VI NEUROFIBROMATOSIS RESEARCH PROGRAM

Neurofibromatosis 1 (NF1) and NF2 are distinct genetic disorders of the nervous system. These disorders usually result in tumors involving nerves anywhere in the body; however, non-nervous tissue such as bone and skin can also be affected. Together, these two genetic disorders affect more than 100,000 Americans of both genders and all ethnic groups. NF1 and NF2 are usually inherited as autosomal dominant disorders. Therefore, a parent with NF has a 50% chance of passing the disorder on to his or her child. However, 30% to 50% of NF1 and NF2 cases arise as a result of a spontaneous genetic change. Tumors that develop in individuals with NF can cause disfigurement, deafness, blindness, bone deformation, learning disabilities, and in some cases death. The tumors that appear in NF patients can vary significantly, even among affected individuals in the same family. Surgical intervention can provide palliative relief; however, at this time there is no cure. NF1, known as Von Recklinghausen's disease or peripheral NF, is the more common type, affecting about 1 in 2,600[1] to 1 in 4,000 individuals. A common characteristic of NF1 is the appearance of flat, pigmented markings on the skin called café-au-lait spots. NF1 is also characterized by neurofibromas, which are small, heterogeneous tumors that develop on or just under the skin. Symptoms of NF often appear at birth and usually by the age of 10. Approximately 50% of people with NF1 have learning disabilities. NF2 is rarer than NF1, only affecting about 1 in 40,000 individuals, and is also known as bilateral acoustic NF (BAN). NF2 is characterized by the growth of tumors on nerves of the inner ear, among other complications. The inner ear tumors in NF2 patients cause hearing loss and can eventually result in deafness. Hearing loss in NF2 patients can appear as early as the teen years.[2]

Schwannomatosis is a rare form of NF that has only recently been discovered. Schwannomatosis is characterized by the growth of multiple, homogeneous tumors, called schwannomas, consisting of Schwann cells or nerve sheath cells. As with NF1 and NF2, schwannomatosis varies greatly among patients. However, more often than not, the first symptom of schwannomatosis is pain.

Vision: To decrease the impact of neurofibromatosis and schwannomatosis.

Mission: To promote research directed toward the understanding, diagnosis, and treatment of NF1, NF2, and schwannomatosis to enhance the quality of life for individuals with those diseases.

Congressional Appropriations for Peer Reviewed Research:

- $110.3M in FY96–03
- $20M in FY04
- $25M in FY05

Funding Summary:

- 117 awards from the FY96–03 appropriations
- 23 awards from the FY04 appropriation
- ~38 awards anticipated from the FY05 appropriation

PROGRAM BACKGROUND

The Congressionally Directed Medical Research Programs (CDMRP) began managing the Department of Defense (DOD) Neurofibromatosis Research Program (NFRP) in response to the fiscal year 1996 (FY96) Senate Appropriations Committee Report No. 104-124,

[1] *Lammert M, Friedmand JM, Kluwe L, and Mautner VF. 2005. Prevalence of neurofibromatosis 1 in German children at elementary school enrollment.* Arch Dermatol *141:71.*

[2] *Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Neurological Disorders and Stroke,* Report on Neurofibromatosis. *1993.*

"I feel truly privileged to have been a member of the NF panel as a consumer reviewer in the CDMRP program. The experience is compelling and heartening: I am allowed to enter into dialogue with dedicated scientists who are devoting their time to the management of the disease that is debilitating so many lives, including members of my family. The organization and planning behind this process are deservedly meritorious, and it is my fervent hope this program continues."

Susan Buono, FY03 & FY05 NFRP Consumer Peer Review Panel Member

which provided $8 million (M) for research in NF.[3] As a leader of NF research funding worldwide, the NFRP has managed $155.3M from FY96 to FY05, and 140 awards have been made through FY04 across the categories of research, research resources, and training/recruitment. The NFRP has supported the development of critical resources and collaborations, innovative basic research, the training of tomorrow's leaders, and preclinical and clinical research in an effort to improve the understanding, diagnosis, and treatment of NF and schwannomatosis and enhance the quality of life of persons with those diseases. (Refer to the storyboard on pages VI-8–VI-11 for a comprehensive review of NF2 research and the contributions of the NFRP to the field.) Appendix B, Table B-3, summarizes the congressional appropriations and the investment strategy executed by the NFRP for FY04 through FY05.

THE FISCAL YEAR 2004 PROGRAM

Congress appropriated $20M to the DOD NFRP in FY04, and the program continued to challenge the scientific community to advance basic research and bring laboratory research to the clinic. Six award mechanisms were offered, five of which were previously established by the program (Clinical Trial Development, Clinical Trial, Investigator-Initiated Research, New Investigator, and Therapeutic Development Awards) and one that was new to the program in FY04 (Concept Awards). The Clinical Trial Development and Clinical Trial Awards were offered in an effort to support clinical trials in NF. The Investigator-Initiated Research Awards resulted in nine basic and clinically oriented research awards relevant to NF and schwannomatosis. Three early-career investigators were supported by the New Investigator Award mechanism to continue their contribution to the field. Finally, the new award mechanism, Concept Awards, resulted in nine awards to explore untested, high-risk questions relevant to NF, and/or schwannomatosis research. Additional summary information about the number of proposals received, number of awards made, and dollars invested for the FY04 NFRP can be found in Table VI-1. (Because of excess funds, one FY03 Idea Award, one FY03 Investigator-Initiated Research Award, and one FY05 NF Consortium Development Operations Center Award were made in FY04.) As illustrated in Figure VI-1, the FY04 NFRP has developed a research portfolio that encompasses basic, clinical, and population-based research.

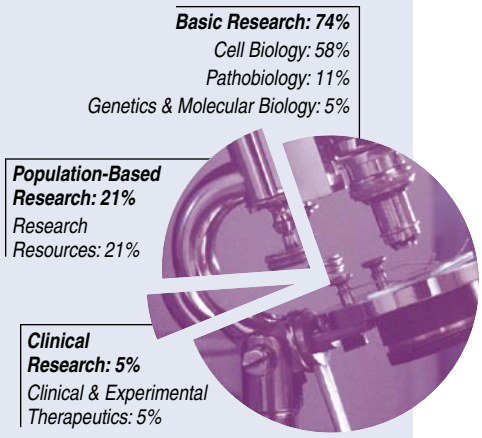


Figure VI-1. FY04 NFRP Portfolio by Research Area

[3] *The U.S. Army Medical Research and Materiel Command, but not the CDMRP, was also responsible for managing congressional appropriations in FY92 for NF research.*

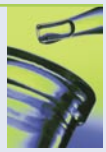
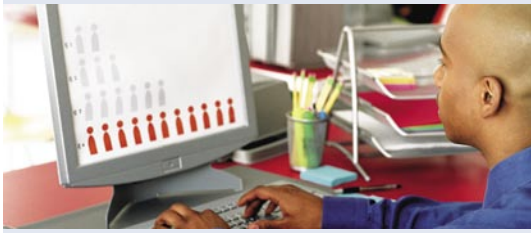


Table VI-1. Funding Summary for the FY04 NFRP

Categories and Award Mechanisms	Number of Proposals Received	Number of Awards	Investment
Research			
Clinical Trial	3	0	0
Clinical Trial Development	0	0	0
Concept	61	9	$0.9M
Investigator-Initiated Research (1 from FY03)	18	9	$12.3M
New Investigator	11	3	$1.8M
Therapeutic Development	2	0	0
FY03 Idea	n/a	1	$0.6M
FY05 NF Consortium Development Operations Center	n/a	1	$2.0M
Total	95	23	$17.6M

"My participation as a consumer in a peer review panel was one of the most uplifting experiences of my life. It gave me an insight to the enormous energy and creativity that exists in the research community, it showed me the seriousness of the selection process to maximize the outcome from limited funds, and it gave me a chance to help the scientists remain close to the main concerns of those affected by the disease."

Miguel Lessing, FY04 NFRP Consumer Peer Review Panel Member

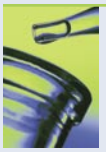


THE VISION FOR THE FISCAL YEAR 2005 PROGRAM

The DOD NFRP was continued through an FY05 congressional appropriation of $25M, marking the largest congressional appropriation to the NFRP since its inception. A total of 125 proposals were received across seven award mechanisms, as detailed in Table VI-2, and approximately 38 awards are anticipated. Five of the award mechanisms were previously established by the NFRP (Clinical Trial, Concept, Investigator-Initiated Research, New Investigator, and Therapeutic Development Awards) and two research resources award mechanisms were introduced in the program in FY05 (NF Consortium Development Operations Center and NF Consortium Development Site Awards). Both of these research resources award mechanisms are intended to support the establishment of an NF1-focused clinical consortium.

Table VI-2. Award Mechanisms Offered and Proposals Received for the FY05 NFRP

Categories and Award Mechanisms	Number of Proposals Received
Research	
Clinical Trial	2
Concept	63
Investigator-Initiated Research	23
New Investigator	14
Therapeutic Development	7
Research Resources	
NF Consortium Development Operations Center	2
NF Consortium Development Site	14
Total	125



"By participating as a consumer reviewer for the U.S. Army's CDMRP program I was able to experience first hand a government program at its finest. As the leader of NF research funding worldwide, the NFRP represents to the many faces of NF encouragement, hope, and promise for the future. This propitious future is advanced by the collaborative effort of dedicated scientists, clinicians, and consumers working together to support the program's vision of decreasing the impact of NF and schwannomatosis. It is hard to imagine where neurofibromatosis research advances, an unfamiliar disorder with a funny name, would be today without NFRP funding. It was my privilege to serve as a peer reviewer and a consumer advocate."

Suzanne C. Earle, FY04 NFRP Consumer Peer Review Panel Member

SCIENTIFIC OUTCOMES AND ADVANCES

The DOD NFRP award outcomes are exciting and present promise for the future. Advances are being made in basic and clinically oriented research in NF and schwannomatosis. The following projects represent a sample of the extraordinary developments that are resulting from research funded by the NFRP to decrease the impact of these diseases. Additional examples of scientific outcomes, products, and technologies resulting from NFRP support can be found in Section III of this annual report.

Phase 2 Trials Under Way for NF1 Treatment

Roger Packer, M.D., Children's National Medical Center, Washington, DC

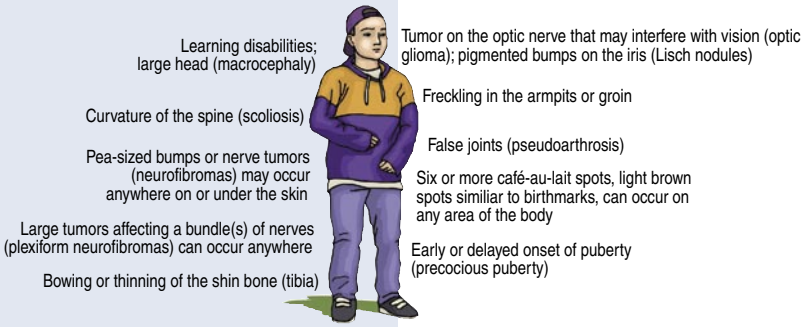
Individuals with NF1 frequently develop plexiform neurofibromas (PNs), tumors arising from the coverings of multiple nerves that can cause disfigurement and severe neurological impairment. There are no effective drugs available for the treatment of PNs, and complete surgical removal of the tumors is often impossible because of their large size. Pirfenidone is a novel oral anti-fibrotic agent that targets growth factors elevated in PNs, suggesting that this drug may inhibit PN development or growth. Dr. Roger Packer, a recipient of a NFRP FY01 Clinical Trial Award, is conducting studies to assess the toxicity and effectiveness of pirfenidone in children with NF1 and progressive PNs. Recently completed Phase 1 safety trials revealed that pirfenidone is well tolerated in children and identified the optimal dose for pediatric patients. Phase 2 trials examining the effects of the drug on tumor progression and quality of life are about to begin. Since there are no effective therapies for progressive PNs other than surgery, Dr. Packer's research has the potential to greatly benefit many children with NF1.

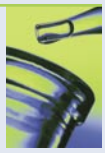
Improving Ras-Targeted Treatment Approaches for NF1 Tumors

David Gutmann, M.D., Ph.D., Washington University, St. Louis, Missouri

NF1 is a genetic disorder characterized by the formation of optic pathway gliomas and other nervous system tumors. Nonsurgical therapies for NF1 tumors are greatly needed. Loss of the *Nf1* gene, neurofibromin, is associated with enhanced activation of growth-stimulating Ras proteins, suggesting that Ras inhibitors may impair NF1 tumor development. However, preliminary human studies with a class of Ras inhibitors known as farnesyltransferase inhibitors (FTIs) have been disappointing. The work of Dr. David Gutmann of Washington University, a recipient of an NFRP FY02

Signs and Symptoms of NF1





Investigator-Initiated Research Award, provides a potential explanation for the relatively low effectiveness of FTIs in NF1 and identifies alternative Ras-targeted approaches that may be preferable for treating NF1 tumors. Dr. Gutmann used primary cell cultures as well as genetically engineered *Nf1* mutant mice to examine the roles of the three types (isoforms) of Ras proteins in the development of NF1 gliomas. Dr. Biplab Dasgupta, a postdoctoral fellow in his laboratory, found that *Nf1* loss in astrocytes, nervous system cells that form gliomas, resulted in preferential activation of K-Ras, but not the other Ras isoforms. Activation of K-Ras in normal astrocytes mimicked the effects of *Nf1* loss on cell growth and migration, and inhibition of K-Ras reversed the abnormalities observed in astrocytes lacking *Nf1*. Additionally, activation of K-Ras in astrocytes of $Nf1^{+/-}$ mice resulted in the formation of optic pathway gliomas, similar to $Nf1^{+/-}$ mice lacking *Nf1* gene expression in astrocytes. These results suggest that K-Ras is the primary target for neurofibromin in astrocytes and that excessive activation of K-Ras plays a critical role in the formation of NF1 gliomas. Importantly, FTIs are known to have minimal effects on K-Ras function, indicating that drugs specifically targeting K-Ras or proteins activated by K-Ras may be more effective for the treatment of NF1-associated brain tumors.

Additional details about this research can be found in the news article that begins on page VI-6 as well as in the following journal articles:

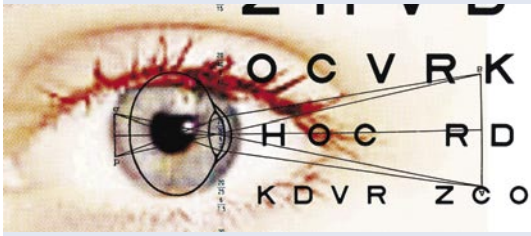
Dasgupta B, Li W, Perry A, et al. 2005. Glioma formation in neurofibromatosis 1 reflects preferential activation of K-RAS in astrocytes. *Cancer Res* 65:236–245.

Bajenaru ML, Hernandez MR, Perry A, et al. 2003. Optic nerve glioma in mice requires astrocyte *Nf1* gene inactivation and *Nf1* brain heterozygosity. *Cancer Res* 63:8573–8577.

Building Collaborations for the Molecular Characterization of Neurofibromas

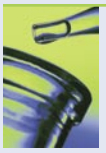
Nancy Ratner, Ph.D., Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

The development of benign neurofibromas and their transformation to malignant tumors in many individuals with NF1 are due to *NF1* gene mutations in a type of nerve cell known as Schwann cells. Currently, very little is known about the molecular changes and pathways involved in neurofibroma formation and progression. Dr. Nancy Ratner of the Cincinnati Children's Hospital Medical Center has received four awards from the NFRP to elucidate the molecular and genetic events leading to neurofibroma development and transformation. Dr. Ratner was awarded an FY96 Investigator-Initiated Research Award in which cell culture models that partially mimic NF1 were developed. Another ongoing study funded by an FY01 Investigator-Initiated Research



"The CDMRP program for neurofibromatosis has become the premier means of supporting ongoing efforts to develop effective treatments for NF1, NF2, and schwannomatosis. This has been accomplished by bringing together committed scientists, clinicians, and lay advocates in a setting that encourages the identification of innovative research projects which have the greatest hope for relieving the suffering of NF patients. Thanks to the partnership established by the CDMRP Neurofibromatosis [Research] Program, we are moving ever closer to a cure for these diseases."

Steven Carroll, M.D., Ph.D., FY05 NFRP Scientific Peer Review Panel Member



Fiscal Year 2005 Integration Panel Members

Jackson Gibbs, Ph.D. (Chair)
Merck Research Laboratories

William Johnson, M.D. (Chair-Elect)
University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School

Cheryl Coffin, M.D.
University of Utah

Brenda Duffy, M.A.
Neurofibromatosis, Inc.

Jonathan Epstein, M.D.
University of Pennsylvania School of Medicine

Robert Finkelstein, Ph.D.
National Institute of Neurological Disorders and Stroke

Nancy Fisher, R.N., M.D., M.P.H.
Washington State Health Care Authority

Tina Young Poussaint, M.D.
Harvard Medical School

Judy Small, Ph.D.

Robert Terrill, M.D.
The Texas Neurofibromatosis Foundation

Award is using mouse models to explore the role of epidermal growth factor receptor, which is overexpressed in many cancers, in the development of neurofibromatosis and malignant tumors. These models met a critical need in the NF community, allowing investigators to study mechanisms of NF1 pathogenesis in cultured cells and in living organisms. With support from an FY00 Idea Award, together with Dr. Shyra Miller, she used microarray analysis to examine gene expression patterns in Schwann cells from normal and *Nf1* mutant mice and determined that brain lipid binding protein was significantly overexpressed in the *Nf1*-mutant cells. That study was expanded when Dr. Ratner received another Investigator-Initiated Research Award in FY03 to compare gene expression profiles in human and mouse NF1 tumors. In collaboration with Dr. Miller and other leading NF researchers world-wide, data from all existing NF1 nerve model systems will be compared to create a database encompassing all of the findings for NF researchers around the world to access. It is hoped these pooled data will identify specific and shared relationships among models. Additionally, identification of gene alterations during neurofibroma development may lead to the discovery of early detection markers in tumors or even in blood samples taken from patients.

For more information about this research, please refer to the following publications:

Ling B, Wu J, Miller SJ, et al. 2005. Role for the epidermal growth factor receptor in neurofibromatosis-related peripheral nerve tumorigenesis. *Cancer Cell* 7(7):65–75.

Miller SJ, Li H, Rizvi TA, et al. 2003. Brain lipid binding protein in axon-Schwann cell interactions and peripheral nerve tumorigenesis. *Mol Cell Biol* 23:2213–2224.

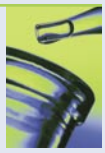
Kim HA, Ling B and Ratner N. 1997. NF1-deficient mouse schwann cells are angiogenic, invasive and can be induced to hyperproliferate: reversion of some phenotypes by an inhibitor of farnesyl protein transferase. *Mol Cell Biol* 17:862–872.

NFRP RESEARCH IN THE NEWS

January 3, 2005
By Gila Z. Reckess, Washington University School of Medicine News and Information
Brain Tumor Study Reveals Why Treatment Efforts Fail in Genetic Disorder

Drugs used to treat the tumors common in people with a disorder called neurofibromatosis 1 rarely work, and scientists now know why. The chemotherapy drugs target a group of related proteins, called RAS proteins, which are thought to be responsible for these tumors. But

(Continued on page VI-12)



DR. JAN DUMANSKI, FY99 AND FY03 INVESTIGATOR-INITIATED RESEARCH AWARD RECIPIENT

The NFRP Investigator-Initiated Research Award, offered each year since the program's inception in FY96, is designed to encourage independent investigators across a broad spectrum of disciplines to conduct basic and clinically oriented research that may provide insight into the molecular mechanisms underlying the development of neurofibromatosis and related diseases. Dr. Jan Dumanski of Uppsala University, a leader in the field of array-based comparative genomic hybridization (CGH), a cutting-edge genetic analysis technology, is one of the most promising investigators funded through the Investigator-Initiated Research Award mechanism. Dr. Dumanski received Investigator-Initiated Research Award grants in FY99 and FY03 to elucidate the molecular and genetic mechanisms of clinical variability in NF2, which differs widely in symptoms and severity among affected individuals. His group found no correlations between specific deletions in the *NF2* gene (also called merlin or schwannomin) and disease severity, suggesting that the observed variability may be caused by other genes, known as "modifier genes," that affect NF2 symptom development. Dr. Dumanski hypothesizes that the gene responsible for the development of schwannomatosis, a disease closely related to NF2, may be an *NF2* modifier gene. A candidate schwannomatosis gene has been identified on the same chromosome as Merlin, and further analysis of this gene is in progress. Other ongoing projects include (1) the identification of genes contributing to the development of NF2 ependymomas, meningiomas, and astrocytomas; (2) analysis of regulatory elements in the *NF2* gene, which may control the timing, location, and amount of Merlin protein produced; and (3) improvement of array-CGH methodology for the diagnosis of NF2. Dr. Dumanski's research will help improve understanding of the molecular mechanisms underlying NF2 and schwannomatosis and provide a foundation for the development of improved therapies for these disorders.

For additional information about this research, please refer to the following list of selected publications:

- Buckley PG, Mantripragada KK, Piotrowski A, et al. 2005. Copy-number polymorphisms: Mining the tip of an iceberg. *Trends Genetics* 21:315–317.
- Buckley PG, Jarbo C, Menzel U, et al. 2005. Comprehensive DNA copy number profiling of meningioma using a chromosome 1 tiling-path microarray identifies novel candidate tumor suppressor loci. *Cancer Res* 65:2653–2661.
- Dhami P, Coffey AJ, Abbs S, et al. 2005. Exon array CGH: Detection of copy-number changes at the resolution of individual exons in the human genome. *Am J Hum Genetics* 76: 750–762.
- Mantripragada KK, Buckley PG, Diaz de Ståhl T, et al. 2004. Genomic microarrays in the spotlight. *Trends Genetics* 20:87–94.
- Hansson CM, Ali H, Bruder CE, et al. 2003. Strong conservation of the human NF2 locus based on sequence comparison in five species. *Mammalian Genome* 14:526–536.
- Mantripragada KK, Buckley PG, Benetkiewicz M, et al. 2003. High-resolution profiling of an 11 Mb segment of human chromosome 22 in sporadic schwannoma using array-CGH. *Int J Oncol* 22:615–622.
- Mantripragada KK, Buckley PG, Jarbo C, et al. 2003. Development of *NF2* gene specific, strictly sequence defined diagnostic microarray for deletion detection. *J Mol Med* 81:443–451.

ENCAPSULATING A FIELD OF RESEARCH: THE NF2 STORYBOARD

Last year's annual report highlighted the NF1 storyboard, part of an ongoing NFRP evaluation effort that depicts key advances in NF1 basic and clinical research on a timeline ranging from before 1995 through the current year. The NFRP has also created an NF2-focused storyboard that covers major breakthroughs across multiple research areas, including molecular biology, animal models, and symptom management. These storyboards, which are featured on the NFRP page of the CDMRP website (http://cdmrp.army.mil/nfrp/default), provide concise yet comprehensive synopses of NF1 or NF2 research in a format that is easily understood by scientists, clinicians, consumer advocates, and members of the general public. The storyboards also assist the NFRP Integration Panel in identifying understudied areas that may benefit from additional research funding. Moreover, the storyboards illustrate the contributions of the NFRP, one of the largest sources of NF funding worldwide, to promoting high-caliber research with the potential to revolutionize the field and reduce the impact of NF1 and NF2. The chart utilizes color-coding schemes to indicate NFRP-funded findings and linked research.

Analysis of the NF2 storyboard reveals that, as with NF1, there have been substantially more advances in basic research than in translational or clinical studies. The efforts of laboratory scientists to unravel the molecular and cellular underpinnings of NF2 have been slow to be translated into the development of new treatments particularly nonsurgical therapies. In response to this critical need, the NFRP has offered the Clinical Trial Award and the Therapeutic Development Award mechanisms since FY99 and FY01, respectively. These award mechanisms were highlighted in last year's annual report. In addition, the NFRP is offering new award mechanisms in FY05 to fund the establishment of a clinical consortium composed of leading NF investigators who will collaboratively develop and conduct clinical studies of new therapies, as well as a central administrative and management core. In its early stages, the consortium will leverage the existing NF1 infrastructure and clinical research groups to form an NF1 network, which can later expand its focus to meet the needs of the NF2 community. Basic research has revealed that many of the same cellular pathways are dysregulated in both NF1 and NF2, indicating that the establishment of the NF1 consortium should facilitate the discovery and development of improved treatments for both disorders.

As its name implies, the storyboard also tells the "story" of the NF2 field, which is still being written by dedicated scientists, clinicians, and consumer advocates within the NF2 community. NF2 was initially described in the 19th century and was clearly distinguished from NF1 when the genes responsible for the disorders were localized to separate chromosomes in 1987. The formal separation of the two diseases ushered in an era of major advances in NF2 molecular and cellular biology. A primary goal of the NFRP is to assist investigators in leveraging the substantial knowledge gained from the work of basic scientists to write the next chapter of the NF2 story, the development and clinical validation of improved treatments for this disorder.

✦ ▲ ◗ ✢ ✱ ▮ ✖ ✪ ● ★ ■ Linked Research $ NFRP Funded Research

Molecular Biology & Genetics

2005

2004

2003
- High-resolution microarray-CGH of an 11 Mb segment of chromosome 22q detected heterozygous deletions in 21/47 (45%) sporadic schwannomas; the NF2 locus was deleted in all but 2 of the 21 cases $ ■

2002
- FERM domain of NF2 contains a 7-residue "blue box" that is highly conserved between human and *Drosophila* Merlin, but not in other ERM proteins $ ●

2001
- High-resolution microarray-CGH found an overall 20.7% detection rate out of 116 NF2 patients with differing severity – Found a high frequency of large chromosome 22 deletions $ ■

2000

1999

1998
- Merlin lacks the conventional C-terminal actin-binding sites but has other actin-binding sites within its FERM domain
- Correlations made between genotype and phenotype: Evans et al.

1997
- NF2 gene product shares similarity with the 4.1 family of cytoskeleton-associated proteins – Specifically the ERM proteins

1996
- Correlations made between genotype and phenotype: Parry et al., Rutledge et al., Kluewe et al. ★

Cellular Biology

2005
- Erbin links Merlin to both adherens junction protein complexes and the MAP kinase signaling pathway

2004
- Merlin forms a ternary complex with magicin and Grb2 $
- Protein kinase A phosphorylates Merlin and promotes Merlin-ezrin heterodimerization $
- Merlin is constitutively localized to lipid rafts and dissociates from the F-actin cytoskeleton at high cell densities $

2002
- Structure of N-terminal FERM domain of Merlin solved – Similiar to domains of moesin and radixin $

2001
- Five multi-allelic complementation groups (including scribbler/brakeless, blistered, and net) identified that alter the subcellular localization of Merlin $
- Regulated overexpression of HGS in rat schwannoma cells has the same effect as Merlin overexpression ✖ $

2000
- Merlin interacts with SCHIP-1, a novel protein that interacts specifically with spliced forms of Merlin $

1998
- Merlin interacts with β-integrin in differentiating Schwann cells
- Merlin co-localizes with F-actin filaments along the membrane
- Merlin interacts with hNHE-RF, which localizes to actin-rich structures

1997
- Truncated forms of Merlin are not detected in NF2 tumors, suggesting protein instability and degradation

1996
- Immunocytochemistry shows Merlin localized to the cell membrane (filopodia, ruffling membrane, and leading edge)

Pathobiology

2005
- Merlin is targeted to the nucleus in glioma and osteosarcoma cells in a cell cycle-dependent manner $
- Merlin inhibits N-WASP-mediated actin assembly $

2004
- Merlin inhibits PI3 kinase activity through binding to PIKE-L $
- A mutation in Merlin that mimics constitutive phosphorylation impairs growth suppressive activity and alters cell shape
- Merlin increases the stability of the p53 tumor suppressor by inhibiting Mdm2-mediated degradation of p53

2003
- NF2-/- cells do not have contact inhibition and lack adherens junctions – Suggests that Merlin organizes adherens junctions and facilitates cell–cell communication (adding Merlin to deficient cells restores cell–cell communication) $

2002
- Paxillin binds Merlin and mediates its membrane localization ▲
- Merlin growth suppression requires HRS expression ✖ $
- Schwannoma cell line developed from NF2 patient – Non-tumorigenic in mice, but alters growth rate and is growth factor-independent ◗ $

2001
- Activation of Rac1 or Cdc42 promotes Merlin phosphorylation (inactivation) $
- HRS interacts with the C-terminus of Merlin in its open form $
- Syntenin specifically interacts with Merlin isoform 1 – Links active Merlin to membrane protein signaling through the actin cytoskeleton

2000
- HRS interacts with Merlin both in vivo and in vitro $

1999
- Method developed for establishing short-term primary schwannoma cells in culture ◗ $

1998
- Merlin indirectly associates with the actin cytoskeleton through an interaction with βII-Spectrin
- Merlin binds Paxillin, which facilitates binding to the cell membrane ▲
- Merlin constitutively degraded by the calpain system in intact cells; N-terminal 35 kD fragment results
- NF2 schwannoma-derived cells have abnormal actin cytoskeletal architecture and proliferation defects
- Naturally occurring mutant NF2 proteins demonstrate altered localizations; C-terminal deletions=cell membrane, N-terminal deletions=perinuclear/cytoplasmic region

1997
- Merlin co-localized with CD44 and the actin cytoskeleton
- Merlin self-interaction (N- to C-terminal) is involved in growth suppression

1996
- Merlin is expressed in the nervous system, smooth muscle, Schwann cells, melanocytes, red blood cells, endothelial cells, and neurons such as Purkinje cells and motor neurons, but not in glial cells

Pathobiology

2005

2004
- NF2 and p53 mutations synergistically promote the development of malignant peripheral nerve sheath tumors ✪ $

2003

2002

2001

2000

1999

1998

1997

1996
- Germline mutations in NF2 found in at least two-thirds of all individuals with typical bilateral vestibular schwannoma

✦ ▲ ◗ ✢ ✱ ▮ ✖ ✪ ● ★ ■ Linked Research $ NFRP Funded Research

Technology/Animal Models

2001
- *Drosophila* Merlin mutants show defects in nuclear migration and mRNA localization in the oocyte ●

2000
- Conditional NF2 knockout mice developed (NF2 disrupted specifically in myelin PO-expressing cells) schwannomas in association with peripheral nerves ✪

1999
- Transgenic mice expressing a mutant *Nf2* that lacks exon 2-3 develop peripheral nerve sheath tumors and Schwann cell hyperplasia ✢
- Transgenic mice expressing the first 314 amino acids of Merlin are normal ✢

1998
- Somatic cell mosaic analysis reveals *Drosophila* Merlin acts as a tumor suppressor ●

1997
- *Drosophila* homologue of NF2 (Merlin) identified ●
- Inactivation of mouse NF2 gene results in embryonic lethality between days 6.5 and 7.0 ✦ $
- NF2 heterozygous (+/-) mice are developed and predisposed to cancer at advanced age, but do not develop the hallmark tumors of NF2 ✦ $
- *Drosophila* NF2 mutations isolated and characterized ●

Imaging, Detection & Diagnosis

2003
- Development of high-resolution NF2-specific diagnostic microarray for the detection of disease-causing gene deletions ■ $
- Schwannomatosis is molecularly and clinically distinct from NF2 $

2000
- Pre-symptomatic diagnosis available for 66% of all classically affected NF2 patients

1997
- Diagnostic criteria for NF2 outlined: bilateral vestibular schwannoma; 1 or more first-degree relatives with NF2+ unilateral vestibular schwannoma at <30 years, or 2 of the following: meningioma, glioma, schwannoma, juvenile posterior lenticular opacities

1996
- Mosaicism at NF2 locus is uncommon and probably underrecognized; unilateral vestibular schwannoma, ipsilateral intracranial tumors, schwannomatosis, and/or asymmetric involvement
- Schwannomatosis described as a separate clinical entity from other forms of NF: multiple schwannomas without evidence of vestibular schwannoma

Epidemiology

2005
- Vestibular schwannoma growth rates are highly variable but tend to decrease with increasing age

2004
- Individuals with constitutional NF2 missense mutations, splice-site mutations, large deletions, or somatic mosaicism have significantly fewer tumors than individuals with constitutional nonsense or frameshift mutations
- Characterization of vestibular schwannoma growth rates in NF2 patients ✱ $
- Newly diagnosed NF2 patients who do not require immediate treatment of vestibular schwannomas are likely to have stable hearing in the untreated ear(s) for 1–2 years ✱ $

2003
- Risk of a mosaic parent with NF2 transmitting the disease to their offspring is lower than anticipated (34% instead of 54%), especially when the mutation cannot be identified by standard techniques
- Molecular analysis of a cohort of 233 NF2 founders revealed mosaicism in 58 cases (24.8%)

2002
- Age at diagnosis, intracranial meningiomas, and type of treatment center (specialty vs. nonspecialty) are informative predictors of the risk of mortality in NF2 patients $

1998
- Preliminary work done on growth rate of vestibular schwannomas $
- Families with splice-site or missense mutations or large deletions of the NF2 gene tend to have fewer tumors and later onset

1996
- Correlations made between genotype and phenotype: Nonsense/frameshift mutations = severe phenotypes; splice-site mutations = variable phenotype within/between families; very few non-truncating mutations detected; mutations not detected by exon-scanning = mild phenotype ★

Experimental Therapeutics

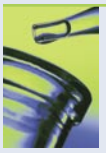
- **2005** — FK228, an anti-PAK1 drug, completely blocks the growth of NF2-deficient cancer cells in vitro
- **2004**
- **2003** — Study of 86 deaf NF2 patients who received auditory brainstem implants found significant improvement in audiological function in 60 patients (70%)
- **2002**
- **2001**
- **2000** — FDA approval of Nucleus 24-Multichannel Auditory Brainstem Implant
- **1999** — Phase 1 trial of SU-101 in children
- **1998** — Strategic radiation therapy (gamma knife): Used in elderly patients with documented tumor growth – Low chance of hearing preservation; Middle fossa internal auditory canal bony decompression: Useful when a change in hearing is documented (for long-term hearing stabilization); Suboccipital approach total tumor removal: Used for smaller, medially based tumors (hearing preservation is unlikely and risk of tumor recurrence is high)
- **1997** — SU-101 tested in Phase 2 trial for patients with recurrent malignant gliomas
- **1996** — Aminoglycosides suppress expression of nonsense mutations of NF2 and modify the neoplastic phenotype of tumor cells in culture

Symptom Management

- **2005**
- **2004**
- **2003** — Retrospective study shows that gamma knife stereotactic radiosurgery controls tumor growth and/or defers the need for surgery in NF2 patients with vestibular schwannomas
- **2002**
- **2001**
- **2000**
- **1999**
- **1998** — Translabyrinthine total tumor removal with auditory brainstem implant: Used for patients with non-useful hearing or large tumors with brainstem compression; MRI annually to screen tumor growth and other intracranial risks + annual audiometric studies to monitor hearing (surgery required when hearing is no longer useful or tumor grows enough to endanger patient)
- **1997**
- **1996**

Important Meetings & Symposia

- **2005**
- **2004**
- **2003** — NINDS Workshop: Developing Therapies for the Neurofibromatoses
- **2002**
- **2001**
- **2000** — NINDS Workshop: Defining the Future of Neurofibromatosis Research
- **1999**
- **1998**
- **1997** — NNFF Clinical Care Advisory Board: Diagnostic Evaluation and Management of NF1 and NF2; House Ear Institute & NNFF workshop on NF2: Reviewed current knowledge and made short-term and long-term goals
- **1996** — DOD NFRP established



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researchers at Washington University School of Medicine in St. Louis found that the disease affects only one member of the protein family, and it happens to be the one form of RAS that does not respond well to these particular treatments.

The study, which will appear in the Jan. 1 issue of the journal *Cancer Research*, suggests where researchers should now look for more promising approaches to treating neurofibromatosis tumors, and may help scientists understand other cancers related to RAS.

"The downside is our study proves we're not using the right therapies for this particular problem," says Principal Investigator David H. Gutmann, M.D., Ph.D., the Donald O. Schnuck Family Professor of Neurology and professor of genetics and of pediatrics. "But there's a chance to make lemonade out of this lemon: We now have a rational reason for why these drugs aren't working, so we should be able to explore new, more effective treatment options."

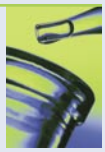
About one in 4,000 newborns has neurofibromatosis 1, in which every cell in the body has one normal and one mutated copy of a gene called *Nf1*. If a cell's normal copy also is mutated, tumors can form. Children with neurofibromatosis 1 are therefore predisposed to developing a variety of serious complications as they grow older, including skin, spine and brain cancers.

Scientists previously found that RAS proteins become overly active when both copies of the *Nf1* gene are abnormal in tumors from patients with neurofibromatosis 1. So physicians have tried treating these tumors with drugs that prevent RAS activity. Unfortunately, the results have been disappointing.

To understand why, Gutmann's team examined whether all forms of RAS are overly active in mouse cells lacking both copies of the *Nf1* gene. They specifically examined support cells in the brain called astrocytes, which often are affected by neurofibromatosis 1. Surprisingly, only one member of the protein family, K-RAS, was significantly affected, suggesting it is an important factor in this disease.

Moreover, when the team activated K-RAS in normal astrocytes, the cells developed many of the same characteristics and activities as those lacking *Nf1*. For example, both types of abnormal astrocytes were round and dense, grew and multiplied at a similar rate and moved around more than normal. They also discovered they could reverse abnormalities in cells without *Nf1* by decreasing K-RAS activity.

K-RAS activation also mimicked *Nf1* loss in live mice. Gutmann's team previously discovered that mice without *Nf1* genes in their astrocytes grow an abnormally large number of astrocytes in their brains, but they don't develop tumors unless all other brain cells are missing at least one copy of the gene. In this study, the researchers found that K-RAS



follows a similar pattern: When the protein was overly active in astrocytes of mice with two normal copies of *Nf1*, the cells multiplied but did not develop into tumors; however, tumors did form when K-RAS was activated in astrocytes of mice lacking one copy of *Nf1* in all cells.

Another form of RAS previously suspected to be linked to neurofibromatosis, called H-RAS, did not mimic loss of the *Nf1* gene in tissue culture or in live animals.

"Collectively, these results suggest that K-RAS activation, specifically, is the biological equivalent of *Nf1* loss in astrocytes," Gutmann says. "If we can understand what K-RAS does that's unique, we should be able to develop targeted therapies."

The research team already has made progress toward that goal. Too much RAS and too little *Nf1* are both known to result in a cascade of events, including activation of another protein called Rac1, which in turn activates LIM kinase. Gutmann and his colleagues found that that effect could be mimicked in normal astrocytes by selectively activating K-RAS. Activating H-RAS did not trigger the cascade.

"Though K-RAS doesn't respond well to available chemotherapy drugs, one of the proteins it interacts with might," Gutmann says. "By showing that K-RAS activates a pathway that is unique from the pathways activated by other RAS molecules, our findings may lead us to a variety of better treatment targets."

Funding from the National Institutes of Health and the United States Department of Defense supported this research.

BOTTOM LINE

Since 1996, the DOD NFRP has been responsible for managing $155.3M in congressional appropriations, which has resulted in 140 awards for FY96 through 04. The NFRP is dedicated to improving and enhancing quality of life of persons with NF and schwannomatosis, and NFRP-supported studies offer the potential to revolutionize the management of these diseases. Research highlights, award data, and abstracts of funded NFRP proposals can be viewed on the CDMRP website (http://cdmrp.army.mil).

"I consider it a true privilege to have had the opportunity to serve as Chair, IP for Army NF money. It is a highly professional panel combining basic research, clinical expertise, and consumer input that is dedicated to funding the best research devoted to ideas and therapeutic approaches that often fall outside the immediate scope of traditional funding venues. During my time on the panel, I have seen increasingly creative applications, a growth in applications focused on translating research into therapeutics, and the beginnings of more broadly organized clinical efforts. These are exactly the efforts needed by the NF community for the discovery of therapeutics that will benefit the patients of this disease."

Jackson Gibbs, Ph.D., FY05 NFRP Integration Panel Chair