

Neurofibromatoses: part 1 – diagnosis and differential diagnosis

Das neurofibromatoses: parte 1 – diagnóstico e diagnóstico diferencial

Luiz Oswaldo Carneiro Rodrigues¹, Pollyanna Barros Batista¹, Eny Maria Goloni-Bertollo², Danielle de Souza-Costa¹, Lucas Eliam⁶, Miguel Eliam⁷, Karin Soares Gonçalves Cunha³, Luiz Guilherme Darrigo-Junior⁴, José Roberto Lopes Ferraz-Filho², Mauro Geller⁵, Ingrid F. Gianordoli-Nascimento¹, Luciana Gonçalves Madeira¹, Leandro Fernandes Malloy-Diniz¹, Hérika Martins Mendes¹, Débora Marques de Miranda¹, Erika Cristina Pavarino², Luciana Baptista-Pereira¹, Nilton A. Rezende¹, Luíza de Oliveira Rodrigues¹, Carla Menezes da Silva¹, Juliana Ferreira de Souza¹, Márcio Leandro Ribeiro de Souza¹, Aline Stangherlin¹, Eugênia Ribeiro Valadares¹, Paula Vieira Teixeira Vidigal¹

ABSTRACT

Neurofibromatoses (NF) are a group of genetic multiple tumor growing predisposition diseases: neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2) and schwannomatosis (SCH), which have in common the neural origin of tumors and cutaneous signs. They affect nearly 80 thousand of Brazilians. In recent years, the increased scientific knowledge on NF has allowed better clinical management and reduced complication morbidity, resulting in higher quality of life for NF patients. In most cases, neurology, psychiatry, dermatology, clinical geneticists, oncology and internal medicine specialists are able to make the differential diagnosis between NF and other diseases and to identify major NF complications. Nevertheless, due to its great variability in phenotype expression, progressive course, multiple organs involvement and unpredictable natural evolution, NF often requires the support of neurofibromatoses specialists for proper treatment and genetic counseling. This Part 1 offers step-by-step guidelines for NF differential diagnosis. Part 2 will present the NF clinical management.

Keywords: neurofibromatoses, neurofibromatosis type 1, neurofibromatosis type 2, schwannomatosis.

RESUMO

Neurofibromatoses (NF) constituem um grupo de doenças genéticas com predisposição ao crescimento de múltiplos tumores: tipo 1 (NF1), tipo 2 (NF2) e schwannomatose (SCH). Estas doenças têm em comum a origem neural dos tumores e os sinais cutâneos. Afetam cerca de 80 mil brasileiros. O maior conhecimento científico sobre as NF tem permitido melhor manejo clínico, redução da morbidade das complicações e melhor qualidade de vida. Na maioria dos casos, os especialistas em neurologia, dermatologia, genética clínica, oncologia e medicina interna estão capacitados a realizar o diagnóstico diferencial e identificar suas principais complicações. Devido à sua variabilidade fenotípica, curso progressivo, multiplicidade de órgãos acometidos e evolução imprevisível, as NF frequentemente necessitam de especialistas em NF para o acompanhamento. A Parte 1 deste texto oferece orientações para o diagnóstico de cada tipo de NF e discute os diagnósticos diferenciais com outras doenças. A Parte 2 oferecerá orientações em relação ao manejo clínico das NF.

Palavras-chave: neurofibromatoses, neurofibromatose tipo 1, neurofibromatose tipo 2, schwannomatose.

Individuals with neurofibromatoses (NF) usually present to neurologists with one or more of the following clinical findings: cognitive and developmental deficits, macrocephaly,

deafness, blindness, neuropathic pain, seizures or benign/malign tumors of the central nervous system (CNS). Also, NF affected persons may be referred to psychiatrists due

¹Universidade Federal de Minas Gerais, Belo Horizonte MG, Brazil;

²Universidade de São Paulo, Faculdade de Medicina de São José do Rio Preto, Sao Jose do Rio Preto, Brazil;

³Universidade Federal Fluminense, Faculdade de Medicina, Niteroi RJ, Brazil;

⁴Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Ribeirão Preto, Brazil;

⁵Universidade Federal do Rio de Janeiro, Faculdade de Medicina, Rio de Janeiro RJ, Brazil;

⁶Radiologia Anchieta, Brasília DF, Brazil;

⁷Instituto de Neurologia de Goiânia, Goiania GO, Brazil.

Correspondence: Luiz Oswaldo Carneiro Rodrigues; Centro de Referência em Neurofibromatoses, Hospital das Clínicas, Faculdade de Medicina da Universidade Federal de Minas Gerais; Alameda Álvaro Celso, 55; 30150-260 Belo Horizonte MG - Brasil; E-mail: rodrigues.loc@gmail.com

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to behavior disorders, including attention deficit/hyperactivity disorder (ADHD) and autism and/or learning difficulties.

There are three distinct diseases under NF denomination: neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2) and schwannomatosis (SCH). The NF share the genetic autosomal dominant predisposition to multiple tumor growth and the neural origin of tumors and cutaneous signals. They are progressive, present a great heterogeneity in phenotype expression and have unpredictable natural course. The NF differ profoundly in age of first manifestations, symptoms, evolution and prognosis¹.

NF diseases affect nearly 80 thousands of Brazilian individuals² and if NF is suspected, it is necessary to distinguish NF1 from NF2 and SCH. Figure 1 depicts a stepwise diagnosis pathway to distinguish the three NF diseases. In most cases, the differential diagnosis among the NF diseases is made with certainty. Nevertheless, special NF patients present overlap with some other diseases because of: a) cafe au lait lesions (CAL); b) localized hyper growth syndromes; c) neurofibromas-like tumors; and d) multiple endocrine neoplasia. These particular situations should deserve attention from the NF specialist.

The present Part 1 text offers step-by-step guidelines for differential diagnosis among the neurofibromatosis types

and other diseases. A further Part 2 will present the NF clinical management. Basic information for patients can be found in www.amanf.org.br.

STEP BY STEP DIAGNOSIS OF NF1, NF2 AND SCH

Figure 1 depicts a practical step-by-step guide to differentiate NF1 from NF2 and SCH. The first two questions to be answered are: are there cafe au lait lesions (CAL)? Are there tumors?

STEP 1- ARE THERE CAFÉ AU LAIT LESIONS?

Yes - CAL are usually multiple and present or develop at birth or in early infancy and are observed in 99% of people with NF1 by age of 3 years. They are macules with uniform ovoid shape and smooth outline, varying of light to dark brown color (Figure 2A). Its size varies from 5 mm (infancy) to 30 mm (adulthood), but they can be bigger than 20 cm and involve an entire anatomic region⁴. The CAL size is proportionate to body growth and they are randomly distributed, although scalp, palms, and soles are spared⁵. Many

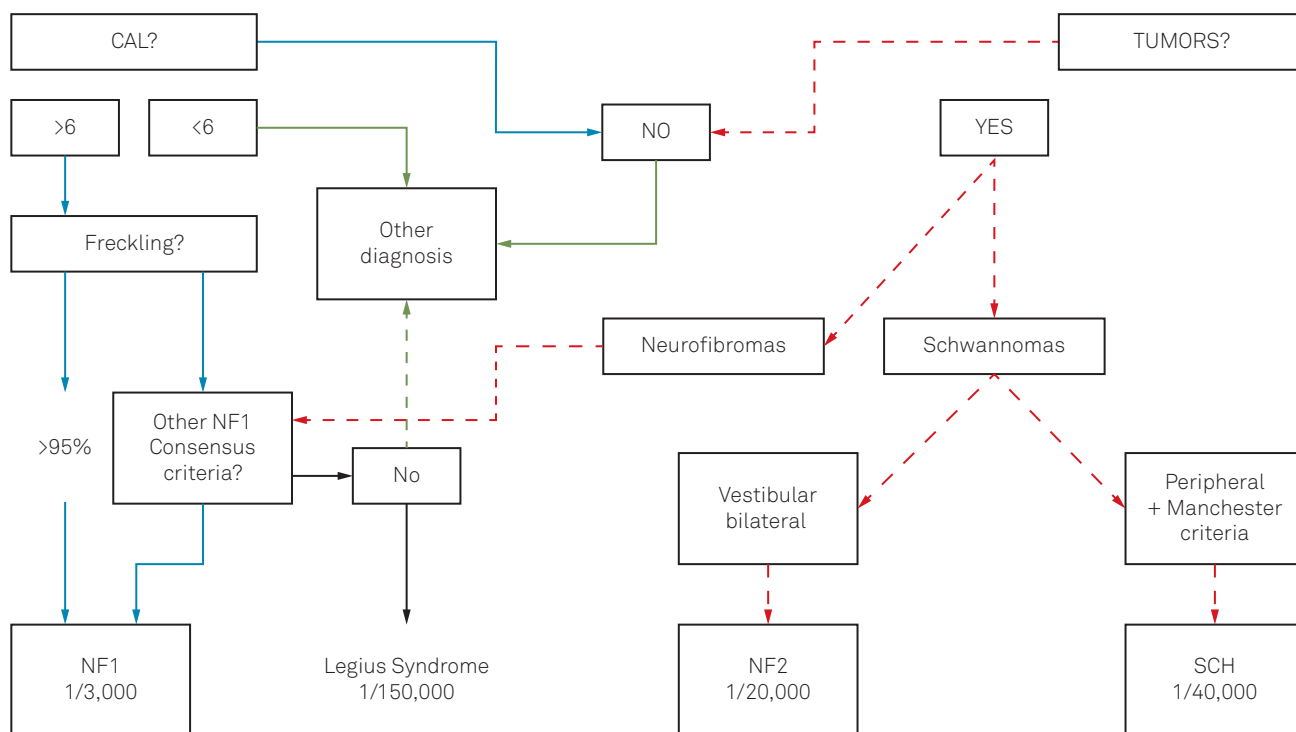


Figure 1. Practical flowchart to differentiate NF diseases: neurofibromatosis type 1 (NF1), type 2 (NF2) and schwannomatosis (SCH). The main questions are: 1) How many cafe au lait lesions (CAL) (Figure 2A) are there? Are there freckling (Figure 2B)? The pathways are shown by blue continuous lines and arrows; 2) Are there tumors: neurofibromas (Figure 2C) or schwannomas? The pathways are shown by red dashed lines and arrows for CAL, freckling and other NF1 criteria (Table 1). Manchester criteria for NF2 and SCH (Table 2), neurofibromas and schwannomas characteristics are described in the text. Main differences between NF1, NF2 and SCH are presented in Table 3. Legius syndrome, special NF1 cases (Table 4) and other diagnoses (green lines) are presented in Table 5. Adapted from Huson et al.³.

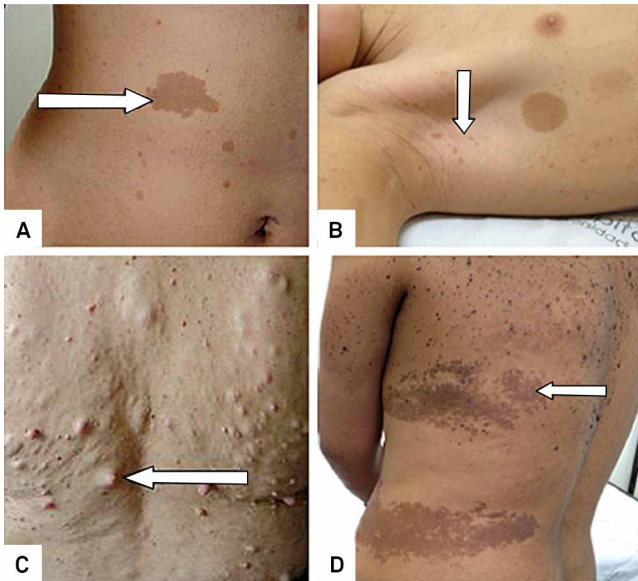


Figure 2. Cutaneous lesions (arrows): Multiple cafe au lait lesions (CAL) (A); freckling and two CAL (B); Discrete neurofibromas (C); Pigmented skin lesions (epidermal nevus) misdiagnosed as CAL and freckling in a non-NF1 patient (D). Pictures made with patient's permission at the *Centro de Referência em Neurofibromatoses*.

other hyperpigmented skin lesions are commonly misdiagnosed as CAL, such as the lesion in Figure 2D.

Six or more typical CAL are a strong sign (95%) of NF1. NF1 is the most common condition associated with multiple CAL and patients with a higher number of CAL are more likely to develop NF1, but CAL can also be seen in other syndromes (Table 5). On the other hand, the presence of more than three CAL is detected in only 0.3% of children with no evidence of genetic disorders⁶.

STEP 2 – ARE THERE AXILLARY AND INGUINAL FRECKLING?

Yes - Axillary and/or inguinal freckling are hyperpigmented spot macules (1-3mm) and the second most common feature in NF1 (Figure 2B). Their appearance is similar to solar-induced freckling, but in NF1 they occur typically in areas with minimal to none sun exposure. Freckling generally appears between 3 and 5 years of age in the axillae and/or inguinal region. Other sites include neck and breasts, around the lips, and even the trunk in adults, but in these sites they are not a diagnostic criterion⁶.

Until recent years, the presence of CAL associated with freckling was enough to the NF1 diagnosis. Nevertheless, the discovery of the Legius syndrome (LEGIUS), which has in common with NF1 the CAL, the freckling, macrocephaly and cognitive deficits, made required a third criterion to make the NF1 diagnosis certain⁷. LEGIUS could be considered the fourth NF disease³.

STEP 3 – ARE THERE OTHER NF1 CONSENSUS CRITERIA?

The diagnostic criteria for NF1 have been established by a National Institute of Health consensus of experts in 1987. A person is thought to have NF1 if they have two or more of the Table 1 criteria.

STEP 4 – ESTABLISHED NF1 DIAGNOSIS (PREVIOUS VON RECKLINGHAUSEN'S DISEASE)

NF1 is the most prevalent human monogenetic disease (incidence of 1:2,500 live births)¹⁴, it is an autosomal dominant disorder caused by hereditary or new mutations in the neurofibromin protein codification gene, located in Chromosome 17 (locus 17q11.2), which results in neural and other tissues dysplasia and increased tumor formation of neural origin¹⁵.

Table 1. National Institute of Health NIH consensus criteria⁸ and the approximate Brazilian prevalence of the criteria⁹⁻¹¹, which are similar to international literature, except for tumors (recent review prevalence presented in bold parenthesis¹²). The descriptions of cafe au lait (CAL), freckling, neurofibromas, optical glioma (OPG) and osseous dysplasia are presented in the text. Lisch Nodules are hamartomas in the iris, which are specific of Neurofibromatosis (NF1). Many features of NF1 increase in frequency with age. At age 1, only 50% of patients fulfill the NIH criteria, but at age 8, almost all patients have fulfilled the diagnostic criteria¹³.

Criteria	Description	Prevalence
CAL	Six or more CAL > 5 mm in the larger diameter in prepubertal individuals and > 15 mm in the larger diameter in post pubertal individuals	95%
Freckling	Two or more freckles in the axillary or inguinal regions	87%
Neurofibromas	Two or more of any type (cutaneous, subcutaneous, spinal)	75% (40 - 60%)
	Or one plexiform	35% (60%)
Glioma	Optical nerve or optical pathway tumor (astrocytoma) diagnosed by nuclear magnetic resonance	6% (15-20%)
Lisch nodules	Two or more nodular hamartomas in the iris, diagnosed by the ophthalmologist	78%
Osseous dysplasia	A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex with or without pseudoarthrosis	5%
Familial history	A first-degree relative (parent, sibling, or offspring) with established NF1 by the above criteria	50%

STEP 5 – ARE THERE TUMORS?

Most NF cases are associated with some CAL; therefore, its absence suggests other diseases. Nevertheless, considering that some special NF patients do not have CAL, the next step is to search for tumors.

The common types of tumors associated with NF1 are neurofibromas (*nfbs*), plexiform neurofibromas (PNF), malignant peripheral nerve sheath tumors (MPNST) and optic glioma (OPG) (see below). The presence of two or more of these tumors is strongly suggestive of NF1 and other NIH criteria must be investigated (STEP 4). If no other criterion is found, this situation deserves a NF specialist support. On the other side, the presence of two or more schwannoma tumors (see below) is suggestive of both NF2 and SCH.

NF1 ASSOCIATED TUMORS

Although a specialized physician usually recognizes the different types of neurofibromas, the diagnostic certainty needs further histological studies. Below, we present a brief description of NF1 commonest tumors^{2,16} and detailed histological information can be found elsewhere¹⁷.

NEUROFIBROMAS (WHO GRADE I)

Neurofibromas, the hallmark tumors of NF1, are benign tumors of the peripheral nerve sheath. They exhibit extensive cellular heterogeneity (Schwann cells, perineural cells, mast cells, fibroblasts and axons in an extracellular matrix) and it can be classified into two major types: localized and PNF.

Localized neurofibromas are the most prevalent type of tumor in NF1. They tend to develop from skin sensory nerves and usually present as a cutaneous and/or subcutaneous tumor that remains associated with a single nerve ending. The *nfbs* are more commonly found on the skin, but other sites can also be affected, such as the spinal roots, heart, stomach, larynx, bladder, bowels and oral mucosa.

Plexiform neurofibromas are almost always associated with NF1 and they are classified as benign peripheral nerve sheath tumors that involve multiple nerve fascicles or large branches of a major nerve. PNF are the first and foremost source of morbidity in NF1, due to their tendency to grow to large sizes and their capacity to cause significant deformity and compression of adjacent structures. Some may be present at birth, but most of them usually become apparent during the first two years of life and, if not present by then, rarely develop after adolescence. The growth rate associated

with these neoplasms is unpredictable, with periods of rapid growth followed by periods of relative inactivity.

MALIGNANT PERIPHERAL NERVE SHEATH TUMOR (WHO GRADE II, III OR IV)

MPNST commonly derive from pre-existing PNF. The NF1 patient lifetime risk of developing MPNST is 8-13% (being the double in the NF1 microdeletion subtype). Because MPNST develop from malignant progression of pre-existing PNF, the risk of developing MPNST increases to almost 50% in patients with NF1 and PNF¹⁸. MPNST occurrence in childhood and adolescence is uncommon. Incidence is higher among adults in the third to sixth decade of life. MPNST should be suspected when the patient develops persistent pain with no other explanation and/or unexplained neurological deficit associated with changes in texture and enlargement of the PNF. Histological differentiation between a PNF with atypical features and a low grade MPNST is difficult, since these lesions actually represent a histological continuum and it is possible to find lesions with areas of atypical features adjacent to areas of frank MPNST.

GLIOMAS

Gliomas are the predominant CNS tumor type associated with NF1 and they may occur in all parts of the nervous system, with a preference for the optic pathways, brainstem and cerebellum. Frequent NF1-associated gliomas include optic pathway gliomas (OPG), other pilocytic astrocytomas, diffuse astrocytomas and glioblastomas.

OPG (WHO GRADE 1)

The majority of OPG in NF1 patients are pilocytic astrocytomas that are located within the optic nerve. Macroscopically most pilocytic astrocytomas are a relatively circumscribed, slowly growing soft-grey mass. Intra or paratumoural cyst formation is common. Optic nerve tumors also often show collar-like involvement of the subarachnoid space. Histopathologically, this astrocytic tumor of low to moderate cellularity exhibits an often biphasic pattern with varying proportions of compacted bipolar cells with Rosenthal fibers and loose-textured multipolar cells with microcysts and granular bodies/hyaline droplets. Rare mitosis, hyperchromatic and pleomorphic nuclei, glomeruloid vascular proliferation, infarct-like necrosis and infiltration of leptomeninges are compatible with the diagnosis of pilocytic astrocytoma and are not signs of malignancy¹⁹. Bilateral growth, when present, is characteristic of NF1.

DIFFUSE ASTROCYTOMAS (WHO GRADE II)

That is a diffusely infiltrating astrocytoma that typically affects young adults and is characterized by a high degree of cellular differentiation and slow growth; the tumor occurs throughout the CNS but is preferentially supratentorial and has an intrinsic tendency for malignant progression to anaplastic astrocytoma and, ultimately, glioblastoma.

GLIOBLASTOMA (WHO GRADE IV)

The most malignant neoplasm with predominant astrocytic differentiation; histological features include nuclear atypia, pleomorphic cells, mitotic activity, vascular thrombosis, microvascular proliferation and necrosis. It typically affects adults and is preferentially located in the cerebral hemispheres. Most glioblastomas manifest rapidly de novo, without recognizable precursor lesions (primary glioblastoma). Secondary glioblastomas develop slowly from diffuse WHO grade II astrocytoma or anaplastic astrocytoma (WHO grade III). Due to their invasive nature, glioblastomas cannot be completely removed and, despite progress in radio/chemotherapy, less than half of patients survive more than one year, with older age being the most significant adverse prognostic factor.

OTHER NF1 ASSOCIATED TUMORS

NF1 patients present with a higher risk of developing other tumors such as:

SARCOMAS

Gastrointestinal stromal tumor (GIST)

NEUROENDOCRINE/NEUROECTODERMAL TUMOURS

Pheochromocytomas
Carcinoid tumor
Medullar thyroid carcinoma
C-cell hyperplasia

HEMATOPOIETIC TUMORS

Juvenile chronic myeloid leukemia
Juvenile xanthogranuloma
Acute lymphocytic leukemia
Non Hodgkin lymphoma

STEP 6 – ARE THERE BILATERAL VESTIBULAR SCHWANNOMAS (BVS)?

Yes – The presence of BVS is a hallmark of NF2. NF2 is an autosomal dominant disorder caused by hereditary or new mutations in the merlin protein codification gene located in chromosome 22 (locus 22q12.2). NF2 presents with BVS, multiple meningiomas, juvenile subcapsular cataract and peripheral schwannomas and other neural tumors.

SCHWANNOMAS (WHO GRADE I)

Schwannomas are benign true nerve sheath neoplasms composed entirely of Schwann cell proliferation, well circumscribed, nodular or ovoid, usually encapsulated dermal, subcutaneous and central nerves tumors².

The prevalence of NF2 vary from 1:25,000-33,000 individuals²⁰ and it presents with deafness and/or tinnitus (50%), balance dysfunction (5%), focal weakness or sensory change (18%), seizure (5%), ocular symptoms (7%) and genetic or radiographic diagnosis of an asymptomatic family member (10%), during the second decade or early adulthood²¹.

Nevertheless, some special cases of NF2 are less evident. The Manchester (modified NIH) diagnostic criteria for NF2 have been expanded to include patients with no family history who have multiple schwannomas and or meningiomas, but who have not yet developed bilateral vestibular nerve tumors²². The diagnosis is best confirmed using high quality MRI of the brain.

If there are two or more painful schwannomas, the present step is to complete the diagnosis of SCH with the following criteria²³. Table 2 presents the consensus criteria for NF2 and SCH diagnosis.

SCH typically becomes symptomatic during the third decade of life and presents a prevalence of 1:40,000 individuals. The molecular biology of SCH has not been well established yet and a candidate gene for SCH, called INI1, was identified, but the NF2 gene and possibly other genes may also be involved. Unlike NF1 and NF2, SCH does not have a clear pattern of inheritance.

The Table 3 summarizes the main clinical differential characteristics of NF1, NF2 and SCH.

NF1 VARIATIONS

There are genetically related (allelic) disorders, where apparently pathogenic NF1 mutations have been demonstrated in a few individuals or families who do not have NF1 according to the NIH Diagnostic Criteria⁴. They have specific phenotype and clinical course and are presented in Table 4 and its management will be discussed in Part 2 of this text.

Table 2. Neurofibromatosis type 2 (NF2) and schwannomatosis (SCH) Manchester consensus diagnosis criteria^{22,23}.

NF2	
Confirmed diagnosis	Bilateral masses of the vestibular (eighth cranial nerve) seen with appropriate imaging techniques (e.g., CT or MRI) plus family history of NF2 or unilateral vestibular schwannoma plus any two of the following: neurofibroma, meningioma, glioma, schwannoma, juvenile posterior subcapsular lenticular opacity.
Probable diagnosis	Multiple meningiomas (two or more) plus unilateral vestibular schwannoma or any two of the following: neurofibroma, glioma, schwannoma and cataract.
SCH	
Confirmed diagnosis	Age over 30 years and two or more schwannomas (at least one with histological confirmation) and no evidence of a 8 th nerve tumor on high-quality MRI scan and no known constitutional NF2 mutation or one pathologically confirmed non-vestibular schwannoma plus a first-degree relative who meets the above criteria.
Presumed diagnosis	Age under 30 years and two or more schwannomas (at least one with histological confirmation) and no evidence of a 8 th tumor on high-quality MRI scan and no known constitutional NF2 mutation or age over 45 years and two or more non-intradermal schwannomas, at least one with histological confirmation and no symptoms of 8 th nerve dysfunction and no known constitutional NF2 mutation OR radiographic evidence (images scans) of a non-vestibular schwannoma plus a first-degree relative meeting the criteria for definite schwannomatosis.

MOSAICISM OR SEGMENTAL NF

Occasionally NF1, NF2 (rarely) and schwannomatosis (often) occur in mosaic forms. Mosaicism in NF1 is observed more frequently and results from somatic mutations. Early

somatic mutations cause generalized disease, clinically indistinguishable from no mosaic forms. Later somatic mutation gives rise to localized disease often described as segmental. In individuals with mosaic or localized manifestations of NF1 (segmental NF1), disease features are limited to

Table 3. Clinical differential characteristics of neurofibromatosis (NF1, NF2) and schwannomatosis (SCH).

Features	NF1	NF2	SCH
First signs and symptoms	Infancy	Beginning of adulthood	Adulthood >30 years
Typical findings	Multiple CAL and neurofibromas	Deafness and balance dysfunction	Pain
Ocular findings	Lisch Nodules	Juvenile posterior subcapsular cataract	None
Growth	Macrocephaly, short stature, cognitive deficits	Normal	Normal
Tumors	Neurofibromas: (cutaneous, plexiform and intraneural - 60%) Gliomas: benign (optic pathways 15-20%) and malignant (0,8%) Pheochromocytomas (0,1-13%)	Schwannomas: bilateral vestibular (>95%), other cranial (24-51%), cutaneous (59-68%), peripheral nerve (42%) Meningiomas: intracranial (50%) Ependymomas (33-53%) Mesothelioma	Schwannomas: peripheral and painful
Prevalence	1:2,500 a 1:7,800	1:20,000 a 1:40,000	1:40,000
Cancer risk	Increased: MPNST (8-13%); GIST (5-30%), leukemia (1%), rhabdomyosarcomas (1-6%), breast cancer 8,4%)	Habitual	?
Osseous problems	Dysplasias	None	None
Inheritance	Autosomal dominant with complete penetrance	Autosomal dominant with variable penetrance	Not well understood
Gene	Chromosome 17 (locus 17q11.2)	Chromosome 22 (locus 22q12.2)	Chromosome 22 (locus 22q12.2) + SMARCB1 (or INI1)?

CAL: cafe au lait lesions; MPNST: malignant peripheral nerve sheath tumor; GIST: gastrointestinal stromal tumor; prevalence of tumors¹².

Table 4. Neurofibromatosis (NF1) variations. The four identified NF1 subtypes with its main clinical characteristics⁴.

Subtype	Main characteristics
Watson syndrome	CAL, Lisch, freckling, pulmonary stenosis, short stature, macrocephaly, cognitive deficits, neurofibromas.
Gene deletion (5%)	NF1 gene large deletions – dysmorphic facial features: hypertelorism, downslanting palpebral fissures, broad fleshy noses, “coarse” face becoming more marked with age, overgrowth with tall stature and large hands and feet, Pectus excavatum, broad neck, excess of tissue in hands and feet, joint hyperflexibility, muscular hypotonia, bone cysts, pes cavus, dermal (cutaneous) neurofibromas occurring at early age and in increased numbers, including spinal neurofibromas, double lifetime risk of MPNST than NF1 population, significant delay in cognitive development, learning difficulties, congenital heart disease and scoliosis (Huxon, The Neurofibromatoses: Differential Diagnosis and Rare Subtypes, 2011).
Mosaicism (1%)	Two or more NF1 criteria present only in specific body part (see below).
Spinal form	Few CAL, normal or higher stature, few or absent cutaneous neurofibromas, but multiple spinal neurofibromas, usually bilateral and involving all 38 spinal nerve roots. MPNST higher risk.

CAL: cafe au lait; MPNST: malignant peripheral nerve sheath tumor.

the affected area, which varies from a narrow strip to one quadrant and occasionally to one half of the body. Distribution is usually unilateral but can be bilateral, either in a symmetric or asymmetrical arrangement.

DIFFERENTIAL DIAGNOSIS

The main characteristics of other diseases, which present differential diagnosis with NF1, are summarized in Table 5.

WHEN ORDERING GENETIC TESTS

Approximately 50% of individuals with NF1 or NF2 have not inherited the disease from a parent and are result of a *de novo* mutation. Individuals who meet the clinical diagnosis criteria for NF1, NF2 and SCH are diagnosed with confidence in most cases, and genetic tests are not required for diagnostic confirmation. Therefore, genetic testing may be helpful in specific cases. The decision to order or not a genetic test remains a physician/patient (family) discussion. Genetic testing for NF is clinically available in just a few laboratories around the world. For an updated list of laboratories that offers genetic tests for NF, please refer to www.genetests.org.

NF1 VERSUS LEGIUS SYNDROME

The NF1 gene is large (280 kb and 60 exons) and most mutations are unique to a particular family, what turns the molecular diagnostic testing laborious and expensive. A definite diagnosis of NF1 can be made in most children by age four using only the NIH criteria²⁴. Confirmatory diagnostic testing can detect the mutation in 95% of cases and is indicated for suspected NF1 individuals, which do

not fulfill the NIH diagnostic criteria, most often in young children, if it can affect clinical management.

The presence of only pigmented lesions at early age is a diagnostic dilemma between NF1 and LEGIUS (caused by *SPRED1* gene mutations). The distinction between NF1 and LEGIUS in young children, in the absence of NF1 family history and neoplastic or osseous manifestations can only be achieved by genetic testing. It is cost saving and easier to start by investigation of *SPRED1* gene than by NF1, because *SPREAD1* contains only 8 exons⁷.

It is important to emphasize that currently there are only two important genotypes that correlate to phenotype in NF1. One of these is the microdeletion syndrome, in which a large part of or the whole *NF1* gene is absent and is associated with a more severe phenotype²⁵. The other genotype is the mutation involving the deletion of three base pairs in exon 17, which correlates with a mild phenotype²⁶. Therefore, discarding these two specific genotypes, it is not possible to predict the severity of the disease based on NF1 genetic testing.

NF2

It is expected that 25% to 33% of those who have NF2 and are a single occurrence in the family (simplex cases) are mosaic for NF2 mutation. The mutation detection rate by sequencing and deletion/duplication analysis is around 92% for familial cases and 72% in simplex cases. NF2 genetic testing may be helpful in specific cases and, although some genotype-phenotype correlations in NF2 are known, it cannot yet predict the severity of the disease²⁷.

a. Pre-symptomatic testing of at-risk patients

Because detection of tumors at an early stage is effective in improving the clinical management of NF2 patients, the screening for NF2 in children of affected patients can start

Table 5. Differential diagnosis in neurofibromatosis (NF).

Group		Main characteristics
Other CAL conditions	Isolated CAL	10% of general population (one or two CAL)
	Familial CAL	Isolated CAL, autosomal dominant.
	Legius Syndrome	Multiple CAL, freckling, cognitive deficits and macrocephaly, autosomal dominant. Without Lisch nodules, neurofibromas or CNS tumors
	DNA Repair Deficiency Syndrome	CAL, tumors, hematological malignant diseases, hereditary colon cancer without multiple polyposes, autosomal recessive.
	NF1-Noonan Syndrome	CAL, ocular hypertelorism, down-slanting palpebral fissures, low-set ears, webbed neck, short stature, and congenital heart disease. 12% of individuals with NF1, autosomal dominant.
	Piebaldism	Areas of cutaneous pigmentation and depigmentation, white forelock; autosomal dominant.
	Bloom Syndrome	Erythematous and sun-sensitive skin lesion of the face; severe pre- and postnatal growth deficiency; autosomal recessive.
Skin disorders with CNS tumors	Fanconi Anemia	CAL or skin hypopigmentation; short stature; malformations of the thumbs, forearms; progressive bone marrow failure; autosomal recessive.
	Tuberous Sclerosis Complex	Hypomelanotic skin macules, facial angiofibromas, shagreen patches, fibrous facial plaques, ungual fibromas, SNC tumors, seizures, cognitive deficits, behavioral disorders; autosomal dominant.
	McCune-Albright Syndrome	CAL, polyostotic fibrous dysplasia, precocious puberty; sporadic.
	Ataxia-telangiectasia	Slurred speech, truncal ataxia, and oculomotor apraxia beginning between ages one and four years, telangiectasias of the conjunctivae, low immunity; autosomal recessive.
Maculae conditions misdiagnosed with CAL	Gorlin Syndrome (nevroid basal cell carcinoma syndrome)	Risk of developing cancerous and noncancerous tumors, most often basal cell carcinoma, less often medulloblastoma during childhood; autosomal dominant.
	Sturge-Weber syndrome (encephalotrigeminal angiomatosis)	Congenital facial cutaneous port-wine stain with associated CNS (developmental delay, seizures) and ocular abnormalities (buphtalmos, eye hemangiomas); unclear etiology.
	LEOPARD Syndrome	Multiple lentigines, hypertelorism, sensorineural deafness, hypertrophic cardiomyopathy; autosomal dominant.
	Neurocutaneous melanosis	Large or multiple congenital melanocytic nevi and benign or malignant pigment cell tumors of the leptomeninges; sporadic.
Locally excessive growing syndromes	Peutz-Jeghers Syndrome	Gastrointestinal polyposis, mucocutaneous pigmentation, and cancer predisposition; autosomal dominant.
	Klippel-Trenauney-Weber Syndrome	Large cutaneous hemangiomata with hypertrophy of the related bones and soft tissues; sporadic.
Tumor conditions misdiagnosed with neurofibromas	Proteus Syndrome	Progressive, segmental or patchy postnatal overgrowth of diverse tissues (skeleton, skin, adipose and SNC), linear verrucous epidermal nevus; sporadic.
	Lipomatosis	Trunk and limb multiple lipomas; autosomal dominant.
	Banayan-Riley-Ruvalcaba Syndrome	Multiple lipomas and hemangiomas, macrocephaly, pigmented macules of the glan penis, cognitive deficit; autosomal dominant.
	Congenital generalized fibromatosis	Multiple nodules of fibrous tissue, congenital or apparent before age of 2 years, in muscle, bone, subcutaneous tissue and viscera, fatal or undergoing spontaneous remission.
	Endocrine multiple neoplasias type 2B (MEN2B)	Medullary thyroid carcinoma, pheochromocytomas, mucosal neuromas, sometimes CAL; autosomal dominant.

CAL: cafe au lait patches; CNS: central nervous system.

Table 6. Transmission risk of neurofibromatosis (NF2) to offspring for isolated cases of Neurofibromatosis type 2 before and after blood genetic test. Adapted from Evans and Wallace, 2008²⁹.

Age of diagnosis of vestibular schwannoma	Transmission risk pre blood genetic test	Transmission risk post negative blood testing
<20 BVS	45%	29%
<20 UVS	33%	15%
20-29 BVS	36%	15%
20-29 UVS	19%	6%
30-39 BVS	28%	11%
30-39 UVS	12%	6%
40+ BVS	22%	9%
40+ UVS	10%	5.5%

BVS: bilateral vestibular schwannoma; UVS: unilateral vestibular schwannoma.

at birth with the search of cataracts²⁸. If no cataracts are found, pre-symptomatic genetic testing, if possible, should be considered.

b. Determining the diagnosis of patients who fall short of the Manchester criteria, including mosaic patients

In cases of patients that fall short of the Manchester criteria, genetic testing may be helpful in establishing the diagnosis. The diagnosis of NF2 is usually a challenge when mosaicism is present. Mutations with mosaicism levels greater than 10% can be detected in blood DNA, otherwise its identification requires testing of tumor material. In these cases, the diagnosis is established if two NF2 identical mutations are found in separate tumors, but is not present in blood²⁶.

c. Estimating the transmission risk for mosaic patients

The transmission rate of NF2 is 50% in the second generation, but the risk of transmission in apparently isolated patients with NF2 is less than 50% due to mosaicism. Transmission risk of NF2 decreases significantly after the proband has had negative genetic testing using a blood sample (Table 6)²⁷.

References

- Riccardi VM. Historical background and introduction. In: Friedman JM, Gutmann DH, MacCollin M, Riccardi VM (Eds). Neurofibromatosis - Phenotype, Natural History, and Pathogenesis. 3 ed. Baltimore: The Johns Hopkins University Press, 1999:1-28.
- Geller M, Bonalumi Filho A. Neurofibromatose: clínica, genética e terapêutica. 1 ed. Rio de Janeiro: Guanabara Koogan, 2004.
- Huson SM. The neurofibromatoses: differential diagnosis and rare subtypes. In: Ferner RE, Huson SM, Evans DG (Eds).

SCHWANNOMATOSIS (SCH)

Currently, it is known that germline mutations in *SMARCB1* (or *INI1*) gene are associated to SCH. Nevertheless, they are found in only 40-50% of the familial cases and in 8-10% of the sporadic cases of SCH. For SCH cases unrelated to *SMARCB1* germline mutations, the genetic alterations are not known²⁷.

SMARCB1 gene testing may be ordered for individuals with multiple schwannomas without evidence of vestibular tumor and no NF2 mutation detected in the blood. Nevertheless, there is a great chance of not detecting a *SMARCB1* genetic alteration and the diagnosis of SCH cannot be ruled out. *SMARCB1* gene testing is also indicated for early detection of at-risk patients (because of family history) for management reasons.

Tumorigenesis of schwannomas associated to SCH is believed to occur through a four-hit model, involving not only mutations in *SMARCB1* alleles (hits 1 and 2), but also mutations in NF2 alleles (hits 3 and 4)³⁰. Differently from NF2-associated schwannomas, SCH-associated schwannomas of the same individual usually carry different somatic NF2 mutations. Therefore, mutational analysis of schwannoma cells is also highly relevant for the diagnosis, even in the absence of *SMARCB1* mutation in blood. The finding of different NF2 somatic mutations in different schwannomas of the same individual, although not indicative of the presence of a germline *SMARCB1*, at least rules out the diagnosis of NF2 mosaicism³¹.

GENETIC TESTING FOR PREIMPLANTATION GENETIC DIAGNOSIS (PGD) OF NF

Another application of NF genetic test is for the PGD. It comprises a set of procedures to test a specific genetic alteration and selecting unaffected embryos for transfer to the uterus. It is a highly specialized method performed in a few centers and is available for couples when the parental mutation has already been identified. It has been shown as a promising and useful method, being the only one that prevents transmission of the mutated gene from one parent to their children³². For an updated list of centers that perform PGD for NF, please refer to www.genetests.org

- Neurofibromatoses in Clinical Practice. 1 ed. London: Springer-Verlag London Limited, 2011:1-46.
4. Ferner RE. Neurofibromatosis 1. In: Ferner RE, Huson SM, Evans DG (Eds.) Neurofibromatoses in clinical practice. 1 ed. London: Springer-Verlag London Limited, 2011:1-46.
 5. Boyd KP, Gao L, Feng R, et al. Phenotypic variability among cafe-au-lait macules in neurofibromatosis type 1. *J Am Acad Dermatol* 2010;63:440-447.
 6. Nunley KS, Gao F, Albers AC, Bayliss SJ, Gutmann DH. Predictive value of cafe au lait macules at initial consultation in the diagnosis of neurofibromatosis type 1. *Arch Dermatol* 2009;145:883-887.
 7. Brems H, Pasmant E, Van Minkelen R, et al. Review and update of SPRED1 mutations causing Legius syndrome. *Hum Mutat* 2012;33:1538-1546.
 8. National Institutes of Health Consensus Development Conference Statement: neurofibromatosis. Bethesda, Md., USA, July 13-15, 1987. *Neurofibromatosis* 1988;1:172-178.
 9. Trovo-Marqui AB, Goloni-Bertollo EM, et al. High frequencies of plexiform neurofibromas, mental retardation, learning difficulties, and scoliosis in Brazilian patients with neurofibromatosis type 1. *Braz J Med Biol Res* 2005;38:1441-1447.
 10. Darrigo Jr LG, Geller M, Bonalumi Filho A, Azulay DR. Prevalence of plexiform neurofibroma in children and adolescents with type I neurofibromatosis. *J Pediatr (Rio J)* 2007;83:571-573.
 11. Souza JFd, Toledo LLD, Ferreira MCM, Rodrigues LOC, Rezende NAd. Neurofibromatose tipo 1: mais comum e grave do que se imagina. *Rev Assoc Méd Bras* 2009;55:394-399.
 12. Lin AL, Gutmann DH. Advances in the treatment of neurofibromatosis-associated tumours. *Nat Rev Clin Oncol* 2013;10:616-624.
 13. DeBella K, Poskitt K, Szudek J, Friedman JM. Use of "unidentified bright objects" on MRI for diagnosis of neurofibromatosis 1 in children. *Neurology* 2000;54:1646-1651.
 14. Huson SM, Harper PS, Compston DA. Von Recklinghausen neurofibromatosis. A clinical and population study in south-east Wales. *Brain* 1988;111:1355-1381.
 15. Ferner RE, Huson SM, Thomas N, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. *J Med Genet* 2007;44:81-88.
 16. Cunha KS, Geller M. Advances in neurofibromatosis research. 1 ed. New York, USA: Nova Science Publishers Inc, 2012:1-273.
 17. Carranza AC, Salinas Martín MV, Polo R, Córdoba JC, González-Cámpora R. Problemas diagnósticos en tumores del nervio periférico (I and II). *Rev Esp Patol* 2011:97-116.
 18. Park SJ, Sawitzki B, Kluwe L, Mautner VF, Holtkamp N, Kurtz A. Serum biomarkers for neurofibromatosis type 1 and early detection of malignant peripheral nerve-sheath tumors. *BMC Med* 2013;11:109.
 19. HO Classification of Tumours of the central nervous system. 4 ed: WHO; 2007:1-312.
 20. Blakeley JO, Evans DG, Adler J, et al. Consensus recommendations for current treatments and accelerating clinical trials for patients with neurofibromatosis type 2. *Am J Med Genet A* 2012;158:24-41.
 21. MacCollin M. Neurofibromatosis 2: clinical aspects. In: Friedman JM, Gutmann DH, MacCollin M, Riccardi VM (Eds). *Neurofibromatosis - phenotype, natural history, and pathogenesis*. 3 ed. Baltimore: The Johns Hopkins University Press, 1999:299-326.
 22. Baser ME, Friedman JM, Wallace AJ, Ramsden RT, Joe H, Evans DG. Evaluation of clinical diagnostic criteria for neurofibromatosis 2. *Neurology* 2002;59:1759-1765.
 23. MacCollin M, Chiocca EA, Evans DG, et al. Diagnostic criteria for schwannomatosis. *Neurology* 2005;64:1838-1845.
 24. Friedman JM. *Neurofibromatosis 1*. Seattle (WA): University of Washington, Seattle; 1993-2013; 1998 (Updated 2012) [cited 2013]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK1109/>.
 25. Pasmant E, Sabbagh A, Spurlock G, et al. NF1 microdeletions in neurofibromatosis type 1: from genotype to phenotype. *Hum Mutat* 2010;31:1506-1518.
 26. Upadhyaya M, Huson SM, Davies M, et al. An absence of cutaneous neurofibromas associated with a 3-bp inframe deletion in exon 17 of the NF1 gene (c.2970-2972 delAAT): evidence of a clinically significant NF1 genotype-phenotype correlation. *Am J Hum Genet* 2007;80:140-151.
 27. Nunes F. Research advances in mutational analysis of the NF2 gene. In: Cunha K, Geller M, editors. *Advances in neurofibromatosis research*. 1 ed. New York, USA: Nova Science Publishers Inc, 2012:165-80.
 28. Evans DG, Raymond FL, Barwell JG, Halliday D. Genetic testing and screening of individuals at risk of NF2. *Clin Genet* 2012;82:416-424.
 29. Evans DG, Wallace A. An update on age related mosaic and offspring risk in neurofibromatosis 2 (NF2). *J Med Genet* 2009;46:792-800.
 30. Plotkin SR, Blakeley JO, Evans DG, et al. Update from the 2011 International Schwannomatosis Workshop: From genetics to diagnostic criteria. *Am J Med Genet A* 2013;161:405-416.
 31. Papi L. Schwannomatosis: a recently recognized form of neurofibromatosis. In: Cunha K, Geller M (Eds). *Advances in Neurofibromatosis Research*. 1 ed. New York, USA: Nova Science Publishers Inc, 2012:258-267.
 32. Bonalumi Filho A, Darrigo Jr L, Oliveira L, Azulay D, Cunha K. Pre-implantation genetic diagnosis of neurofibromatosis. In: Cunha K, Geller M (Eds). *Advances in Neurofibromatosis Research*. 1 ed. New York, USA: Nova Science Publishers Inc, 2012:258-267.