF1 diagnostic criteria use specific sizes for inclusion, which is measured across the maximum diameter of the lesion: in prepubertal lesion spots of >5 mm are counted and in postpubertal >1.5 cm.
- The spots tend to be 2–5 cm in diameter but can be larger. If a patient has a much larger area it needs to be monitored as this can be a marker for an area where a plexiform will develop.

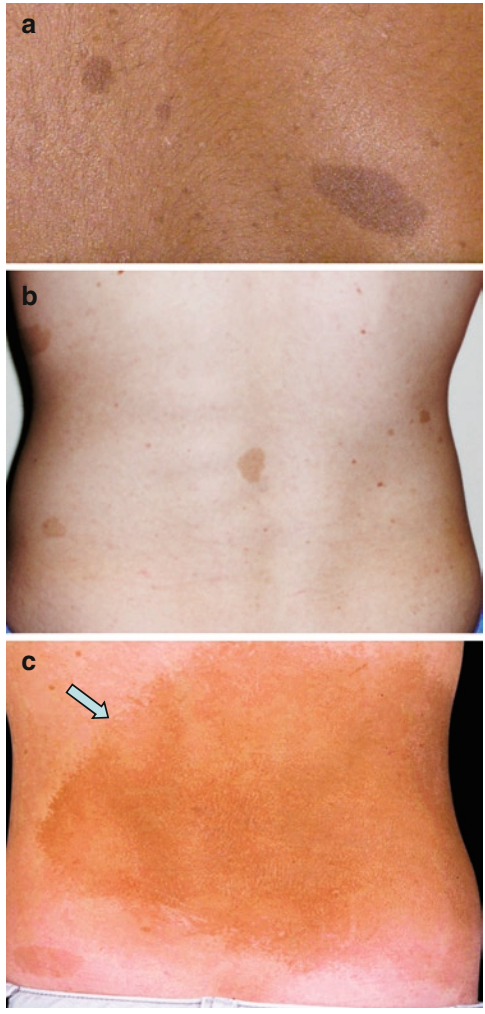

FIGURE 3.2. Different kinds of CAL pigmentation (**a**) Typical NF1 CAL spots in an Asian patient: note relatively smooth outline and even pigmentation. (**b**) CAL spots in a patient with Legius syndrome, exactly the same as in NF1 (reproduced with permission from Spurlock et al.[10]). (**c**) Large CAL with irregular outline and hypertrichosis overlying a large internal plexiform neurofibroma. (**d**) Atypical CAL lesion in an Asian child with ataxia telangiectasia: note irregular outline and uneven depth of pigmentation.

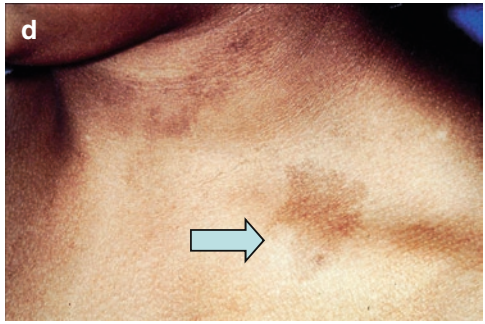

FIGURE 3.2. (continued).

- The NF1 diagnostic criteria require 6 or more CAL spots and NF1 children often have many more; 10% of the general population have one or two CAL spots. When children present with 3–5 CAL spots (which is uncommon) I usually monitor them through childhood because of the rare possibility they have NF2 or are one of the rare cases of NF1 with <6 CAL.
- NF1 CAL spots have no distinguishing pathological features; biopsy is not a helpful aid to diagnosis.
- CAL spots become paler and can disappear with age.
- CAL spots are not at risk of malignant change and their number does not correlate with disease severity in any way.
- Although patients with NF2 have CAL spots more frequently than the general population, it is very unusual for them to have six or more (1%) and they do not get skin fold freckling.

Skin Fold Freckling

Why NF1 patients develop freckles in very specific areas of the body remains unexplained but clinically it is an important aid to diagnosis. The freckles, like the CAL spots, never do any harm but are an important diagnostic feature. They develop after the CAL, usually from around the 3rd birthday and can affect the axillae, base of the neck, and groins. Women may develop them below the breasts and overweight

people in skin folds. Some patients develop freckles over their whole trunk.

Peripheral Nerve Tumors in NF: Clinical Clues to Diagnosis

Neurofibromas Versus Schwannomas

The important thing to remember in assessing lesions in patients referred as "query form of NF," is that it is not possible to distinguish individual neurofibromas and schwannomas clinically (Fig. 3.3). As reviewed in Chap. 1, the appearance and problems associated with neurofibromas are dependent on where in the nervous system they develop (dermal (cutaneous),

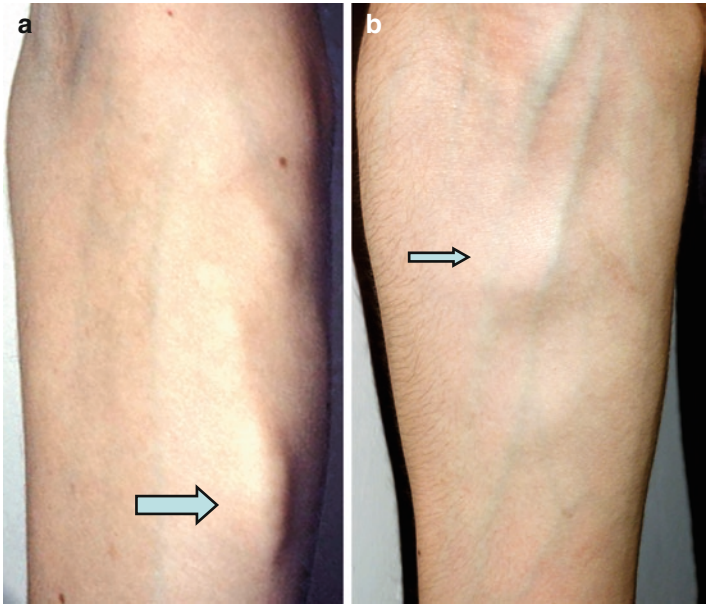

FIGURE 3.3. Peripheral nerve lesions (arrowed) in the forearm: (**a**) The patient has NF1 (neurofibromas); (**b**) the patient has NF2 (schwannomas) – clinically they are indistinguishable.

peripheral nerve, or spinal nerve root). Schwannomas in NF2 at the different sites present very much like neurofibromas. The only tumor that virtually always has a unique appearance is the NF2 plaque lesion described in the next section.

The problem in distinguishing neurofibromas and schwannomas clinically is usually not an issue as other diagnostic features of the type of NF will be present. The times when it becomes important are in assessing patients with segmental/mosaic phenotypes presenting with just localized nerve involvement when histology is usually necessary. The other cases that can cause confusion are cases with NF2 and marked skin involvement. These tend to be people with severe NF2 with no family history where dermal or peripheral nerve lesions present before cranial or spinal lesions. Although schwannomas are not seen in NF1, neurofibromas can occasionally occur in NF2 or tumors with a mixed neurofibroma/schwannoma picture are reported. Rare patients with severe NF2 can develop plexiform lesions in childhood and these too are indistinguishable from those in NF1 clinically, although are usually plexiform schwannomas histologically. The clues clinically are the reduced number or lack of CAL spots, specific eye signs, and identification of NF2 plaques if present.

NF2 Plaques

These are the one specific cutaneous lesion that are an invaluable clinical clue.[11] Initially they can look like small CAL spots – the skin has a brown–orange color; the difference is that the skin is slightly thickened and there is often excessive hair growth (see Fig. 2.10, Chap. 2). They rarely grow beyond 1–2 cm in diameter. Histologically they are usually described as having "schwannomatous elements." When a child presents with an NF2-related eye problem, isolated peripheral nerve amyotrophy or NF2-related cranial or spinal tumor, clinical examination for NF2 plaques is essential. The youngest patient I have seen with one was 18 months; they presented with unilateral amblyopia and a dysplastic optic disk which was recognized by an Ophthalmologist familiar with NF2.

Eye Features of NF

The eye features in the main forms of NF are summarized in Table 3.1. As mentioned above, the critical thing here is to ensure that patients are being assessed by an Ophthalmologist familiar with the different forms of NF.[8] Although a few NF1 patients have so many Lisch nodules that can be seen by ophthalmoscope, one cannot rely on just ophthalmoscopic examination to say they are absent.

NF1 Differential Diagnosis: The NF1/NF2 Overlap

The reasons NF1 and NF2 were lumped together historically are because of overlapping skin features and the difficulty in distinguishing, both clinically and radiologically, neurofibromas and schwannomas. It is very unusual for people with NF1 to be diagnosed as having NF2, but "?NF1" is often the initial diagnosis in children presenting with NF2-related skin changes. The key things to check here are:

- Are there any NF2 plaques?
- An ophthalmic examination
- Histology review of any lesions removed if described as neurofibromas

Why early recognition of NF2 is important is because the NF2-related VS can get to a considerable size before causing hearing loss. I have seen numerous patients diagnosed in their late teens, presenting with significant brain stem compression, who have either had ophthalmic review for NF2-related problems in early childhood or who have had skin lesions removed over the years, the significance of which were not appreciated.

When I began working with NF families in the 1980s, misdiagnosis between NF1 and NF2 was common. Fortunately with increased awareness and improved neuroimaging the situation has improved. Despite this, within the last 5 years, patients under our care have been misinformed by colleagues. For

example, a man with NF2 and multiple dermal (cutaneous) schwannomas was told he must have NF1 and NF2 and the possibility of NF2 was queried in a girl with severe spinal nerve root involvement and NF1. The patient was so alarmed by this that we had to undertake genetic testing to provide reassurance.

Case History: Delay in Diagnosis in a Child with NF2

A 10-year-old boy was referred with a diagnosis of possible NF2. There was no family history. He had been reviewed for various cutaneous lesions since the age of 5 in dermatology, pediatrics, and plastic surgery. He only had 2 CAL spots and NF1 had been thought to be unlikely. The lesions removed were initially reported as neurofibromas; however, at the age of 9 a lesion was removed and was thought to be atypical for a neurofibroma. Expert review diagnosed a plexiform schwannoma. The possibility of NF2 was considered.

Cutaneous examination showed several NF2 plaques and nodular (subcutaneous) peripheral nerve lesions. His first cranial scan showed bilateral vestibular schwannomas of significant size; despite this, he had only just started to notice any hearing problems. Mutation testing identified a nonsense mutation in the NF2 gene.

Key Points

- Dermal (cutaneous) and peripheral nerve lesions in the absence of ≥6 CAL spots make NF2 a real possibility.
- The vestibular schwannomas in NF2 can grow to a considerable size and not affect hearing.
- The NF2 plaque is an invaluable diagnostic feature in childhood.
- If the tumor histology does not fit the clinical picture, ask for review.

NF1 Subtypes

Introduction

These are all caused by germline or somatic mutations in the *NF1* gene except Legius syndrome, which is caused by mutations in another RasMAPK gene, *SPRED1*. The importance of recognizing the different types is that they have either specific genetic implications (e.g., the much lower recurrence risk in segmental NF1) or a different natural history (e.g., very mild in the CAL-only phenotypes, consistently more severe in microdeletion patients and spinal NF1). At most these subtypes probably account for around 10% of the NF1 group. In the majority of families NF1 is extremely variable in its manifestations even WITHIN the family.

Segmental/Localized NF1

The term segmental or localized NF1 is used to describe the patients with disease features limited to one or more body segments. The estimated disease prevalence is between 1 in 36,000–40,000 individuals in the general population.[12] Most patients are asymptomatic and seek medical opinion because of the unusual appearance of the skin. In the majority of patients the area involved is unilateral and varies in size from a narrow strip to one quadrant and occasionally one half of the body. Some patients have more than one segment involved on both sides of the midline, either in a symmetrical or asymmetrical arrangement. Within the affected area the patients either have NF1-related pigmentary changes, neurofibromas alone, or both. Patients may also present with isolated plexiform neurofibromas and no other disease features.

NF1-related pigmentary changes are the most common phenotype. In a number of patients the whole segment of affected skin is darker and within this CAL spots and freckles develop. The segment of pigmented skin may be the presenting feature in infants and the NF1 changes develop within the segment with time.

The frequency of NF1 complications is much lower in segmental cases (only 7% in one series[12]). If the phenotype includes neurofibromas on major peripheral nerves or a major plexiform there is still a risk of malignant change.

In the Manchester clinic we offer annual review until late teens and then adjust follow-up according to phenotype. If there is internal involvement of significance we follow as we would in generalized NF1. We advise uncomplicated patients that the risk of associated problems is low, but if unusual symptoms develop always to ensure the Doctor they are seeing is aware of their segmental NF1 diagnosis.

Genetically, the phenotype results from somatic mutation in the NF1 gene, with the manifestations depending on the timing of mutation in embryonic development. The importance of recognizing this group is for their different natural history and because they have much lower recurrence risks in offspring. In my own practice I use an empiric recurrence risk of 5% at most, unless the portion of the body affected is particularly large. It is exceptional to find a mosaic gene mutation on analysis of lymphocytes and it is usually necessary to perform NF1 mutation analysis in schwann cells derived from neurofibromas or melanocytes from the CAL spots of the affected segment to identify the causal mutation.[13] From a clinical viewpoint, patients with segmental NF1 sometimes find the small, but definite risk of a child with generalized NF1 too big a risk. In these cases mutation analysis on affected tissue can define the mutation so that prenatal testing can be offered.

When counseling parents of a child with newly diagnosed NF1 about recurrence risks, it is my practice to examine the skin and irides of the parents. Most affected parents report their skin changes, even though they may not have been formally diagnosed before. However, I have very occasionally found areas of segmental NF1 change the significance of which the parent had not appreciated. If the parent's examination is completely normal, then I give a recurrence risk of much less than 1%. There have been very few reported cases of pure gonadal mosaicism in NF1[4]; in my own practice I have seen only one family with two affected children and we found they had different NF1 mutations.

Generalized Mosaic NF1

As Professor Evans reviews in his chapter on NF2, up to one third of sporadic cases of NF2 are mosaics, presenting with mild disease which is more usually generalized than limited to a body area. One of the ways the significance of mosaicism in NF2 was highlighted was that the number of affected children born to sporadic cases was less than the expected 50%.[14] Although early NF1 studies such as my own[15] found no evidence of this in NF1, the general awareness of NF1 at the time was so limited that one would only expect sporadic cases with very obvious NF1 to be diagnosed. The other pointer to a higher frequency of mosaic cases is a lower mutation detection rate in sporadic cases than in the second generation of familial cases; although NF1 series have given conflicting results to date (reviewed in Kehrer-Sawatski and Cooper[4] and Ruggieri and Huson[12]) this could still be due to only obviously affected sporadic cases being tested. Mosaicism in sporadic NF1 microdeletion cases, particularly type two deletions, is well recognized.[16] With improved mutation detection techniques and awareness of the importance of recognizing mosaicism for genetic counseling, it is likely more cases of nondeletion sporadic NF1 will be found to be mosaics. Muram-Zborovski et al.[17] report a father and son with only CAL spots who they thought may have Legius syndrome. Molecular analysis showed no SPRED1 mutations, that the boy had an NF1 mutation which his father was mosaic for on lymphocyte analysis.

The NF1 Microdeletion Syndrome

Up to 5% of NF1 mutations are large deletions of both the *NF1* and a variable number of flanking genes. The clinical importance of the deletions is that they are associated with a more consistently severe phenotype. Clear delineation of genotype/phenotype has been hampered as some reports contain detailed molecular analysis with limited clinical detail and vice versa. Fortunately with larger studies[18,19] and studies which include a review of all previously

Table 3.2. Clinical features associated with the common type one NF1 microdeletion.

Feature	Frequency/comment
Dysmorphic facial features: hypertelorism, downslanting palpebral fissures, broad fleshy nose, "coarse" face becoming more marked with age	26/29[19]
Overgrowth with tall stature and large hands and feet	13/28[19]
Other dysmorphic features	
Pectus excavatum	9/29[19]
Broad neck	9/29
Excess soft tissue in hands and feet	12/24
Musculoskeletal features	
Joint hyperflexibility	21/29[19]
Muscular hypotonia	13/29
Bone cysts	8/16
Pes cavus	5/29 (only reported in Mautner series[19])
Neurofibroma burden	Dermal (cutaneous) neurofibromas consistently reported to occur at an earlier age and in increased numbers Mautner et al. report increased frequency of all types of neurofibroma compared with general NF1 population including spinal neurofibromas
MPNST	6/29[19]; De Raedt et al.[23] estimate double lifetime risk of general NF1 population
Learning and development	Significant delay in cognitive development 14/29 with IQ < 70 in 8/21[19] Learning difficulties 13/29 Mean IQ lower than general NF1 population by 12.5 points (76 in microdeletions compared with 88.5[24])
Other features which may occur in excess	Congenital heart disease[21,22] Scoliosis[19]

Source: Data compiled from Mautner et al.,[19] Venturin et al.,[21] Mensink et al.,[22] De Raedt et al.[23] and Descheemaeker et al.[24]

published cases[20-22], the phenotype, particularly for the common, type one, deletion is evolving (Table 3.2, Fig. 3.4). There have also been additional studies comparing one particular aspect of the deletion phenotype with the general NF1 population – IQ, growth, and frequency of MPNST.[23-25]

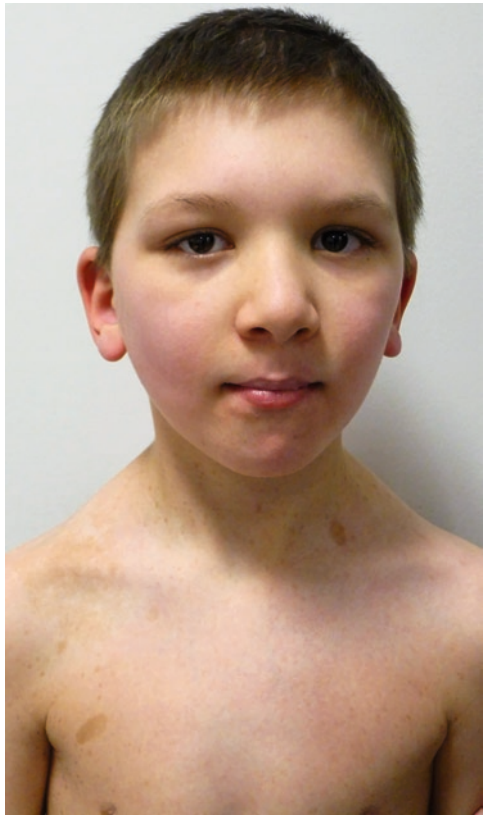

FIGURE 3.4. A child with a NF1 microdeletion: note the broad nasal bridge, slight downslanting palpebral fissures, low set ears, and high trapezius insertion giving appearance of broad neck.

Molecular Basis of Deletions

At a molecular level there are three recurrent deletions.[18] The most common type one deletion is a 1.4 Mb deletion with 14 additional genes deleted, caused by nonallelic homologous recombination between two regions of low copy repeats; these deletions are rarely mosaic. Type two deletions are often mosaic, and there is a 1.2 Mb deletion with 13 additional genes. The deletion is caused by recombination between the *SUZ12* gene and its pseudogene which are on opposite sides of the NF1 gene. The smallest recurrent deletions (type three) have only recently been identified[18] and are only 1 Mb in size with eight additional deleted genes and the breakpoints lie within the same distal but a different proximal region of low copy repeats as the type one deletions. In addition there are a number of patients reported with atypical, unique deletions of varying sizes. Laboratory-based studies estimate microdeletion account for 5–10% of NF1 patients but this may represent overascertainment of severely affected cases.

The Clinical Phenotype

The most consistent phenotype is seen in the common type one deletion.[18-22] Mosaicism is common in type two deletions and can result in a milder phenotype. The phenotype of the three initial type three cases included facial dysmorphism. The value of these and other atypical cases will be in assessing which flanking gene contributes to a particular disease feature. The most consistent features associated with deletions are:

- *Facial dysmorphism*: Three large series[19,21,22] have reported a much increased frequency of facial dysmorphism in microdeletion cases than in the general NF1 population (52–78% compared with 5–15%). However, all these series included either cases from multiple clinicians or a literature review and mainly combine data from all kinds of deletion. In a large series of type one cases from a single clinic, Mautner et al.[19] report facial dysmorphism in 26/29 cases

(90%). The main features are downslanting palpebral fissures, hypertelorism, ptosis, and a broad fleshy nose (Fig. 3.4). The overall dysmorphic facial appearance is described as coarse and this becomes more marked with age.

- *Developmental delay and learning problems*: Microdeletion patients are often ascertained because their degree of developmental delay and subsequent intellectual development is more severe than in NF1 as a whole. The NF1 deletion children often exhibit delayed early motor milestones, which is unusual in nondeleted patients. One study[24] looked specifically at type one deletion patients and found a full scale IQ difference of 12.5 points compared with a nonmicrodeletion group (mean IQ 76 in microdeletion group and 88.5 in nondeletion group). In their series of type one patients, Mautner et al.[19] reported significant delay in cognitive development in 14/29, with a further 13 patients having learning problems. Of the 21 who had formal IQ measurements, the mean IQ was almost the same as in the Belgium study (76.9), with 8/21 (38%) having an IQ <70. They also report a possible increase in muscular hypotonia (45%) and speech difficulties (48%).
- In series where CNS imaging is available there has been a suggestion of an increased frequency of structural brain anomalies.[19,26]
- *Excessive neurofibroma burden and MPNST*: From the earliest reports of deletions one of the major features was that patients tended to have early onset of appearance and excessive numbers of neurofibromas.[22] This has always been my clinical impression but data from two recent large series are conflicting. Mautner et al.[19] report an increased frequency of all types of neurofibromas: cutaneous, subcutaneous, plexiform, and spinal. Whereas Pasmant et al.[18] reporting on a large multicentre cohort found no significant increase compared with nondeleted patients. My own clinical experience would support Mautner's findings, in that 3 of 15 cases ascertained through the Oxford NF clinic had had surgery for cervical nerve root neurofibromas and our adult microdeletion patients have all had a large dermal neurofibroma burden.

- Patients with the common microdeletions are also at an elevated risk of MPNST. The lifetime risk of MPNST in NF1 as a whole is in the region of 8–13%; De Raedt et al.[23] estimated that the deletion patients have double this risk. In the Mautner series,[19] 6/29 (21%) type one deletion cases had developed MPNST.
- *Cardiovascular abnormalities*: A variety of congenital heart problems have been reported in cases with typical deletion sizes, including atrial and ventricular septal defects, patent ductus arteriosus, pulmonary stenosis, dilated aortic valve, hypertrophic cardiomyopathy, and mitral valve prolapse.[21] However, no one specific lesion has emerged as being more frequent in the larger series and so it is difficult to know their significance.[18,19] However, there is enough concern to warrant careful cardiac examination, even in the absence of symptoms. One of the patients reported by Mensink et al. developed bacterial endocarditis in previously undiagnosed mitral valve prolapse.[22]
- *Stature*: Another feature that makes many deletion cases stand out in the NF1 clinic is the fact they are taller than average. In my Welsh population study, 31.5% of patients were at or below the third centile for height and we showed that the NF1 children were 7–8 cm shorter than their affected siblings. In contrast 19/114 reported deletion cases[22] had tall stature/overgrowth. In the Mautner series,[19] 46% of the patients were ≥94th centile for height. Spiegel et al.[25] showed the growth of their cohort (19 with a type one and two with a type two deletion) of deleted patients showed a distinct pattern of childhood overgrowth. They speculated that a gene associated with overgrowth lay within the deletion. This was subsequently proven by the finding of mutations in *RNF135* in individuals with significant overgrowth who did not have NF1.[27] In the Pasmant series the four patients whose deletion excluded RNF135 were not overgrown.[18]
- *Other skeletal and connective tissue features*: The patients with deletions who were tall in the Mautner series also had large hands and feet.[19] In the same series 5/29 patients had pes cavus which had not previously been reported. The other probable new association was of an increased

frequency of bone cysts. In their case series and literature review, Mensink reported large hands and feet in 25/114 cases.[22] The palms of the hands were soft and fleshy with an excess of connective tissue in half of Mautner's series. Joint hypermobility also seems to be commoner in the deletion cases (72 and 58% frequency in two large series[19,22]). Other skeletal features that probably occur more frequently in the deletion patients are pectus excavatum and a broad neck with downslanting shoulders.[19,22]

- Scoliosis may also be more common in deletion cases than in the general NF1 population although the data, even from large series, are conflicting.

Should All NF1 Patients Be Tested for Deletions?

In the Manchester clinic at the present time we do not offer routine mutation testing. If a patient has any dysmorphic or other clinical features which suggest a deletion we recommend testing. However, the series of Mautner et al.[19] highlights that even within the same type one deletions there is variability and a case could be made that all newly diagnosed patients should be checked for a deletion. I endorse the conclusions of Mautner et al.[19] that once identified this group of NF1 patients require increased clinical and psychological support.

Spinal NF1

The importance of recognition of this rare NF1 subtype[28-36] is because of the consistent presence of multiple spinal neurofibromas, usually bilateral and involving all 38 spinal nerve roots, which is extremely uncommon in ordinary NF1 (Fig. 3.5). In most NF1 families, just one person will develop a symptomatic spinal tumor, whereas in spinal NF1, the phenotype has largely been consistent in reported families. Furthermore, in spinal NF1 there may be marked involvement of major peripheral nerves – MPNST have been reported both in spinal and peripheral nerve lesions in these patients.[28,33]

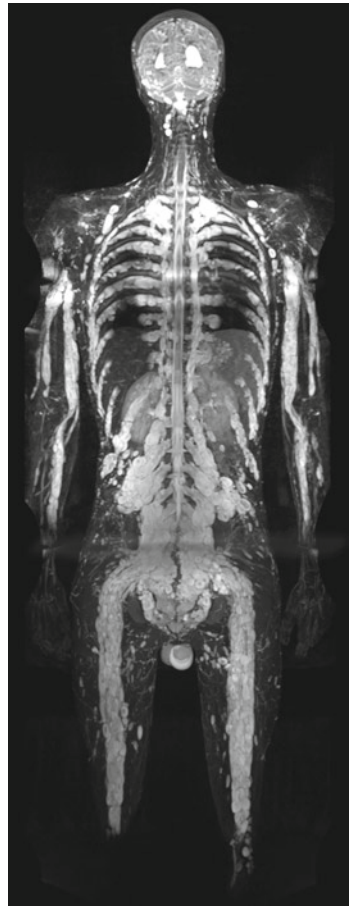

FIGURE 3.5. A maximum intensity projection reformat of a coronal STIR whole body acquisition whole body MRI of a patient with spinal neurofibromatosis: note extensive involvement of spinal nerve roots and major peripheral nerves.

The other consistent feature in the reported families has been that other major NF1 features and complications are usually absent. The exception to this is café au lait spots, although some cases have ≤6 and no skinfold freckling. Dermal (cutaneous) neurofibromas are usually absent. However, peripheral nerve involvement can be marked with patients having multiple nodular (subcutaneous) neurofibromas along their course – this can be particularly obvious along the intercostal nerves.

In our own clinic we do whole-body MRI in patients with a suggestive phenotype, proceeding to spinal MRI in those with the phenotype. These patients require close neurological monitoring and have open access to our clinic for any new/changing symptoms. The role of follow-up scans is being determined; at present we are doing two yearly scans or earlier if new signs/symptoms develop.

The molecular basis for this phenotype is still to be elucidated; its existence suggests that the requirement for development of dermal (cutaneous) and spinal lesions may be different. However, consistently, in the reported cases there has been an excess of splice site and missense mutations.[32,35,36] One hypothesis proposed was that these mutations may result in abnormal protein production with a "dominant-negative effect."[30] This cannot be the sole explanation as a number of similar mutations have been reported in patients with typical NF1.[36] Other suggestions have been that of a closely linked modifier gene.[34] The recent report of excess numbers of spinal neurofibromas in microdeletion patients adds some support to this.[19,36] There has also been one family which did not map to the NF1 locus suggesting genetic heterogeneity.[28]

Watson Syndrome

Watson[37] described autosomal dominant inheritance of pulmonary stenosis, multiple café au lait spots, and intelligence at the lower end of the normal range. At that time pulmonary stenosis was not recognized as an NF1 complication and all family members had mild learning problems which is unusual in NF1. A few similar families have since been reported. Follow-up of the original Watson patients confirmed that their phenotype had remained distinct from NF1.[38] Although a few individuals had Lisch nodules on slit lamp examination and some had developed neurofibromas, both of these features were present at a very much lower frequency than is usually seen in NF1. Since then three different mutations in the NF1 gene have been reported in Watson syndrome (an 80-kb deletion, an inframe tandem duplication in exon

28, and the exon 17 3-bp deletion discussed below[39,40], respectively). This suggests that the NF1 mutation alone is not sufficient to explain this distinctive phenotype.

CAL-Only Phenotypes

Riccardi[41] first described families with CAL spots in numbers comparable to NF1, but without Lisch nodules and neurofibromas, although pectus excavatum and nonspecific learning problems did occur. Clinically therefore one can only make the diagnosis in the presence of two or more affected generations. Even then there is the possibility that a mildly affected sporadic parent could be a mosaic. This was therefore a phenotype for which molecular genetic testing has long been awaited.

Two specific and separate genetic mechanisms have now been identified. One is a specific mutation in exon 17 of the *NF1* gene[42] (exon 17 using NF consortium nomenclature; exon 22 using National Center for Biotechnology Information nomenclature) and the other mutations in another gene in the same cellular pathway, SPRED1 on chromosome 15[43] have now been identified associated with this phenotype. The CAL-only phenotypes are relatively uncommon, even in a large NF1 clinic. The importance of recognition for families is the much better natural history, with the removal of most of the concerns associated with NF1 (e.g., How many dermal neurofibromas will develop? What complications can happen?).

Legius Syndrome

Legius syndrome is the most important cause of the "familial CAL" phenotype identified to date. No affected individuals have been reported with any form of neurofibroma or Lisch nodules. Affected individuals have a higher frequency of learning problems than the general population but these have been milder and less frequent than seen in NF1. No consistent association with specific malignancies has emerged so far; the reported tumors are listed in Table 3.3.

Table 3.3. Summary of clinical features of reported cases of Legius syndrome.

Feature	Frequency/comment/references if not compiled from all
Age (years)	
≥18	54/106
≥20	14/40 (Messiaen et al.[44] use a 20 year cut-off)
Familial/sporadic/unknown	129/13/4
CAL spots	
Present	142/146
Adults with ≥6 CAL >1.5 cm diameter	39/61[10, 43-46]
Children with ≥6 CAL >0.5 cm	60/64[43-47]
Skinfold freckling	62/146
Neurofibromas of any sort	0
Lisch nodules	0
Macrocephaly	9/100; two papers record true and relative macrocephaly in 10/55[44,46]; in one series head circumference on higher centile than height in 20/24[46]
Learning and behavior	
Learning difficulties	26/142 (data from Denayer et al.[46])
Delayed psychomotor development (mostly confined to speech delay)	13/142
Hyperactivity, attention problems, or ADHD	14/142
Dysmorphic features	
Noonan-like facies	13/146
Pectus excavatum	13/146
Postaxial polydactyly (unilateral)	3/146
Excess periorbital pigmentation	1/146 (only reported in one case[10])

(continued)

Table 3.3. (continued).

Feature	Frequency/comment/references if not compiled from all
Tumors	
Lipomas	19/146 plus 1 angiolipoma
Single case tumors of uncertain significance as yet	Childhood acute myeloblastic leukemia; abdominal wall desmoid; vestibular schwannoma (patient aged >50); tenosynovial giant cell tumor; ovarian dermoid tumor; nonsmall cell lung cancer; childhood renal cancer (possibly Wilms); colon adenoma (45 years)
Other reported features which can occur in NF1	Scoliosis – four cases reported in series of Denayer et al.[46] but no detailed description Congenital pulmonary stenosis and mitral valve prolapse (same patient[44]) T2 hyperintensities on cranial MRI – reported in a 39 and 11-year-old[46]
Other reported features which can be seen in other RASopathies (one case each)	Inguinal hemangioma[10]; temporal venous anomaly[44]; vascular anomaly leg[44]

Source: Data compiled from Brems et al.,[43] Spurlock et al.,[10] Pasmant et al.,[45] Messiaen et al.,[44] Muram-Zborovski et al.[47] and Denayer et al.[46]

The syndrome was first reported as an "NF1-like syndrome"[43] but has now been named Legius syndrome to reflect its clinical and molecular characterization by the group of Professor Eric Legius and the absence of neurofibromas. In the large Leuven NF1 clinic Professor Legius identified five families with CAL spots, axillary freckling, macrocephaly, and Noonan-like facies in some individuals. No neurofibromas or Lisch nodules were present. *NF1* mutations were not identified in these families and linkage studies

in the two largest families mapped the locus to chromosome 15. In this region *SPRED1* was recognized as an ideal candidate, as it negatively regulates MAPK signaling like neurofibromin. Mutations were found in all five families. They then extended their studies to 86 unrelated patients who had negative NF1 testing and CAL spots +/– freckling only and found 7/86 (8%) had *SPRED1* mutations.

The consistency of the phenotype and the significance of Legius syndrome as a cause of multiple CAL has since been determined through reports from several centers[10,44-47] (Table 3.3). The CAL spots and freckles seen in Legius are exactly the same in appearance and age of onset as in NF1 (Fig. 3.2). A small proportion of individuals with the mutation have had <6 CAL spots; the majority of these cases were adults and four cases have had none (3 adults aged 60, 58, and 37 and a child aged 2 years[43,44,46]). Therefore on assessing families it is important to test both parents of sporadic cases and in any family to offer testing to at-risk individuals with any CAL spots.

Of the 146 reported cases none have had any form of neurofibroma and those examined have not had Lisch nodules. Learning problems, speech delay, and ADHD are associated but at a lower frequency than in NF1. It should be noted that one report of a SPRED1 mutation in a child with an orbital plexiform and sphenoid wing dysplasia has subsequently been retracted as the child did not have a mutation.[48] No other tumors have occurred in more than one patient to date except lipomas. However, we need to wait until larger numbers of people with Legius have been reported until possible associations with a low incidence can be confidently excluded. Messiaen et al.[44] estimate that to exclude rare complications with a prevalence of 1%, data from 250 well-characterized, preferably adult, patients are needed.

The majority of reported cases have been familial. The highest chance of finding a *SPRED1* mutation is in familial cases with ONLY NF1 pigmentary changes. In a cohort of sporadic patients with only CAL +/– freckling but no other NF1 mutations, Messiaen et al.[44] found a *SPRED1* mutation

in 13/414 (1.3)%. However, in a familial cohort they detected 19% to have *SPRED1* mutations (18/94). In both cohorts they found more NF1 mutations (414/957, 44%, in sporadic group and 69/94, 73%, in familial group).

In terms of follow-up of Legius syndrome, given the association with learning problems, we currently follow children annually until the age of 7 years, but thereafter annual review seems unnecessary. In their most recent publication the Legius group suggests 3 yearly review.[46] However, the families should be asked to report unusual medical problems to exclude any association with the syndrome.

NF1 Exon 17 3-bp Inframe Deletion (c.2970_2972delAAT)

Upadhyaya et al.[42] reported 21 unrelated probands (14 familial and 7 sporadic) with the same c.2970–2972 del AAT (p.990delM) mutation but no cutaneous neurofibromas and no clinically obvious plexiform Neurofibromas. Of the total cohort ($n=47$), only one had had a symptomatic spinal neurofibroma removed. Thirty of the forty-seven individuals had axillary freckling. There was a different frequency of complications, with a much lower frequency of learning problems, macrocephaly, and short stature; a similar frequency of scoliosis but with an increased frequency of pulmonary stenosis than in an ordinary NF1 cohort. The main importance of the phenotype was the lack of dermal neurofibromas in adult patients.

Since the initial publication, there has been one further report[17] of a child with NF1 pigmentary changes only. This child was identified when a cohort of 151 patients satisfying the NF1 diagnostic criteria, and followed in a primarily pediatric NF1 clinic, were tested initially for SPRED1 (2 patients identified) and then exon 17 sequenced in the reminder. In our own clinic we have found no further patients with the deletion when testing adults with CAL only. It is a less common cause of the CAL phenotype than *SPRED1* mutations.

Neuro-Cardio-Facial Cutaneous (NCFC) Syndromes and Their Overlap with NF1

Introduction

The overlap of clinical features, particularly in facial appearance, learning disability, short stature, macrocephaly (true and relative), and cardiac involvement, between NF1, Noonan, LEOPARD, cardio-facio-cutaneous (CFC), and Costello syndromes has been recognized for some years. There is also overlap in the kind of malignancies which can occur. The conditions have now been shown to be caused by mutations in genes in the same molecular pathway.[49,50] The RAS mitogen-activated protein kinase (RAS/MAPK) pathway has been most studied because of its critical role in cancer pathogenesis; the fact that the same genes cause these syndromes highlights their key role in developmental processes. It also raises the prospect that drugs developed to control the pathway in cancer may be effective in their treatment.

The two most common conditions, NF1 and Noonan syndrome are notable for their extreme variability, even within families. The identification of genes in the same pathway in this group of conditions with overlapping features raises the possibility that functional polymorphisms in different pathway genes affect the expression of causative mutations in others.

The term NCFC syndromes is used by some as a collective term for the group of conditions,[49] others simply refer to "RASopathies."[50] In this section there is a brief clinical description of each disorder. The key features and causative genes are summarized in Table 3.4 and the pathway itself is illustrated in Fig. 3.6. Inheritance in all is autosomal dominant. Other than NF1 and Noonan syndrome, the syndromes are rare.

In clinical practice, the only pathway syndrome that cannot be distinguished clinically from NF1 is Legius syndrome. Although CAL spots are reported as features of Noonan and LEOPARD syndrome, there are nearly always sufficient

Table 3.4. Summary of features of other neuro-cardio-facio-cutaneous syndromes which overlap with NF1 and Legius syndrome.

	Noonan	LEOPARD	Cardio-facio-cutaneous	Costello
Year of first description	1965	1969	1986	1977
Overlapping cutaneous features	Café au lait spots described more frequently than general population	CAL spots reported in 70–80% and usually precede lentigines; lentigines develop in skin-folds	CAL spots reported but not confirmed in recent survey of 61 individuals; multiple melanocytic nevi common	Although generalized increased pigmentation reported, CAL are not. Skin tends to be loose and soft, particularly on palms – possible overlap with NF1 microdeletion cases
Congenital heart disease	Pulmonary stenosis 20–50%	Pulmonary stenosis 25%	Pulmonary stenosis: frequency uncertain	Pulmonary stenosis in ~30%
Hypertrophic obstructive cardiomyopathy (HOCM) – if this is associated with NF1 at all is uncertain	20–30% presenting at birth or developing in childhood	Detected in up to 70% usually developing in infancy	HOCM: frequency uncertain	30–47% develop HOCM in infancy or early childhood
Psychomotor development	25% have learning problems and 10–15% require special education	Mild learning problems in approximately 30%	Majority have moderate to severe developmental problems	Mild to moderate developmental delay in all children

Associated tumors	Increased risk of Juvenile myelomonocytic and acute myeloid leukemias; also giant cell lesions (benign tumor-like lesions frequently affecting jaws but also other bones or soft tissues).	Individual case reports ,[51] of myelodysplasia, acute myelogenous leukemia, neuroblastoma, malignant melanoma, and bilateral choristomas (a congenital corneal tumor)	Single cases: acute lymphoblastic leukemia, hepatoblastoma (patient immunosuppressed), non-Hodgkin lymphoma, and large B-cell lymphoma	Overall childhood tumor frequency 17%. Rhabdomyosarcoma most frequent tumor of early childhood followed by neuroblastoma; transitional cell carcinoma of bladder occurs in adolescents

Source: Data compiled from Burkitt Wright and Kerr,[49] Sarkozy et al,[51] Allanson,[52] Gelb and Tartaglia,[53] Rauen[54] and Gripp and Lin[55]

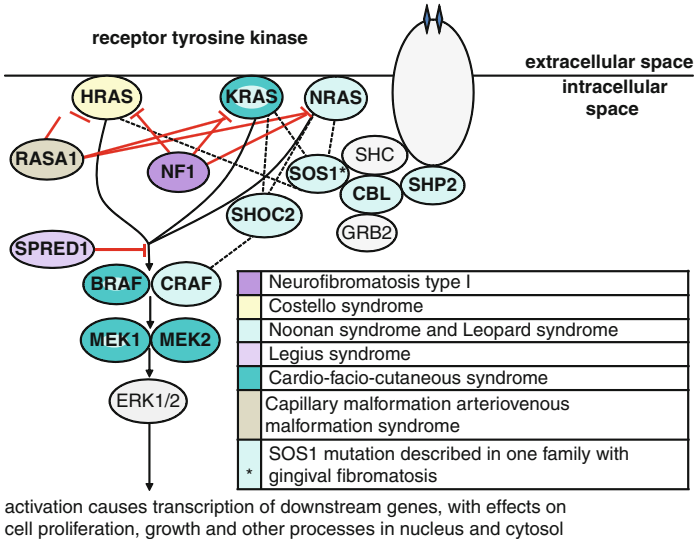

FIGURE 3.6. The RasMAPK pathway and associated syndromes.

distinguishing features from NF1 and skinfold freckling does not occur – although the lentigines in LEOPARD do involve the skin folds. The only condition I have seen misdiagnosed as NF1 is LEOPARD syndrome. The other syndromes have all been delineated much more recently than NF1 and it may be that more overlapping complications will emerge as adults with the different syndromes are followed. Given the underlying overlap in pathogenesis we need to be alert in the clinic for the occurrence of similar rare tumors or other problems. A good example of this is in Legius syndrome; there have been two cases reported with vascular anomalies, not something we would associate with NF1, but because of the association of mutations in RASA1 in capillary malformation–AV malformation syndrome, it becomes a possible true association.[10,44,50]

Noonan Syndrome

Noonan syndrome is at least as common as NF1.[52] The main clinical findings are short stature, pectus abnormalities, congenital heart defects (usually pulmonary stenosis and hypertrophic cardiomyopathy), learning disorders, and a characteristic facial appearance (ptosis, posteriorly rotated ears and hypertelorism). CAL spots are reported to occur more commonly in Noonan syndrome but no other overlapping skin features.

Is There a Neurofibromatosis Noonan Syndrome?

It has long been debated whether there is a specific syndrome which combines the features of NF1 and Noonan.[56,57] However, when cohorts of patients with NF1 have been systematically surveyed no evidence for a specific syndrome has emerged,[58] and this has always been my impression. Some people with NF1 had facial features which overlap with Noonan's but these did not routinely segregate in families.[58] The other overlapping features are pectus abnormalities and pulmonary stenosis. When mutation analysis has been done in cohorts of individuals with NF1 but Noonan-like facies mutations in the NF1 gene alone have been found.[59]

LEOPARD Syndrome

The name LEOPARD is an acronym for the common disease features: multiple **L**entigines, **E**lectrocardiographic conduction abnormalities, **O**cular hypertelorism, **P**ulmonary stenosis, **A**bnormal genitalia, **R**etardation of growth, and sensorineural **D**eafness.[51,53] In practice the overlap with NF1 mainly arises because of the lentigines and occasional CAL spots – the lentigines can develop in skin folds causing further confusion. In distinction from the freckles in NF1, the lentigines in LEOPARD are consistently darker and, in my experience, slightly raised above the skin (Fig. 3.7).

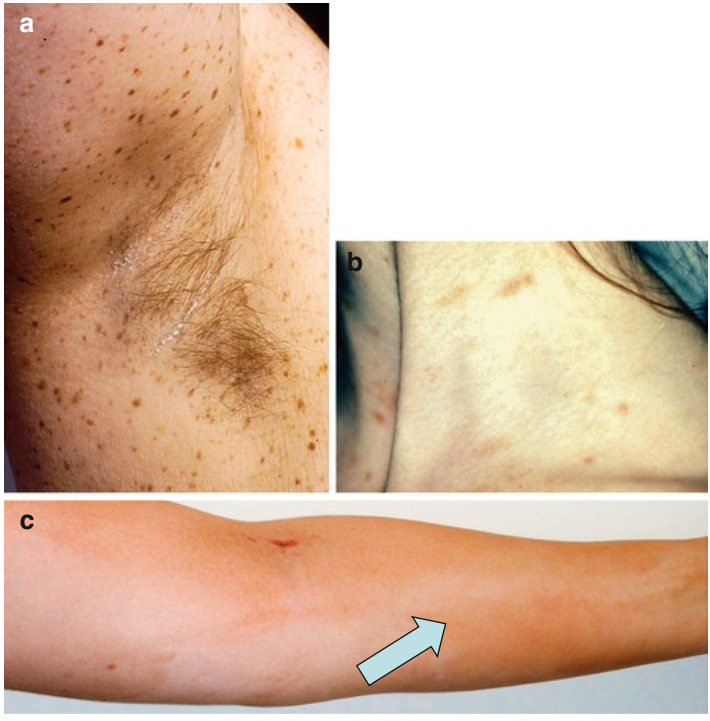

FIGURE 3.7. The differential diagnosis of NF1 skin lesions: (**a**) The lentigines in LEOPARD syndrome; note axillary involvement-patient originally diagnosed as NF1. (**b**) Urticaria pigmentosa: note the *orangey-brown* skin lesions with superficial resemblance to CAL spots; however, the lesions are slightly raised. (**c**) Fake tan giving impression of segmental CAL pigmentation.

Costello and Cardio-Facio-Cutaneous (CFC) Syndromes

These are the most severe of the RASopathies.[49,54,55] Affected children usually present in infancy with severe feeding problems and failure to thrive; nearly all cases are significantly developmentally delayed. As these are not usually seen in

NF1, the conditions are rarely confused. The other feature common in Costello and CFC, but not usually seen in other NCFC syndromes, is abnormal hair. The facies in both conditions may be relatively normal at birth but become coarse with age.

Costello Syndrome

Features which suggest Costello syndrome include neonatal atrial arrhythmias, excess skin which darkens with age, papillomas (usually after age 2 years), and ulnar deviation of the hands with deep palmar creases.[49,55] Approximately 15% of patients develop solid tumors particularly embryonic rhabdomyosarcomas, neuroblastomas, and bladder carcinoma (from teenage years onward).

CFC Syndrome

In CFC, more severe developmental problems and underlying brain abnormalities often predominate the clinical picture, with 50% of patients developing seizures which may present with infantile spasms.[49,54] Ectodermal abnormalities are also often a predominant feature with absent eyebrows (ulerythema ophryogenes) and keratosis pilaris. Whether there is a risk of associated malignancy remains to be established; there has been single cases of hepatoblastoma (in an immunosuppressed patient after cardiac transplantation), acute lymphoblastic leukemia, nonHodgkin's lymphoma, and large B cell lymphoma.

Other Pathway Disorders

The other two pathway disorders (Capillary malformation–AV malformation syndrome caused by mutations in *RASA1*, and the form of multiple hereditary gingivomatosis caused by mutation in *SOS1*) have no major overlapping features with NF1.

Constitutional Mismatch Repair Deficiency (CMMR-D): A Rare But Important Cause of a Phenotype Overlapping with NF1

This syndrome is rare but many of the reported cases were initially diagnosed as NF1 and it has therefore become an important condition to be aware of when assessing children with ?generalized/mosaic NF1. The syndrome is characterized by the development of childhood cancers, mainly hematological malignancies and/or brain tumors, as well as early onset colon cancers; some authors refer to CMMR-D as "Childhood cancer syndrome" or by the acronym (CoLoN), Colon tumors or/and leukemia/Lymphoma or/and Neurofibromatosis features.[60-63]

Inheritance is recessive and the syndrome is caused by biallelic mutations in one of four mismatch repair genes (*MLH1, MSH2, MSH6, PMS2*) – heterozygous mutations in the genes are associated with dominantly inherited nonpolyposis colon cancer (HNPCC). However, only approximately half the reported cases have a significant history of familial cancer. This is particularly the case for families with *PMS2* mutations probably related to the higher age of onset and reduced penetrance of heterozygous *PMS2*.

Wimmer and Kratz[61] recently reviewed the reported cases and added three more ($n=92$). These patients had had a total of 132 malignancies: 30 hematological (lymphoma/leukemia), 44 Brain Tumors (mainly glioblastoma and other astrocytic tumors), and 51 cases of HNPCC-associated cancers but with a much lower age of onset. There were single cases of neuroblastoma, Wilms tumor, rhabdomyosarcoma, ovarian neuroectodermal tumor, infantile myofibromatosis, breast cancer, and sarcoma.

The overlap with NF1 arises because 63/92 reported cases have café au lait macules. Some reports[63,64] have emphasized that the CAL in CMMR-D are atypical with irregular outlines and patchy pigmentation; areas of skin hypopigmentation are also reported. However, a proportion of the cases have other significant NF1 features including skin fold freckling, Lisch

nodules, and pseudarthrosis and satisfy NIH diagnostic criteria. In addition, in some of the cases the skin changes were segmental in distribution suggesting a somatic NF1 mutation. One of the cases with CMMR-D due to homozygous MLH1 mutations has been shown to have an NF1 truncating mutation.[65] There is also evidence that the NF1 gene is a mutational target of MMR deficiency. It therefore seems that at least in some CMMR-D cases there has been somatic NF1 mutation giving rise to the children having the additional phenotype of generalized or segmental NF1.

Although rare, these cases are important to recognize because of the more severe cancer phenotype than normal NF1, the 25% recurrence risk for sibs, and the increased colon cancer risks in the heterozygous parents and their extended families.

When to Think About CMMR-D in a Child Presenting with Multiple CAL Spots or Segmental NF1

- Consanguinous parents
- Sibling or cousin (in consanguineous families) with childhood cancer
- If child has had one of the CMMR-D related malignancies – the majority of which would be unusual in ordinary NF1
- Family history of colon cancer or other HNPCC-associated tumors

CAL in Other Mismatch Repair Disorders

This group of conditions includes ataxia telangiectasia, Fanconi's anemia, Bloom syndrome, and Nijmegen break syndrome. They are all recessively inherited and usually the other presenting features mean that the differential of NF1 is

never considered. They are not associated with typical CAL but can have multiple atypical lesions with irregular outlines and variable depth of pigmentation.[9]

NF1: Differential Diagnosis – Other Conditions

Introduction

The conditions which tend to get misdiagnosed as NF1 fall into three categories – those with pigmentary features that have CAL spots or patchy skin pigmentation, those with pigmentary features misdiagnosed as CAL, and those with tumors misdiagnosed as neurofibromas. A fourth, extremely rare group is tumor predisposition syndromes in which both CAL and tumors that can be mistaken for neurofibromas occur. The first two tend to present in childhood and the clinical clues come from assessing whether other problems the child has are typical for NF1 and in the assessment of the patches themselves. The conditions with other tumors tend to present in adults and here the lack of CAL spots in childhood or other common NF1 childhood problems, like learning disability, are usually the pointers in the history.

The two commonest misdiagnoses are through overinterpretation of variation of normal skin variation in childhood and lipomas in adults. The other conditions are all extremely rare and even in specialist practice I have only ever seen one or two cases of each – in all cases there were major clinical clues to the fact this could not be typical NF1. For this reason I have just given a brief description of each condition based on three main reference sources.[66-68] Shah[9] has recently reviewed the diagnostic and clinical significance of CAL spots and the syndromes with which they are strongly/weakly associated. I have only included conditions which I have seen personally, as I presume this means they are very rarely confused with NF1.

Conditions with CAL Spots and Other Patchy Skin Pigmentation Changes

Variation of Normal Skin Pigmentation

When I first started an NF clinic, this group of patients probably represented the one which caused me most confusion and the families' unnecessary concern. Over the years I have gradually learned about the different normal pigmentary patterns one sees in different ethnic groups. For example, in black skin I have seen children with marked pigmentation after scarring (e.g., after an insect bite or chicken pox); if they have one or two CAL as variation of normal and the pigmented scars are then counted, they can be mistakenly labeled as having NF1.

When we are assessing possible mosaic skin changes, then the clue are segments of skin with increased/decreased pigmentation often with well-demarcated borders. The other reason large areas of pigmentation are important in NF1 is they may be the first clue in early childhood to an area where a plexiform neurofibroma may develop; in this case there may also be excessive hair growth. However, there are some natural pigmentary demarcation boundaries that should not be confused. These lines are often more obvious in darkly skinned individuals – the one that has most often caught my attention in the ?NF1 setting is the line which runs down the anterolateral line in the upper arm.[68] The other thing that has caught me out has been use of artificial tanning – the patients having to point out to me the cause of their apparently "affected" segment (Fig. 3.7)!

The final group in this category is that which my NF mentor, Professor Vic Riccardi, refers to as "Pigmentary miscegeny." These are the skin changes seen when children have parents with very different skin coloring, usually from different ethnic groups but also if one parent has very pale skin and the other very dark. These children can have a mixture of hypo- and hyperpigmented patches – the latter tend to have very irregular outlines and depth of pigmentation. In our own

clinic we see several children a year referred as ?NF1 where this is the cause. If the children have any other problems, one needs to ensure there is not an alternative diagnosis which might produce pigmentation with irregular depth/outline such as the DNA repair disorders.

Rare Disorders with Typical CAL Spots

These are the two disorders where I have seen cases with absolutely typical NF1-like CAL spots. In both the other problems the children had were not typical for NF1 and the distinction from NF1 had already been made when I reviewed them. Typical CAL are also reported in Russell Silver syndrome.

1. *Ring chromosome syndromes*: Ring chromosomes occur when part of one end of the chromosome is deleted and the two ends then "stick" together. Problems occur as the ring structure is unstable in mitosis. CAL spots have been reported in a variety of ring chromosome cases (chromosomes 7, 11, 12, 15 and 17[9]). The other clues to diagnosis are usually more profound development problems than in NF1, shorter stature, microcephaly, and dysmorphic facial features.
2. *Schimke immunoosseus dysplasia*: This is an autosomal recessive condition characterized by growth retardation, renal failure, recurrent infections, cerebral infarcts, and skin pigmentation beginning in childhood. The majority of cases are caused by mutations in the *SMARCAL1* gene. I have only seen one case but the skin changes were typical NF1-like CAL with skin fold freckling.

McCune Albright Syndrome

The major features of this disorder are polyostotic fibrous dysplasia, precocious puberty and other endocrinopathies, and large, segmental areas of CAL pigmentation. It is sporadic and caused by postzygotic mutations in the *GNAS1* gene.

The distinction from NF1 is usually straightforward as the areas of CAL are much larger than normal with no associated smaller CAL. They also tend to follow the lines of Blaschko.[68] The CAL in McCune Albright characteristically have a jagged outline said to resemble "the coast of Maine" compared with the smooth contours in NF1 a likened to the "coast of California." This is however, not universal as sometimes the large CAL overlying plexiforms can have a jagged edge (Fig. 3.2). The more reliable diagnostic aids are the presence of multiple much smaller lesions in NF1 and the other features in McCune Albright.

Conditions with Skin Lesions Misdiagnosed as CAL Spots

Urticaria Pigmentosa

This is the most common variant of childhood mastocytosis. The skin lesions usually develop in the first year of life, are slightly elevated, and their color can be brown–red or yellow. As they develop the lesions, when brown can be confused with CAL (Fig. 3.7). However, as they develop they become either plaque-like or popular and this distinguishes them from CAL. The clue to etiology is elicited by "Darier's sign": when a lesion is scratched a marked urticarial reaction is usually elicited.

Congenital Melanocytic Nevi

When congenital melanocytic nevi are particularly large and cover a major part of the body (e.g., bathing trunk distribution), they can be confused with the skin changes seen over some plexiform neurofibromas. Both may first appear as just flat pigmented lesions, and then the lesion becomes thickened with time – in the case of NF1 with plexiform change histologically. The congenital nevi are usually much darker than NF1-associated CAL and carry a risk of melanomatous change not seen in NF1.

Conditions with Tumors

Multiple Lipomatosis

Multiple lipomas are the "commonest" misdiagnosis we see in adults in NF1, even this accounts for only a handful of cases a year at most. Inheritance is autosomal dominant and my impression is that penetrance may not be 100%. The lipomas present as subcutaneous swellings, which are usually painless (in contrast to peripheral nerve neurofibromas/schwannomas), and usually grow to several centimeters in diameter or larger. They tend to cluster on the forearms, thighs, lower chest wall, and abdomen. The distinction from nerve tumors is the lack of pain and they are usually softer on palpation. The other major distinction from NF1 is the lack of associated pigmentary changes.

Steatocystoma Multiplex

This is a rare dominant disorder caused by mutations in the keratin 17 gene. Affected individuals develop painful cutaneous swellings, arising from the sweat glands, from childhood onward (Fig. 3.7). The lesions have a yellowish color and firm consistency which on biopsy show disordered sebaceous gland elements.

Proteus Syndrome

The most famous misdiagnosis of NF1 historically was the Elephant man, Joseph Merrick. In 1986, Tibbles and Cohen[69] suggested the alternative diagnosis of Proteus syndrome and this is now widely accepted. Proteus is an extremely rare disorder characterized by asymmetrical overgrowth of almost any part of the body, associated with epidermal and connective tissue nevi, dysregulated growth of fatty tissue (lipomas or regional absence), bony hyperostosis, and vascular malformations.

The overlap with NF1 is because some of the overgrown areas can resemble plexiform neurofibromas. I have seen two cases where plexiform neurofibromas affecting the feet were initially thought to represent the connective tissue nevi of Proteus, with the "moccasin sole" appearance. The distinguishing feature clinically is that the plexiforms are usually soft in consistency whereas the connective tissue nevi in Proteus are firm on palpation.

Rare Autosomal Dominant Tumor Predisposition Syndromes

This group is summarized in Table 3.5. In my experience they are easily distinguishable from NF1 clinically. However, as both CAL and cutaneous lesions which can be mistakenly labeled as neurofibromas occur I have included them. The important thing is to remember them when assessing patients that "don't fit" for NF1.

NF1 Diagnostic Criteria: Pitfalls

The NIH NF1 diagnostic criteria agreed at a 1987 consensus meeting[70] are:

The clinical diagnosis is made when at least two of the following are present:

- A first-degree relative with NF1
- Six or more café au lait patches >0.5 cm in children and >1.5 cm in adults
- Axillary or groin freckling
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Two or more Lisch nodules (iris hamartomas)
- Optic pathway glioma
- Bony dysplasia of the sphenoid wing
- Thinning of the long bone cortex with or without pseudarthrosis of the long bones

Table 3.5. Rare dominant tumor predisposition syndromes where a diagnosis of a form of NF may initially be considered.

Name	Key features	Overlap with neurofibromatosis
PTEN hamartoma tumor syndrome (includes Cowden syndrome, Banayan-Ruvalcaba-Riley Syndrome (BRRS))	Cowden phenotype: a multiple hamartoma syndrome, high risk of benign and malignant tumors of the thyroid, breast, and endometrium. Affected individuals usually have macrocephaly, trichilemmomas, and papillomatous papules and present by the late 20s	CAL spots reported
	BRRS is a congenital disorder characterized by macrocephaly, intestinal hamartomatous polyposis, lipomas, and pigmented macules of the glans penis; up to 50% have developmental/learning problems	Misdiagnosis of skin or GI lesions (ganglioneuromas can occur)
	PTEN mutations found in some individuals presenting with autistic spectrum and macrocephaly	Macrocephaly: in PTEN syndromes head circumference nearly always ≥ 97th centile, whereas in NF1 may just be relative macrocephaly

Carney complex Has also been referred to by two acronyms: NAME – nevi, atrial myxomas, ephelides and LAMB – lentigines, atrial myxoma, blue nevi	Pale brown to black lentingines: develop in increasing numbers from early childhood, occur anywhere, particularly affect face around the eyes, nose, and mouth Myxomas: cutaneous, cardiac, and breast predominantly Endocrine tumors: including primary pigmentary nodular adrenocortical disease, testicular tumors, and thyroid adenomas Psammomatous melanotic schwannoma (PMS): PMS may occur anywhere in the central and peripheral nervous system; it is most frequently found in the nerves of the gastrointestinal tract (esophagus and stomach) and paraspinal sympathetic chain	CAL spots reported Skin myxomas misdiagnosed as neurofibromas PMS pathology very specific but presentation could be same as a lesion in NF1/NF2
Multiple endocrine neoplasia syndrome type one and 2B	MEN1 associated with parathyroid adenoma, pituitary adenoma, pancreatic islet cell adenoma, lipoma, gingival papules, facial angiofibromas, collagenomas MEN2B associated with mucosal neuromas, pheochromocytoma, medullary thyroid cancer, parathyroid adenoma, and marfanoid habitus	CAL spots reported in both Mucosal neuromas misdiagnosed as neurofibromas in MEN2B Pheochromocytoma in MEN2B

In the majority of cases the diagnosis of NF1 is straightforward and the NIH diagnostic criteria have stood the test of time well until recently. However, caution now needs to be made because the recognition of Legius syndrome and CMMR-D as follows:

1. Individuals with CAL and axillary freckling but nothing else may have Legius syndrome, particularly if there is a family history of the same phenotype.
2. The term first-degree relative includes parents, children, and siblings. As CMMR-D is autosomal recessive they could be diagnosed with NF1 on the grounds of CAL spots and an affected sibling and the much more serious diagnosis, with a different inheritance pattern, missed.
3. It is possible for people with segmental NF1 to have ≥6 CAL and unilateral skinfold freckling in an affected segment BUT they do not have generalized NF1 and the importance of the distinction is the lower frequency of complications and offspring recurrence risk.

NF1 Genetic Testing Indications

The fact that the clinical diagnosis is usually straightforward, combined with little demand for prenatal testing, the large gene size, and lack of recurrent mutations all contributed to little use of NF1 gene testing in routine clinical practice until relatively recently. With improved mutation detection (95% using most complete methods[3]), the recognition of clinically useful genotype–phenotypes, and Legius syndrome this is now changing.

At the current time our clinic testing criteria are:

1. Those who may have deletions on clinical grounds
2. Those with an atypical phenotype for diagnostic clarification
3. Families with two or more generations with isolated pigmentary changes (*NF1* and *SPRED1*)
4. Children with no family history and isolated pigmentary changes (*NF1* and *SPRED1*)
5. Someone considering prenatal/preimplantation diagnosis

NF2: Differential Diagnosis and Related Conditions

Introduction

Had NF1 and NF2 not been historically lumped together as one disease, it would be more appropriate for NF2 and the related disorder schwannomatosis to be classified as different types of "schwannomatoses." With improved imaging techniques and molecular testing the diagnosis of NF2 is usually straightforward and there are a very limited number of true differentials. In both schwannomatosis and multiple meningiomas the diagnosis can only be made after exclusion of NF2.

Schwannomatosis

Patients with schwannomatosis develop peripheral nerve and spinal root schwannomas almost exclusively, but with no skin tumors. Cranial nerve involvement is rare. There is no eye involvement, ependymomas have not been seen, and meningiomas occur very rarely.[71-73] The appearance of the tumors is clinically and radiologically the same as in NF2. However, the tumors in schwannomatosis are usually associated with more persistent pain than just the transient paraesthesia in response to pressure that most peripheral nerve lesions cause. The problem is that if a sporadic patient presents with peripheral and/or spinal lesions there is no way of knowing if this is mosaic NF2 or schwannomatosis. Diagnostic criteria for Schwannomatosis have been proposed[73] (Table 3.6). Patient assessment includes a thorough cutaneous and eye examination for signs of NF2, full MRI neuroaxis imaging, and NF2 mutation testing.

The majority of cases of schwannomatosis are sporadic. The risk to offspring of sporadic cases is much less than 50%. In familial cases inheritance is dominant but expression is variable and incomplete penetrance is recorded.[73] Some patients present with multiple lesions localized to one body part suggesting a mosaic genetic mechanism; whether it is mosaic NF2 or Schwannomatosis can only be evaluated by molecular analysis in tumors.

Table 3.6. Diagnostic criteria for schwannomatosis proposed by MacCollin et al.[73]

Definite schwannomatosis
- Age >30 years AND
- ≥2 nonintradermal schwannomas, at least one with histologic confirmation AND no evidence of vestibular tumor on high-quality MRI scan AND no known constitutional NF2 mutation

OR

- One pathologically confirmed nonvestibular schwannoma plus a first-degree relative who meets above criteria

Possible schwannomatosis
- Age <30 AND
- ≥2 nonintradermal schwannomas, at least one with histologic confirmation AND no evidence of vestibular tumor on high-quality MRI scan AND no known constitutional NF2 mutation

OR

- Age >45 years AND ≥2 nonintradermal schwannomas, at least one with histologic confirmation AND no symptoms of 8th nerve dysfunction AND no known constitutional *NF2* mutation

OR

- Radiographic evidence of a nonvestibular schwannoma and first-degree relative meeting criteria for definite schwannomatosis

Segmental schwannomatosis
- Meets criteria for definite or possible but limited to one limb or ≤5 contiguous segments of spine

The genetic mechanisms underlying schwannomatosis are gradually being elucidated. The gene has been localized to chromosome 22 proximal to *NF2* and mutations in the *SMARCB1* tumor suppressor gene reported in 2007.[74] Subsequent reports suggest that between 33 and 66% of familial cases[75,76] and 7%[75] of sporadic cases have germline *SMARCB1* mutations. Mutations in the same gene also cause inherited predisposition to rhabdoid tumors, the tumors developing after a somatic "second hit." The major question is therefore why are the two phenotypes so

different? Tumor analysis has shown a complex mechanism of tumorigenesis in schwannomatosis which requires somatic mutation in both copies of the *NF2* gene as well as in *INI1*.[75,77] Two families with both meningiomas and schwannomas with SMARCB1 mutations have also been reported.[78,79]

In the clinical setting tumor analysis can be used to determine if a sporadic case, with normal lymphocyte mutation testing for *NF2* and *SMARCB1* represents mosaic NF2 or schwannomatosis. In mosaic NF2 each separate tumor will share one mutation in common, whereas in schwannomatosis the *NF2* mutations are different in each tumor.

Multiple Meningiomas

Multiple meningiomas can occur as part of NF2 or as a separate genetic entity dominant inheritance of multiple meningiomas and no other features; this is a very rare entity. Linkage to NF2 was excluded in one family and other genes have not yet been identified.[80] As for schwannomatosis, the diagnosis of familial non-NF2 meningiomas can only be made with a clear family history. In sporadic cases, the causes include NF2 mosaicism, or noncontiguous spread of a single sporadic tumor or new mutation in the as yet unidentified gene(s) responsible for non-NF2 familial meningiomas.

The clinical approach to the patient with multiple meningiomas is like that for schwannomas; NF2 must be excluded initially.

Misdiagnosis of Other Cerebello-Pontine (CP) Angle Tumors as Vestibular Schwannomas

This is an extremely rare event but most NF2 clinics have had one or two cases referred where other tumors in the CP angle are initially diagnosed as vestibular schwannomas. These have included choroid plexus papillomas (which can further mimic NF2 by seeding down the spine) and lymphoma.[81]

Acknowledgments I am grateful to Dr. Rick Whitehouse for providing Fig. 3.5, Dr. Emma Burkitt Wright for Fig. 3.6, and to the patients who have allowed me to use their pictures and clinical histories.

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