

# Neurofibromatosis 2

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## Purpose of review

Recent clinical and molecular research on neurofibromatosis 2 (NF2) is reviewed, and the implications for clinical practice and research are discussed.

## Recent findings

NF2 patients who are treated in specialty centers have a significantly lower risk of mortality than those who are treated in non-specialty centers. Vestibular schwannoma growth rates in NF2 are generally higher in younger people but are highly variable, even among multiple NF2 patients of similar ages in the same family. Radiation therapy is best reserved for NF2 patients who have particularly aggressive tumors, those who are poor surgical risks, those who refuse surgery, or those who are elderly. In-vivo studies have demonstrated that leptomenigeal cell activation of *Nf2* in mice results in leptomenigeal hyperplasia and meningioma formation. In-vitro studies have identified molecules that interact with the *NF2* product (merlin or schwannomin), some of which (e.g., CD44 and paxillin) may play critical roles in merlin growth regulation.

## Summary

NF2 patients should be referred to specialty treatment centers for optimal care. Clinical management of multiple patients in NF2 families cannot be based on the expectation of similar vestibular schwannoma growth rates, even when other clinical aspects of disease severity are similar. The availability of accurate mouse models of human NF2-associated tumors and the identification of molecules involved in merlin growth regulation now provide an opportunity to design targeted treatments for schwannomas and meningiomas.

## Keywords

neurofibromatosis 2, NF2, schwannoma, merlin, mouse model

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## Abbreviations

ERM ezrin, radixin and moesin  
NF2 neurofibromatosis 2

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## Introduction

Neurofibromatosis 2 (NF2) is an autosomal dominant disease caused by inactivating mutations of the *NF2* gene [1,2], and is characterized by the development of nervous system tumors, ocular abnormalities, and skin tumors. In 1992, the population-based birth incidence of NF2 was estimated as 1 in 33 000–40 000, and the population-based symptomatic prevalence as 1 in 210 000 [3]. It is likely that the prevalence is higher due to asymptomatic *NF2* mutation carriers and increased patient survival from improvements in early diagnosis and treatment. Previous cross-sectional studies described the clinical spectrum of NF2 [4–6], but it is only recently that longitudinal studies have characterized the natural history of the disease. In-vitro and in-vivo approaches are ongoing to clarify the function of the *NF2* product (merlin or schwannomin). These approaches include the identification of merlin binding partners, the generation of mouse models of NF2, and investigations of the role of merlin in Schwann and meningeal cell biology and tumorigenesis.

## Natural history

Vestibular schwannomas (usually bilateral) occur in about 95% of adult patients with NF2, and adult-onset disease usually manifests with vestibular symptoms [4–6]. In contrast, children with NF2 often present with non-8th-nerve tumors and non-vestibular symptoms [7,8]. Recent studies have highlighted the occurrence of mononeuropathy in NF2 [9,10]. Peripheral neuropathy is common in NF2 patients with severe disease; in these patients, axonopathy can be caused not only by tumor growth, but also by multiple tumorlets and proliferation of Schwann and perineurial cells [11\*,12\*].

Vestibular schwannoma growth rates in NF2 are generally higher in younger patients but are extremely variable, both between patients and over time in the same patient [13\*,14\*]. Growth rates are highly variable even among multiple NF2 patients of similar ages in the same family [13\*]. This suggests that stochastic processes (random processes operating over time) or as-yet-unknown factors influence vestibular schwannoma growth rates in NF2. For this reason, clinical management of multiple patients in NF2 families cannot be based on the expectation of similar vestibular schwannoma growth rates, even when other clinical aspects of disease severity are similar.

Age at diagnosis of NF2, intracranial meningiomas, type of treatment center, and type of constitutional *NF2*

mutation are informative predictors of the risk of mortality [15\*]. Age at diagnosis is, by far, the strongest single predictor of the risk of mortality, and therefore is a useful index for patient counseling and clinical management. It is encouraging to note that NF2 patients who are treated in specialty centers have a significantly lower risk of mortality than those who are treated in non-specialty centers.

### Genotype–phenotype correlations

Since the mid-1990s, many studies have found genotype–phenotype correlations in NF2, most recently for intradural extramedullary spinal tumors [16]. In general, people with constitutional nonsense or frameshift *NF2* mutations have severe disease, those with missense mutations, in-frame deletions, or large deletions have mild disease, and those with splice-site mutations have variable disease severity. The variable disease severity in people with splice-site mutations may be associated with the location of the mutation [17]. Most genotype–phenotype correlation studies have been cross-sectional, but NF2 patients with missense mutations have a lower risk of mortality than those with other types of mutations [15\*].

In longitudinal studies, the type of constitutional *NF2* mutation has not been found to be a significant predictor of vestibular schwannoma growth rates, although these studies have had relatively few patients [13\*,14\*]. Since cross-sectional genotype–phenotype correlation studies have found strong associations between mutation type and age at onset or diagnosis, and age at onset or diagnosis predicts vestibular schwannoma growth rates, a logical question is why mutation type is not a stronger predictor of vestibular schwannoma growth rates. A possible explanation is that age at onset or diagnosis and vestibular schwannoma growth rates each reflect a composite of disease-influencing factors, while mutation type is only one of these factors.

Multiple NF2 patients in the same family often have similar disease severity, but specific disease features and disease progression differ even between monozygotic twins with NF2, probably due to processes such as the stochastic inactivation of the second *NF2* allele [18]. There are significant intrafamilial correlations in clinical indices of disease severity (age at onset of symptoms, age at onset of hearing loss, and number of intracranial meningiomas), both in NF2 families as a whole and in NF2 families with specific types of *NF2* mutations [19]. This is consistent with the effects of both allelic and non-allelic familial factors (such as modifying genes) on clinical variability in NF2.

### Diagnosis

Four sets of clinical diagnostic criteria have been proposed for NF2. To establish the diagnosis, the 1987

and 1991 US National Institutes of Health (NIH) criteria each require bilateral vestibular schwannomas or a family history of the disease plus other characteristic disease features. Yet, half of all patients with NF2 do not have a family history of the disease [4], and patients can present with intracranial meningiomas, spinal tumors, peripheral nerve tumors, or ocular abnormalities long before the appearance of a vestibular schwannoma [4–6,7,8]. The Manchester criteria (Table 1) [4] and criteria proposed by a group organized by the National Neurofibromatosis Foundation [20] each expanded the NIH criteria to permit the diagnosis of NF2 in people who do not have a family history of NF2 or bilateral vestibular schwannomas, but who do have multiple schwannomas or meningiomas. This change increases sensitivity while maintaining high specificity [21,22]. The Manchester criteria are the most sensitive of the four sets of criteria [23].

For about a decade, linkage analysis using tightly-linked genetic markers has been available to determine *NF2* mutation carrier status in at-risk individuals in families with living NF2 patients from two or more generations. At-risk children of parents with new mutations (people who are the first in their family to have the *NF2* mutation) can be tested for constitutional *NF2* mutations if an identical mutation is found in two tumors from the affected parent [22,24]. Using standard mutation identification techniques, the efficiency of constitutional *NF2* mutation detection is lower for new mutations than for inherited cases. This reflects the fact that approximately 20% of new mutations are somatic mosaics; other causes are large deletions and chromosomal rearrangements at the *NF2* locus. The implications of mosaicism in NF2 have been recently reviewed [25].

### Management

Patients with NF2 should be referred to specialty treatment centers, where they can be managed by multidisciplinary teams of neurosurgeons, otolaryngologists, neuroradiologists, ophthalmologists, geneticists, and audiologists. NF2 patients who are managed at specialty treatment centers have a significantly lower risk

**Table 1. Manchester clinical diagnostic criteria for NF2**

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- (A) Bilateral vestibular schwannomas
  - (B) First-degree family relative with NF2 and unilateral vestibular schwannoma or any two of the following: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
  - (C) Unilateral vestibular schwannoma and any two of the following: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
  - (D) Multiple meningiomas (two or more) and unilateral vestibular schwannoma or any two of the following: schwannoma, glioma, neurofibroma, cataract
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Note: 'any two' means two individual tumors or cataracts. Reproduced from [4] with permission.

of mortality than those who are treated at non-specialty centers [15•]. Studies of operative outcomes in vestibular schwannoma surgery have found that the rate of favorable outcomes increases, and the rate of serious complications decreases, with increasing surgical experience [26,27].

It is important to balance the use of microsurgery and radiation treatment, which can have a role for NF2 patients who have particularly aggressive tumors, those who are poor surgical risks, those who refuse surgery, or those who are elderly. Radiation treatment can provide good short-term 'tumor control' [28], but in patients with NF2, this must be balanced against longer-term risks such as a significantly elevated prevalence of malignancy [29,30] and the knowledge that vestibular schwannomas usually grow slowly or episodically [13•,14•]. Somatic *NF2* mutations are common in sporadic malignant mesotheliomas, and asbestos-exposed people with an inactivated constitutional *NF2* allele may be particularly susceptible to mesothelioma because only a single hit is needed to functionally inactivate the *NF2* gene [31].

### The *NF2* gene

The *NF2* gene on chromosome 22q codes for merlin, a 595-amino-acid protein that contains three predicted structural domains [1,2]. Structurally, merlin is most closely related to a family of proteins that link the actin cytoskeleton to cell-surface molecules important for cellular remodeling and growth regulation. This family of structurally similar molecules includes ezrin, radixin and moesin (ERM proteins) [32]. Merlin, like the ERM proteins, contains an amino-terminal Protein 4.1 cell-surface glycoprotein-binding domain (FERM domain; residues 1–313) followed by a predicted alpha-helical region and a non-conserved carboxy-terminal domain. Unlike ERM proteins, the merlin carboxy-terminal domain lacks conventional actin-binding sequences (Fig. 1a). Recent studies on the FERM domain of merlin have demonstrated that it is composed of three subdomains that may mediate specific interactions with critical protein binding partners [33•,34•].

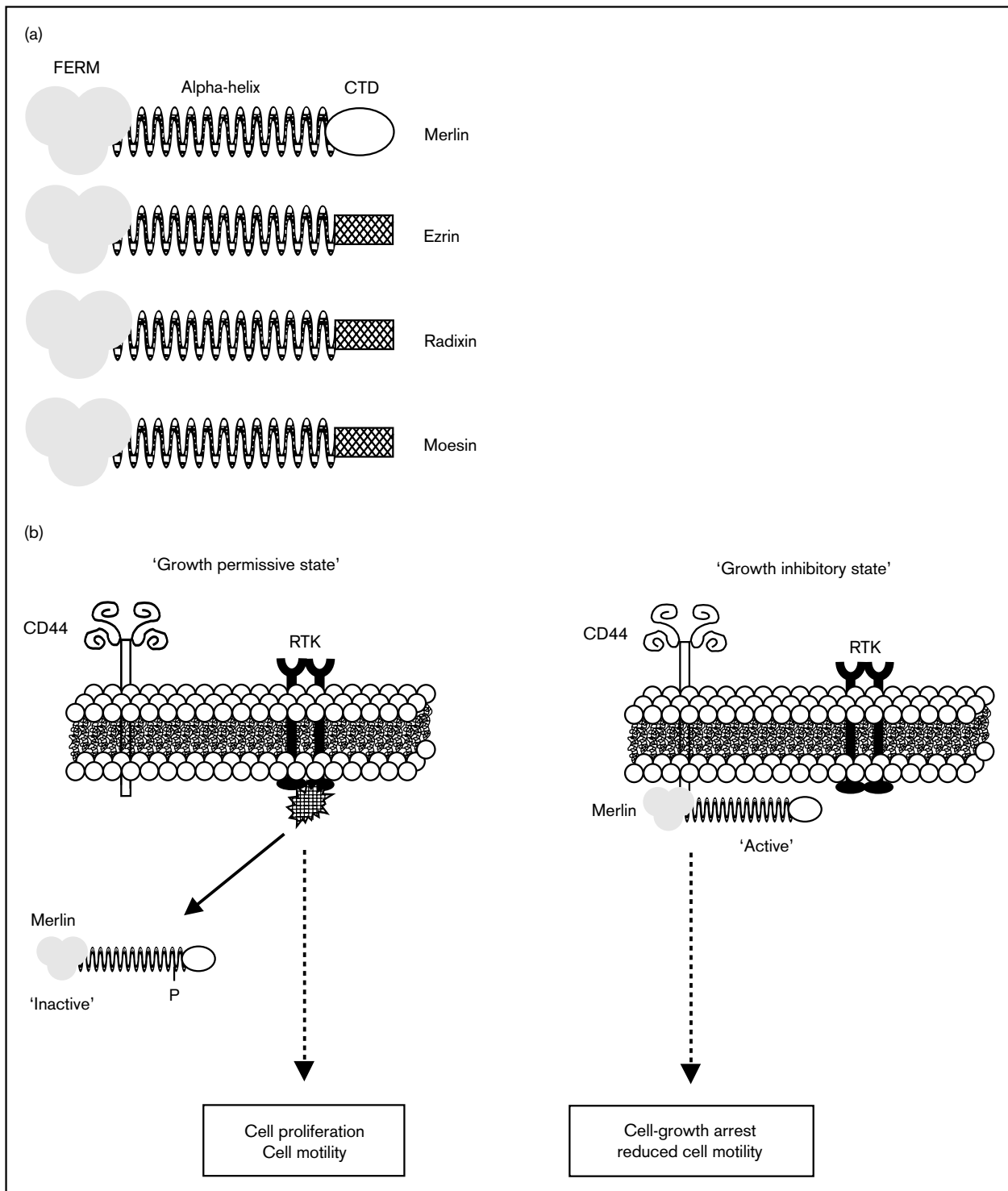
Merlin is expressed in a variety of tissues relevant to the clinical features of NF2. High levels of merlin expression are detected in a large number of tissues during embryonic development. In adult tissues, significant merlin expression is detected in Schwann cells, meningeal cells, lens, and nerve, accounting for the development of schwannomas, meningiomas, and lenticular opacities seen in individuals affected by NF2. Within cells, merlin appears to be localized in the cell membrane at regions involved in cell–cell contact and motility.

With the identification of the *NF2* gene, intense study has focused on determining how this putative tumor suppressor functions in normal cells, and how its loss predisposes to tumor formation. Several complementary approaches have been taken in recent years to clarify how merlin operates: these have included (1) the identification of merlin binding partners, (2) the generation of mouse models of NF2, and (3) investigation of the role of merlin in Schwann and meningeal cell biology and tumorigenesis.

### Identification of merlin binding partners

Despite the structural similarity to ERM proteins, the predicted *NF2* protein sequence, when analyzed, provides minimal clues as to how merlin functions as a negative growth regulator. One approach for deducing the function of merlin is to identify proteins that associate with merlin. A large number of merlin-interacting proteins have been identified in recent years. One class of merlin-interactors includes cell-surface proteins that bind FERM-containing proteins such as CD44 and  $\beta$ 1-integrin [35•,36]. Another class of merlin-binding proteins is represented by molecules involved in cell-cytoskeleton dynamics ( $\beta$ II-spectrin, paxillin, actin, and syntenin) [37–40]. Lastly, several molecules have been identified that may be important for regulating ion transport (sodium hydrogen exchange regulatory factor; NHE-RF) [41] and endocytosis (hepatocyte growth factor-regulated tyrosine kinase substrate; HRS) [42]. While some of these molecules may hold the key to deciphering merlin's function as a tumor suppressor, it is not yet clear which of these proteins is required for merlin to operate *in vivo*.

Recent studies on several of these interactors have suggested a possible role for selected binding partners in merlin growth regulation. The cell-surface glycoprotein CD44 has been implicated in cell proliferation and cell motility. The cytoplasmic tail of CD44 binds to merlin at the cell membrane, and appears to be important for providing a growth-arrest signal [35•,43]. This CD44–merlin association is tightly regulated by protein phosphorylation [35•,44•–46•]. In addition, the association between merlin and the actin cytoskeleton is important for localizing merlin to the proper subcellular location. The merlin–actin cytoskeleton interaction may be mediated by several merlin-binding molecules, including actin,  $\beta$ II-spectrin, syntenin, and paxillin. Recent results have suggested that the association between merlin and paxillin is critical for the proper subcellular localization of merlin [47•]. Lastly, merlin binds to HRS, a molecule implicated in endocytosis and growth factor receptor recycling. It is intriguing that the consequence of regulated over-expression of HRS in rat schwannoma cells is

Figure 1. Structure and potential function of the *NF2* gene product, merlin

(a) Merlin is structurally related to the ezrin, radixin and moesin (ERM) family of molecules. It contains three distinct predicted domains, including a FERM domain (an amino-terminal Protein 4.1 cell-surface glycoprotein-binding domain), an alpha-helical domain, and a unique carboxy-terminal domain. The alignment between merlin and other ERM proteins is depicted. The regions of greatest sequence similarity are found in the FERM and alpha-helical regions. The shaded carboxy-terminal domains shown for ezrin, radixin and moesin denote the conventional actin-binding domains. (b) On the basis of evidence from a number of studies, it is likely that merlin functions as a growth and motility regulator by inhibiting the transduction of mitogenic and motogenic signals from the extracellular milieu. In this putative model, merlin acts to suppress cell growth and motility only under growth-inhibitory conditions when it is 'active' and able to associate with cell-surface proteins, such as CD44. Under growth-permissive conditions, merlin may be phosphorylated ('P') by receptor tyrosine kinase (RTK) activation and rendered 'inactive'. CTD, carboxy-terminal domain; RTK, receptor tyrosine kinase.

remarkably similar to that of merlin [42]. Further studies are required to determine how these merlin-interactors facilitate merlin function.

### Mouse models for NF2

Since individuals affected with NF2 develop tumors at an increased frequency, the *NF2* gene has been hypothesized to function as a tumor-suppressor gene. In support of this notion, inactivation of the *NF2* gene and loss of merlin expression have been demonstrated in NF2-related tumors. Surprisingly, a large proportion of sporadic schwannomas and meningiomas also have *NF2* inactivation and merlin loss, suggesting that the *NF2* gene is an important growth regulator for Schwann cells and leptomeningeal cells relevant to the development of schwannomas and meningiomas, respectively [48–50]. In contrast, no alterations in ERM protein expression have been reported in schwannomas or meningiomas.

To directly address the role of the *NF2* gene as a tumor suppressor, several research groups have generated mice with targeted defects in the mouse *Nf2* gene. Mice in which both copies of *Nf2* are inactivated die during early embryonic development [51], while those with only one mutated *Nf2* gene (*Nf2*<sup>+/-</sup> mice) are prone to cancer [52]. Unfortunately, the cancers that arise in these mice are not schwannomas or meningiomas. In an effort to generate improved models of NF2-related tumors, Giovannini *et al.* have developed mice in which the *Nf2* gene is conditionally inactivated in either Schwann cells or leptomeningeal cells. Schwann cell-restricted inactivation of *Nf2* results initially in Schwann-cell hyperplasia and then schwannoma formation [53]. Similarly, leptomeningeal loss of merlin is associated with leptomeningeal hyperplasia and meningioma formation [54•]. Collectively, these results argue that loss of *Nf2* in the appropriate tissues is sufficient for tumorigenesis.

### Role of merlin in Schwann and meningeal cell biology and tumorigenesis

As a member of the ERM family, merlin would be predicted to have a role in actin cytoskeleton-associated processes. Several independent observations support this prediction. First, the cancers that arise in *Nf2*<sup>+/-</sup> mice are highly motile and metastatic [52]. Second, loss of merlin in primary cultures of schwannomas is associated with abnormal actin-cytoskeleton organization that can be reversed upon the re-expression of merlin [55]. Lastly, regulated overexpression of merlin in rat schwannoma cells results in disorganization of the actin cytoskeleton, abnormalities in the initial phases of cell spreading, and reduced cell motility [56]. In contrast, regulated overexpression of *NF2* containing missense patient mutations has no effect on actin-cytoskeleton function.

This effect of merlin on cell motility should not be surprising in the light of clinical observations. While it is accepted that schwannomas, as opposed to neurofibromas, grow as discrete masses separable from the associated nerve, there is often tumor infiltration into the nerve. Similarly, meningiomas can migrate along the leptomeninges forming multiple discrete tumors as well as invading brain parenchyma. Loss of merlin in Schwann cells and leptomeningeal cells may promote this motile and invasive behavior.

The role of merlin in actin-cytoskeleton-associated processes also suggests that merlin may regulate cell growth in response to specific cues from the environment. Studies from a number of laboratories have demonstrated that merlin probably has a specific role in growth suppression mediated by activation of transmembrane proteins (e.g. CD44) or cell contact (Fig. 1b). These results suggest that merlin growth regulation occurs in the context of extracellular interactions provided by normal brain or nerve. Loss of merlin might result in an impaired ability to respond to these environmental growth-regulatory cues and culminate in increased cell growth, tumor formation, and tumor-cell infiltration.

### Conclusions and future research

Recent natural history studies have provided information that is useful for the care of patients with NF2. Such patients should be referred to specialty treatment centers for optimal care, and age at diagnosis is a useful index for patient counseling and clinical management. Clinical management of multiple patients in families with NF2 cannot be based on the expectation of similar vestibular schwannoma growth rates, even when other clinical aspects of disease severity are similar. Radiation therapy is best reserved for NF2 patients who have particularly aggressive tumors, those with poor surgical risks, those who refuse surgery, or those who are elderly. The sensitivity of the diagnostic criteria for NF2 can be increased by adding mononeuropathy as a clinical diagnostic criterion and the results of genetic testing; such changes should be empirically evaluated before the revised set of criteria is adopted for use.

The development of mouse models of NF2-associated tumors is the first step towards preclinical evaluation of targeted therapies for schwannomas and meningiomas. In addition, more detailed studies of the mechanism by which merlin regulates cell growth and proliferation may identify specific targets for therapeutic design giving particular efficacy for NF2-associated tumors. The combination of these two approaches will undoubtedly result in improved therapeutic options for individuals affected with NF2.

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