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The Genetic and Molecular Pathogenesis of NF1 and NF2

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Neurofibromatosis types 1 and 2 (NF1 and NF2) are autosomal dominant phakomatoses. The NF1 and NF2 genes encode for neurofibromin and merlin, respectively. These 2 functionally unrelated proteins both act as tumor suppressor genes, possibly through modulation of the RAS/RAC oncogenic pathways. Improved understanding of the mechanisms by which these tumor suppressors act may allow for medical therapies for neurofibromatosis and may offer insights for cancer therapeutics.
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Neurofibromatosis types 1 and 2 (NF1 and NF2) are autosomal dominant genetic phakomatoses without special predilection for race or gender. The genes for NF1 and NF2 are found on chromosomes 17 and 22, respectively, and have high rates of spontaneous mutation, resulting in 50% of cases of each disorder arising de novo.[1-3] Both have essentially 100% penetrance but wide phenotypic variability.[3,4] For both NF1 and NF2, the factors modulating phenotypic variability likely represent both genetic and environmental factors.[5-8] NF1 is fairly common with a incidence of about 1 in 3,000.[2] NF2 is about 1/10th as common, with an incidence of about 1:40,000.[9] Somatic mosaicism can occur in either disorder. Both genes encode for tumor suppressors. Mutations in the *NF1* gene result in loss of function of the protein neurofibromin. Neurofibromin is a guanosine triphosphatase (GTPase)-activating protein (GAP) that helps maintain the proto-oncogene RAS in an inactive form. Loss of neurofibromin results in increased RAS activity, particularly in neurocutaneous tissues, leading to increased proliferation and tumorigenesis.[10] The *NF2* gene encodes for merlin, which may act as a regulator of growth, motility, and cellular remodeling by inhibiting the transduction of extracellular mitogenic and motogenic signals.[11] Recent work has greatly improved our understanding of the function of neurofibromin and merlin and how they relate to the clinical manifestations of NF1 and NF2.

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NF1

The gene for NF1, located on chromosome 17q11.2, was isolated and transcribed in 1990.[12] Subsequently, it was shown that the NF1 product was a protein that acted as a tumor suppressor by inhibiting RAS.[13] It was referred to as neurofibromin and further found to contain a GAP-related domain that acts to downregulate RAS via stimulation of intrinsic GTPase.[14]

The *NF1* gene is large, comprised of over 350 kb of genomic DNA and 60 exons.[15,16] The gene codes for a 360 amino acid sequence homologous to the catalytic domain of GAP and the products of the yeast genes *IRA1* and *IRA2*.[17,18] This region is collectively known as the NF1 GAP-related domain (NF1-GRD). The NF1-GRD is located near the center of the *NF1* gene. Three other genes, *EVI2A*, *EVI2B*, and *OMGP* are embedded within exon 27 of *NF1*, and the pseudogene *AK3* is embedded within intron 37.[19-21] Four separate transcripts of NF1 have been identified and designated: GRD I, GRD II, 3′ALT, and 5′ALT2.[18]

The gene product of NF1 was predicted to be 327 kDa. However, neurofibromin is approximately 220 to 250 kDa.[22-24] There is no evidence of posttranslational glycolsylation or processing of the full-length protein.[25] Neurofibromin has been shown to be located within the cytoplasm[26] and colocalized with cytoplasmic microtubules.[27]

NF1 has been found to be expressed in a wide variety of tissues including human white blood cells, fibroblasts, brain, spleen, lung, muscle, neuroblastoma, thymoma, neurofibromas, colon carcinoma cell lines, and breast cancer cell lines.[28,29] Through immunoprecipitation and Western blot analysis, it has been shown that the gene product neurofibromin is expressed at highest levels in neurons, oligodendro-

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glial cells, the dorsal root ganglia, and nonmyelinating schwann cells and not seen in astrocytes or myelinating Schwann cells. In rat tissues, expression was seen in the brain and spinal cord and to a lesser extent in the liver, spleen, and pancreas. It was not seen in the muscle, lung, kidney, or skin.[26] Other studies have shown neurofibromin in keratinocytes and melanocytes in rats and humans.[30]

Mutations in affected individuals can occur virtually anywhere within the *NF1* gene and can consist of deletions, insertions, nonsense mutations, missense mutations, and intronic mutations.[18,31] Most germline mutations result in frameshift or nonsense mutations. The spontaneous mutation rate of the *NF1* gene is one of the highest known in the human genome and is approximately 100-fold greater than the average mutation rate.[18] As a result, approximately half of patients with NF1 have no family history of the disorder.[32]

There is poor correlation between the specific mutation and disease phenotype except in patients with deletions of essentially the entire gene who tend to have more severe phenotype and higher numbers of neurofibromas. Members of the same family typically show different manifestations of the disorder[33] and unrelated individuals with NF1 with identical mutations may also show different manifestations and severity.[18] The high correlation of severity and manifestations seen in monozygotic twins suggests that there is a strong genetic component to the variability, but the relatively low correlation seen in distant relatives indicates that the specific mutation of *NF1* plays a relatively minor role in comparison to other, unknown modifier genes.[34]

Genetic testing for NF1 became available in the mid-1990s and relied on the use of premature truncation assays. However, the clinical utility of the premature truncation assay was limited by its sensitivity of only about 70%.[35,36] More recently, more sensitive testing has been offered. Initially, a premature truncation test is performed starting from puromycin-treated Epstein-Barr virus (EBV) cell lines and, if no mutation is found, followed with heteroduplex, fluorescent in situ hybridization (FISH), Southern blot, and cytogenetic analysis.[37]

Consistent with the "2-hit" model of tumorigenesis, loss of heterozygosity (LOH) has been shown to occur in NF1-associated malignancies including malignant peripheral nerve sheath tumors,[38] leukemias,[39] pheochromocytomas,[40] and astrocytomas[41,42] as well as some benign dermal and plexiform neurofibromas.[43-45] In neurofibromas, the LOH is seen specifically in involved Schwann cells but not fibroblasts, suggesting that Schwann cells are the cell of origin.[46] LOH has been shown in non–NF-related malignancies such as breast cancer, colon cancer, and neuroblastoma.[47-49]

However, LOH is not seen in all neurofibromas nor is it responsible for many of the other clinical manifestations of *NF1*. The heterozygous state of *NF1* has been shown in and of itself to result in some degree of abnormal cell growth. Haploinsufficiency of *NF1* has been associated with increased mast cell proliferation, survival, and colony formation and enhanced RAS mitogen-activated protein kinase C activity.[50] Astrocytes haploinsufficient for *NF1* show increased glial fibrillary acidic protein (GFAP) expression, indicating increased activation,[51,52] increased proliferation[53] and growth advantage[54] with cytoskeletal abnormalities, reduced cell attachment, and increased motility.[55]

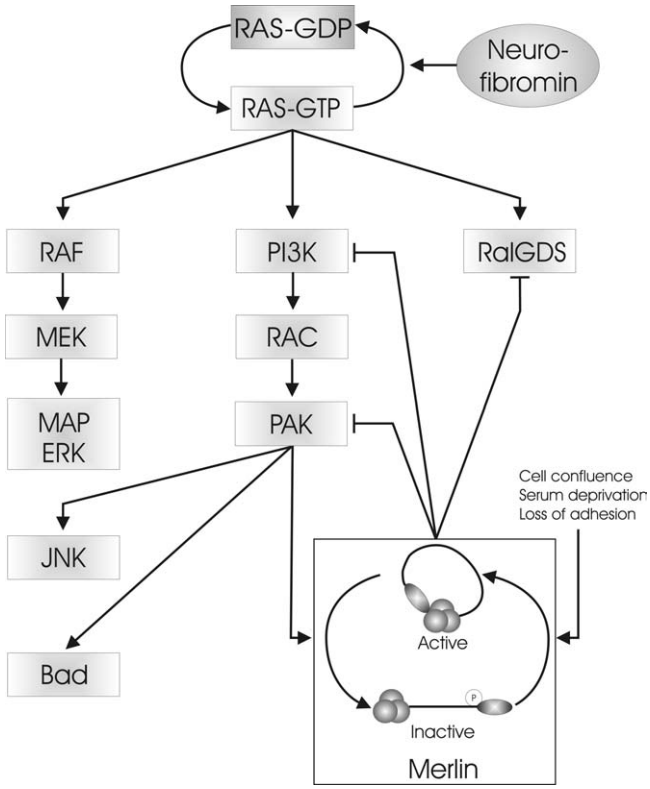

Figure 1 A schematic representation of the interactions of neurofibromin and merlin with the RAS and RAC pathways. Neurofibromin helps to maintain RAS in the inactive GDP form by accelerating the conversion of active GTP-RAS to inactive GDP-RAS. Merlin is a downstream target of PAK that promotes phosphorylation and inactivation of merlin. Active hypophosphoylated merlin inhibits PAK in a feedback loop and may also inhibit PI3K and RalGDS. Factors such as cell density influence merlin, promoting the active form.

Neurofibromin, like other GAP proteins, interacts with RAS.[17] RAS is a guanosine triphosphate (GTP)-binding protein that is active in the GTP-bound state and inactive in the guanosine diphoshate (GDP) bound state. Activating mutations of RAS lead to increased signaling for cell proliferation and have been implicated in the formation of many malignant tumors.[18,56] GAP proteins help maintain RAS in the inactive GDP form by accelerating the conversion of GTP-RAS to GDP-RAS. Loss of neuorfibromin results in the generation of elevated levels of active GTP-RAS, thus stimulating dysregulated cell growth and tumorigenesis[17,57-59] (Fig 1).

There are also suggestions that neurofibromin has functions independent of its effect on RAS. Despite intact RAS-mediated signaling, drosophila homozygous for the null mutation of *NF1* showed reduced larva, pupae, and adult body size that can be rescued by expression of activated adenosine 3′5′ monophosphate dependent protein kinase (p21-activated kinase [cyclic AMP-dependent protein kinase A (PKA)]). This raises the possibility that neurofibromin and PKA may interact in a growth control pathway independent