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NF1 Gene and Neurofibromatosis 1

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Neurofibromatosis 1 (NF1), also known as von Recklinghausen disease, is an autosomal dominant condition caused by mutations of the *NF1* gene, which is located at chromosome 17q11.2. NF1 is believed to be completely penetrant, but substantial variability in expression of features occurs. Diagnosis of NF1 is based on established clinical criteria. The presentation of many of the clinical features is age dependent. The average life expectancy of patients with NF1 is probably reduced by 10–15 years, and malignancy is the most common cause of death. The prevalence of clinically diagnosed NF1 ranges from 1/2,000 to 1/5,000 in most population-based studies. A wide variety of *NF1* mutations has been found in patients with NF1, but no frequently recurring mutation has been identified. Most studies have not found an obvious relation between particular *NF1* mutations and the resulting clinical manifestations. The variability of the NF1 phenotype, even in individuals with the same *NF1* gene mutation, suggests that other factors are involved in determining the clinical manifestations, but the nature of these factors has not yet been determined. Laboratory testing for *NF1* mutations is difficult. A protein truncation test is commercially available, but its sensitivity, specificity, and predictive value have not been established. No general, population-based molecular studies of *NF1* mutations have been performed. At this time, it appears that the benefits of population-based screening for clinical features of NF1 would not outweigh the costs of screening. *Am J Epidemiol* 2000;151:33–40.

neurofibromatosis; neurofibromatosis 1

GENE

The neurofibromatosis 1 (*NF1*) gene is located at chromosome 17q11.2. *NF1* and its protein product, neurofibromin, were characterized in 1990 (1, 2). The gene is large, spanning 350 kilobases of genomic DNA, and contains 60 exons (3). Neurofibromin belongs to a family of proteins that serve as negative regulators of the *ras* oncogene (4). Neurofibromin is believed to act as a tumor suppressor, but the protein has other functions as well. The proposed tumor suppressor function is supported by the findings of somatic "second hit" mutations of the *NF1* gene in benign and malignant tumors from NF1 patients (5, 6).

NF1 is an autosomal dominant condition with virtually 100 percent penetrance by adulthood (7). About 50 percent of NF1 cases result from new mutations. Germline mosaicism has been observed (8) and must be considered when counseling unaffected parents of cases with new mutations. The *NF1* mutation rate is among the highest observed in humans, with estimates ranging from about 1/7,800 to 1/23,000 gametes (7, 9). About 90 percent of new mutations occur on the paternally derived chromosome (10, 11). The exception is large deletions, which are usually of maternal origin (12, 13).

GENE VARIANTS

As of February 1999, the *NF1* Genetic Analysis Consortium documented more than 240 different constitutional *NF1* mutations in its database (http://www.nf.org/nf1gene/). Table 1 summarizes the types of mutations identified thus far. The majority of mutations lead to a truncated protein product; only about 10 percent involve amino acid substitutions, and fewer than 2 percent are 3′ untranslated region mutations. However, it should be noted that the types of mutations identified are largely dependent on the techniques used for mutation detection. This may result in an overrepresentation of mutation types that are more easily identified (e.g., large gene deletions) and an underrepresentation of those that may be more difficult to identify (e.g., mutations in the 3′ untranslated region). None of the methods used for *NF1* mutation detection are capable of identifying all mutation types.

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Abbreviations: NF1, neurofibromatosis 1; NF2, neurofibromatosis 2.

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TABLE 1. Summary of *NF1* mutation types*

Type of mutation	No. of cases
Chromosome abnormality	4
Deletion of entire gene	18
Multi-exon deletion	38
Small deletion	55
Large insertion	3
Small insertion	27
Stop mutation	43
Amino acid substitution	29
Intron mutation	25
3′ untranslated region mutation	4
Total	246

* Reported to the NF1 Genetic Analysis Consortium (http://www.nf.org/nf1gene/) as of February 1999.

Mutations have been identified throughout the gene. While some recur in different families, no true "hotspots" have been found in *NF1*. The most frequently recurring alteration is a nonsense mutation in exon 31 (R1947X) that accounts for 1–2 percent of the *NF1* mutations identified (14).

At this time, no information is available on the frequency of different mutations in different populations and ethnic groups.

DISEASES

Clinical features of NF1

Neurofibromatosis 1 (NF1), also known as von Recklinghausen disease, is the condition most commonly associated with *NF1* gene mutations. Early discussions of NF1 referred to the condition as "neurofibromatosis" and included cases of the much less frequent condition, neurofibromatosis 2 (NF2). However, these conditions are both clinically and genetically distinct. The most characteristic lesions of NF2 are bilateral schwannomas on the vestibular portion of the eighth cranial nerve; such tumors are rarely seen in NF1 patients. NF2 results from mutations in the *NF2* gene on chromosome 22.

Despite advances in understanding of the molecular genetics of NF1, its diagnosis remains a clinical one, based on diagnostic criteria established by a National Institutes of Health consensus conference (15, 16). A diagnosis of NF1 by these criteria requires the presence of two or more of the following: 1) six or more café-au-lait macules more than 5 mm in greatest diameter in prepubertal individuals and more than 15 mm in greatest diameter after puberty; 2) two or more neurofibromas of any type or one plexiform neurofibroma; 3) freckling in the axillary or inguinal regions; 4) an optic pathway tumor; 5) two or more Lisch nodules (iris hamartomas); 6) a distinctive osseous lesion, such as sphenoid wing dysplasia or thinning of the cortex of long bones (with or without pseudarthrosis); or 7) a first-degree relative (parent, sibling, or child) with NF1 diagnosed by the above criteria.

Some of these features, including café-au-lait spots, freckling in non-sun-exposed areas, and iris Lisch nodules, are not of clinical significance beyond their usefulness in making a diagnosis of NF1. Benign cutaneous and subcutaneous neurofibromas are present in nearly all patients with NF1 by adulthood, and their number in an individual varies widely from only a few to hundreds or more. While these lesions are primarily of cosmetic significance, they may be disfiguring and result in significant psychologic distress. In contrast, about 15 percent of individuals with NF1 have plexiform neurofibromas (17). These tumors may extend into contiguous tissues, causing serious functional impairment and even death and appear to be the site of malignant peripheral nerve sheath tumor development. Optic pathway tumors are observed in about 20 percent of the children with NF1, but most such tumors do not cause ophthalmologic or other symptoms (18). Bony changes, such as pseudarthrosis, appear to occur in about 5 percent of the cases (17). Often these changes are benign; however, some patients are severely affected, with long-bone bowing leading to fracture and, in some cases, requiring amputation (19).

Several other features are often associated with NF1, including macrocephaly, scoliosis, short stature, hypertension, and high-T2-signal-intensity lesions on magnetic resonance imaging of the brain (16). Most individuals with NF1 have normal intelligence, but 30–60 percent have learning disabilities (20).

Individuals with NF1 also appear to be at increased risk for malignancy, but the magnitude of this is difficult to estimate, given the paucity of epidemiologic studies. In an investigation of a Danish cohort of 212 NF1 patients followed for 42 years, a relative risk of 4.0 (95 percent confidence interval: 2.8, 5.6) was observed for malignant neoplasms or benign central nervous system tumors among probands. Since the probands had been identified initially through hospitals and might represent a bias toward more severely affected cases, the relative risk was also determined for affected relatives; this risk was 1.5 (95 percent confidence interval: 0.9, 2.4). The risk was greater for females than for males (21).

Certain types of cancers occur more frequently in individuals with NF1. Malignant peripheral nerve sheath tumors, often referred to as neurofibrosarcomas, are the most common malignancy occurring with increased frequency in NF1. These aggressive tumors are relatively resistant to therapy and are often lethal

(22). Central nervous system tumors, including optic pathway tumors, other astrocytomas, ependymomas, medulloblastomas, and others, also occur more frequently in NF1 patients (23). In addition, individuals with NF1 have an increased risk for myeloid leukemias, with over a 200-fold relative risk for chronic myelomonocytic leukemia (24). The increased risk for malignancies in NF1 is compatible with the finding that the NF1 protein serves as a down-regulator of the *ras* oncogene (4). An increased risk for malignancy could be predicted to result from inactivation of this tumor suppressor function through *NF1* mutation.

The presentation of most NF1 features is age dependent. Café-au-lait spots may be present at birth and increase in number in early childhood. Skinfold freckling is most often observed next. Neurofibromas frequently first appear or increase in number between ages 10 and 20 years. Lisch nodules of the iris are often not present in childhood but are seen in nearly all adults with NF1 (17).

Prevalence of NF1

For several reasons, NF1 is a difficult condition for which to determine an accurate prevalence number. First, the wide variability in expression means that mild cases may escape ascertainment in studies dependent on an affected individual coming to medical attention. Second, the age-dependent presentation of most NF1 features means that examination of young children may miss cases that are truly affected with the condition. Third, the increased mortality seen in individuals with NF1 (see Mortality of NF1, below) reduces the prevalence in later adulthood. Prevalence studies are summarized in table 2 and suggest that NF1 is one of the most common autosomal dominant conditions. The prevalence does not appear to differ by gender. The wide variation in prevalence estimates may reflect differences in diagnostic criteria and methods of case ascertainment of the studies; however, the variation may also represent true differences between populations, perhaps due to a founder effect (particularly in smaller populations) or other factors. One study (25) demonstrated differences in NF1 prevalence among various ethnic groups, with a higher prevalence in individuals of North African and Asian origins (1/522 and 1/1,052, respectively) and a lower frequency among individuals of European and North American backgrounds (1/1,562). These differences were statistically significant, and case ascertainment in this study was based on a mandatory physical

TABLE 2. Studies of the prevalence of neurofibromatosis 1

Study site	No. screened	Ethnic origin of population studied	Method of ascertainment	Age of cases ascertained	Estimated prevalence	Reference
Michigan	252,092	Residents of Michigan	Surveys of general hospital admissions and state institutions for the mentally retarded and "epileptic" (estimate extrapolated from these populations)	All ages	1/2,500–1/3,300*	55
USSR	94,000	Primarily "Russian"	Screening examination for 6-café-au-lait spots as part of evaluation for military duty; detailed examination for those initially identified	16 years	1/7,800†	56
Sweden	440,082	Residents of Göteberg, Sweden	Medical record review, letters to medical institutions and physicians, assessment of family members of affected cases	20 years and older	1/4,600	57
Southeast Wales	668,100	Residents of southeast Wales	Medical record review, letters to physicians, assessment of family members of affected cases	All ages	1/4,150‡	7
New Zealand	113,700	British descent with "substantial Scots component"	Medical record review, letters to physicians, assessment of family members of affected cases	All ages	1/2,190	58
Italy	2,375,304	Northeast Italy	Cases from genetics service and from computerized hospital data	All ages	1/6,711	59
Israel	374,440	Primarily from Europe, North America, Asia, North Africa, and Israel	Physical examination as part of evaluation of fitness for military duty	17 years	1/960	25
Finland	732,000	Residents of northern Finland	Medical record review	All ages	1/3,716	22

* Estimated incidence at birth.

† Assumes that about three quarters of the cases of NF1 would be ascertained through mass medical examination for at least six café-au-lait spots.

‡ Corrected estimate based on possible "missed," mildly affected cases, especially in children.

examination for fitness for military service, suggesting that referral bias was not responsible for the observed differences. The question of the true prevalence of NF1 and whether it differs significantly between populations will require further study.

Mortality of NF1

The best available mortality data are from a population-based study of NF1 patients living in Göteberg, Sweden (26). Adults (age 20 years and older) with NF1 were ascertained through multiple medical specialities. The average age at the time of ascertainment was 43.6 ± 15.4 years for the 70 patients followed. Cases were followed for 12 years. Over this time period, 22 of the 70 NF1 patients died; 5.1 deaths were expected on the basis of the general Swedish population. Of these 22 deaths, 13 were women and nine were men, with 1.7 and 3.4 deaths expected in the populations, respectively, leading the authors to suggest that women may be affected more than men. The study showed a significantly reduced life expectancy in patients with NF1 ($p < 0.001$), with a mean age at death of NF1 patients of 61.6 years compared with a life expectancy in the general population of 75 years.

Malignancy was the most common cause of death, occurring in 12 (55 percent) of the patients (26, 27). Hypertension significantly associated with mortality; 10 of 12 patients with high blood pressure died during the observation period.

NF1 risk factors

Paternal age has been shown to be significantly advanced in sporadic cases of several other autosomal dominant disorders, but whether paternal age is advanced in sporadic cases of NF1 is not clear. A study in Texas (28) recently addressed this question. Paternal age was obtained from the birth certificates of cases (identified as NF1 patients seen in two specialty neurofibromatosis clinics) and birth certificates of controls (two per case, chosen at random from the same year and county of birth). Fathers of NF1 patients were 1.5 years older than were fathers of controls at the birth of the child, but this difference was not statistically significant ($p = 0.07$) (28). It appears that the paternal age effect in sporadic cases of NF1 is either small or nonexistent.

ASSOCIATIONS

NF1 is the condition most commonly associated with *NF1* gene mutations. For NF1, the penetrance is believed to be virtually 100 percent by adulthood (29); that is, individuals with an *NF1* gene mutation have clinical manifestations of NF1, usually by age 6 years. Most studies have not found an obvious relation between particular *NF1* mutations and resulting clinical manifestations in a patient. However, attempts at genotype-phenotype correlation in NF1 are confounded by the effect of age, which increases the frequency of disease manifestations and the likelihood of serious complications in all patients. In addition, there is no consensus regarding how to define NF1 severity.

Some studies of patients with large *NF1* gene deletions indicate that they may have earlier onset of cutaneous neurofibromas and more often have dysmorphic facial features and mental retardation than do most NF1 patients (13, 30, 31). However, not all NF1 patients with this phenotype have a large gene deletion (32), and some with large gene deletions have an unremarkable NF1 phenotype (33), raising questions about this genotype-phenotype relation. The presence of a more severe phenotype may be a function of the amount of flanking DNA involved in the deletion rather than of the *NF1* gene deletion itself.

Certain variants of NF1 have been associated either with specific *NF1* mutations or with linkage to the *NF1* gene, at least in some cases. These include Watson syndrome (characterized by pulmonic stenosis, café-au-lait spots, short stature, and cognitive impairment) (34, 35); familial multiple café-au-lait spots (without other NF1 features) (36–38); familial spinal neurofibromatosis (characterized by spinal tumors and, sometimes, café-au-lait spots, but not by other features of NF1) (39, 40); and encephalocraniocutaneous lipomatosis (characterized by unilateral lipomatous growths, ipsilateral ophthalmologic and brain malformations, mental retardation, and seizures) (41). It appears that these variants may be allelic to NF1, at least in some families.

Patients with segmental neurofibromatosis have features of NF1 confined to a particular area of the body (e.g., one side of the body) (42). While it has been postulated that segmental neurofibromatosis results from a somatic mutation in the *NF1* gene, this postulate has not yet been molecularly demonstrated. Somatic mosaicism for the *NF1* gene has been reported in at least four cases (33, 43–45), but all of these cases showed typical NF1, suggesting that the somatic mutation occurred early in embryonic development.

Noonan syndrome is an autosomal dominant condition characterized by webbing of the neck, unusual facies, short stature, and congenital heart disease (often pulmonic stenosis). Features of Noonan syndrome, often without a cardiovascular malformation, have been observed in many patients with NF1. About 13 percent of patients with NF1 specifically examined for Noonan syndrome features had a Noonan syndrome phenotype (46); this frequency of co-occurrence seems

unlikely if NF1 and Noonan syndrome are independent disorders. In some families, NF1 and Noonan syndrome have been shown to segregate as independent autosomal dominant traits, and Noonan syndrome is not linked to the *NF1* locus in families without features of NF1. In other instances, features of both Noonan syndrome and NF1 appear to result from mutations of the *NF1* gene, and these phenotypes segregate together (46). It appears that the concurrence of NF1 and Noonan syndrome may have several different causes (47), but this question awaits further study.

NF1 and the associated clinical presentations discussed above are the only conditions known to be caused by *NF1* gene mutations. No studies of the *NF1* gene in the general population have been performed.

INTERACTIONS

The wide variability of the NF1 phenotype, even in individuals with the same *NF1* gene mutation, suggests that other factors are involved in determining clinical manifestations. These may include other modifying genes, environmental factors, and chance. Thus far, little is known about the relative contribution of these to the NF1 phenotype.

A study of 175 individuals in 48 families, including six monozygotic twin pairs, evaluated variation of the NF1 phenotype with degree of relation (48). The number of café-au-lait spots and of neurofibromas showed a high correlation between monozygotic twins, a lower correlation between first-degree relatives, and the lowest correlation among more distant relatives. The study also looked at the presence or absence of plexiform neurofibromas, optic gliomas, scoliosis, epilepsy, and referral for remedial education. With the exception of plexiform neurofibromas, these traits also showed familial clustering. The authors concluded that much of the phenotypic variation in NF1 is related to trait-specific "modifying genes."

It has been suggested that environmental factors influence NF1 phenotype; however, no convincing evidence has been presented to support the involvement of any particular environmental factor. Riccardi (49) has suggested that mechanical trauma (in the form of injury to the skin) may often precede the development of neurofibromas, but the evidence for involvement of this factor is anecdotal.

The role of stochastic factors (chance) in the occurrence of some NF1 manifestations has also been hypothesized. Chance may be involved in determining which cells are affected by a somatic mutation and at what point in development somatic mutation occurs. Major questions remain about how the NF1 phenotype is determined, but it is likely that the NF1 genotype, modifying genes, environmental factors, and chance all play a role in the clinical manifestations of *NF1* gene mutations.

LABORATORY TESTS

Laboratory testing for *NF1* mutations is difficult. Although a variety of approaches has been used singly or in combination in research laboratories, none has been shown to be appropriate for routine clinical use.

A protein truncation test is available commercially for *NF1* mutation testing, but its sensitivity, specificity, and positive predictive value in a large group of patients have not been reported. In this test, RNA is reverse transcribed, and the complementary DNA product is used to perform in vitro transcription and translation. Truncated neurofibromin proteins are identified by separating the protein products using an sodium dodecyl sulfate-polyacrylamide gel (50). Mutations may then be confirmed by direct DNA sequencing. False-positive results are possible when truncated proteins are not confirmed by sequencing (16). In addition, the protein truncation test cannot detect mutations that do not result in a truncated protein, such as missense mutations and large deletions, or mutations in which the RNA is unstable and, thus, is unavailable for reverse transcription. The ability of the protein truncation test to detect mosaic mutations is unknown (16). However, it appears that the risk for both false positives (when a finding of a truncated protein is not confirmed by DNA sequencing) and false negatives may be significant with this test. Published studies of the sensitivity of the protein truncation test have been small; about 70 percent of the cases meeting NF1 diagnostic criteria (13 of 20 cases in one study (50) and 11 of 15 cases in another (51)) had a positive result on the protein truncation test. Thirty-seven (77 percent) of 48 cases that met NF1 diagnostic criteria referred for commercial testing are reported to have had a positive protein truncation test result (T. Brown, LabCorp, Research Triangle Park, North Carolina, personal communication, 1999). No information is available on the specificity or positive predictive value of the protein truncation test. When the protein truncation test is negative, further molecular studies may be helpful in identifying the mutation, but these studies are currently available only on a research basis.

In familial NF1 cases (when two or more family members are affected), linkage analysis can be performed. The availability of intragenic microsatellite NF1 markers has increased the proportion of families in which linkage studies will be informative and has also increased the diagnostic accuracy (52) to an average of 90 percent.

Given that NF1 is easily diagnosed clinically in most affected individuals over age 6 years, the need for laboratory testing is limited to specific circumstances. One of these is for prenatal diagnosis when one of the

parents has NF1. If the causative mutation has been identified, direct testing for this specific mutation can be performed on chorionic villus or amniotic fluid samples. However, the severity of NF1 cannot be predicted prenatally; only the presence or absence of the mutation can be identified. Because of the wide variability in NF1 clinical expression, many families do not find prenatal diagnosis of NF1 acceptable (16).

In families in which there are multiple affected relatives, linkage analysis can also be used for prenatal diagnosis. Once again, only the presence or absence of the affected allele can be predicted, not the severity of the clinical manifestations.

The other situation in which laboratory testing may be considered is in children at risk for NF1, before clinical diagnostic criteria are met. The child may be at risk because of a family history or because of having some features (typically café-au-lait spots), but not sufficient features to meet the established diagnostic criteria. While the ability to confirm or rule out the diagnosis with a laboratory test would be helpful, these children are at particular risk for possible stigmatization and unnecessary medical intervention if a false-positive test results (16). Therefore, following the child on a regular basis for appearance of NF1 complications and sufficient clinical criteria to assure the diagnosis is likely to be a better option at this time.

POPULATION TESTING

No general, population-based studies using molecular testing to identify *NF1* mutations have been performed. This type of study seems unnecessary since individuals over age 6 years with *NF1* mutations can usually be identified by physical and ophthalmologic examination.

Clinical methods of NF1 ascertainment have been performed to estimate the prevalence of the condition in research studies in different populations (see Prevalence of NF1, above). However, population-based screening of individuals for clinical features of NF1 has not received substantial support. This is, in part, due to the difficulty of the effort: Careful physical examination for NF1 features is time consuming, unlike other population-based screening methods based on a simple laboratory test. In addition, since many NF1 features are age dependent, diagnosis in a child under age 3 years is often challenging. However, most adult individuals with NF1 can be identified as a result of a regular physical examination, even in the absence of a screening program.

An important question is whether an early NF1 diagnosis, achieved through a screening program, would lead to prevention of NF1 complications. Since primary prevention of NF1 complications is not presently possible, this beneficial effect would be confined to the possibility that early recognition of complications may result in improved treatment. Several studies have assessed whether screening of individuals already known to have NF1 for complications is helpful. A recent paper suggests that the vast majority of abnormalities identified through a comprehensive screening program (consisting of ophthalmologic consultation with slit-lamp examination, chest radiograph, abdominal ultrasonography, neuroimaging, and analysis of catecholamine levels) did not result in therapeutic action (53). Studies such as these have led many NF1 experts to suggest that a careful clinical evaluation for NF1 complications on an annual basis (or more often, if necessary) by a physician familiar with NF1 is optimal for affected individuals (16). Regular ophthalmologic examination is also recommended for children with NF1 (18). Unfortunately, no studies are available that address the more general question of whether an earlier NF1 diagnosis, made through a screening program, would lead to improved treatment.

Another valid concern when considering whether a population-based screening program may be beneficial is the effect that early diagnosis may have on family planning (avoidance of future pregnancies or utilization of prenatal diagnosis). In a recent survey, the majority of parents preferred an early diagnosis of NF1 in their child; however, NF1 diagnosis did not usually result in avoidance of future pregnancies, and while prenatal diagnosis was viewed favorably, only a few parents said they would actually terminate an affected pregnancy (54). All of these issues will need to be taken into account in the discussion regarding population-based screening (whether using molecular methods or clinical methods); however, at this time, it appears that the benefits of early diagnosis do not outweigh the potential costs of a population-based screening program.

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APPENDIX 1. INTERNET SITES

General resources

March of Dimes:
http://www.noah.cuny.edu/pregnancy/march_of_dimes/birth_defects/neurofib.html
National Organization for Rare Disorders
http://206.105.18.10/nord/rdb_sum/3.htm

Genetic databases

GeneCards
http://bioinfo.weizmann.ac.il/cards-bin/carddisp?NF1&search=NF1&suff=txt
GeneClinics
http://www.geneclinics.org/profiles/nf1/
Genome Database
http://gdbwww.gdb.org/gdb-bin/genera/accno?GDB:120231
Human Gene Mutation Database
http://www.uwcm.ac.uk/uwcm/mg/search/120231.html
NNFF International NF1 Genetic Mutation Analysis Consortium
http://www.nf.org/nf1gene/
Online Mendelian Inheritance in Man (OMIM).
http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?162200

Educational resources

Massachusetts General Hospital Neurofibromatosis Clinic
http://neurosurgery.mgh.harvard.edu/NFclinic.htm
National Institute of Neurological Disorders and Stroke
http://www.ninds.nih.gov/patients/disorder/neurofib/neurofib.htm

Support groups

National Neurofibromatosis Foundation
http://www.nf.org/
Neurofibromatosis, Inc.
http://nfinc.org/
Neurofibromatosis
http://touch.ch/neurofibromatosis/Mainfr1.html
The Neurofibromatosis Association
http://www.users.zetnet.co.uk/neurofibromatosis/

Other websites

American Academy of Pediatrics Policy Statement: Health Supervision for Children with Neurofibromatosis
http://www.aap.org/policy/00923.html
World Wide Neurofibromatosis Clinicians Forum
http://www.neurofibromatosis.org/md12.htm