

A Summary

The NNFF International Consortium for the Molecular Biology of NF1 and NF2

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by
Dr. Judy A. Small
NNFF Director of Clinical Trials and Technology Transfer

The pace of research continued to amaze all who attended the 2002 meeting of the *NNFF International Consortium for the Molecular Biology of NF1 and NF2*, held in Aspen, CO, in June this year.

Mouse models have become more sophisticated, and can be programmed to develop tumors specific to each disorder. Preclinical studies of promising new drugs or other treatments are being done using these and other animal or cell models.

Natural history studies are ongoing to study the growth rates of plexiform neurofibromas in NF1, and vestibular schwannomas in NF2, and results from these studies will provide information critical to assessing outcomes in clinical trials.

There was also new information about the signaling pathways that control cell growth in both NF1 and NF2. In addition to oral presentations by invited and selected speakers, two poster sessions were held so that all participants had the opportunity to present their work for discussion.

The meeting was chaired and organized by Dr. Peggy Wallace (University of Florida) and Dr. Rick Fehon (Duke University). Two guest speakers, Dr. Rick Kenyon, US Army Medical Research, Department of Defense (DOD), and Dr. Robert Finkelstein, National Institute of Neurological Disorders and Stroke, National Institutes of Health (NIH), spoke about funding programs related to NF research. Sponsors of the meeting included the National Institute of Neurological Disorders and Stroke, the National Cancer Institute, the National Institute of Deafness and Other Communication Disorders, the National Heart, Lung and Blood Institute, and the March of Dimes. There were 4 keynote speakers. Dr. Natalie Ahn (Univ of Colorado, Boulder) spoke about the use of proteomics to understand signal transduction pathways. Dr. Richard Hynes (MIT Center for Cancer Research) spoke about cytoskeletal connections within the Band 4.1/Talin family. Dr. Frank McCormick (UCSF Cancer Center) described his studies on cancer therapy based on ras and p53, including the use of a mek inhibitor and a raf kinase inhibitor. Dr. James Salzer (NY Univ.) described the expression of ERM proteins in myelinated nerves.

Dr. Frank McCormick (UCSF Cancer Center) provided a take-home message about the recent pace and advances in research. Figure 1 shows the information known about cell signaling pathways and cancer about 10 years ago. Figure 2 shows what is known in June 2002. This chart changes almost daily as more information becomes available from many sources. Knowledge is good, but complexities arise as the search for answers continues to yield more questions. Scientists must continue working together across fields of expertise if the answers are to be found.

Figure 1. The Ras Pathway, circa 1992

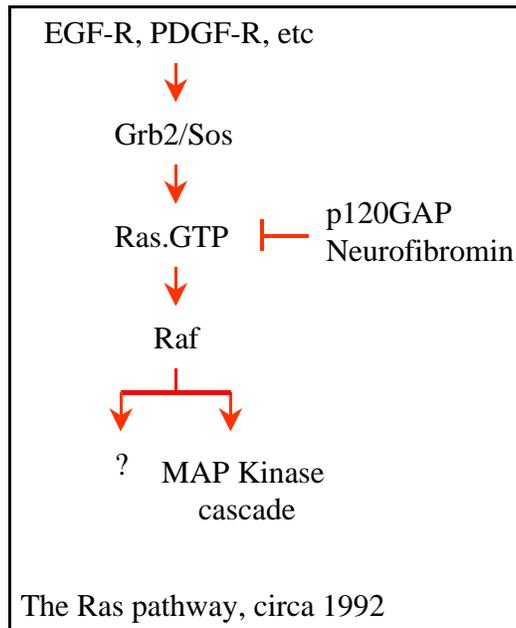
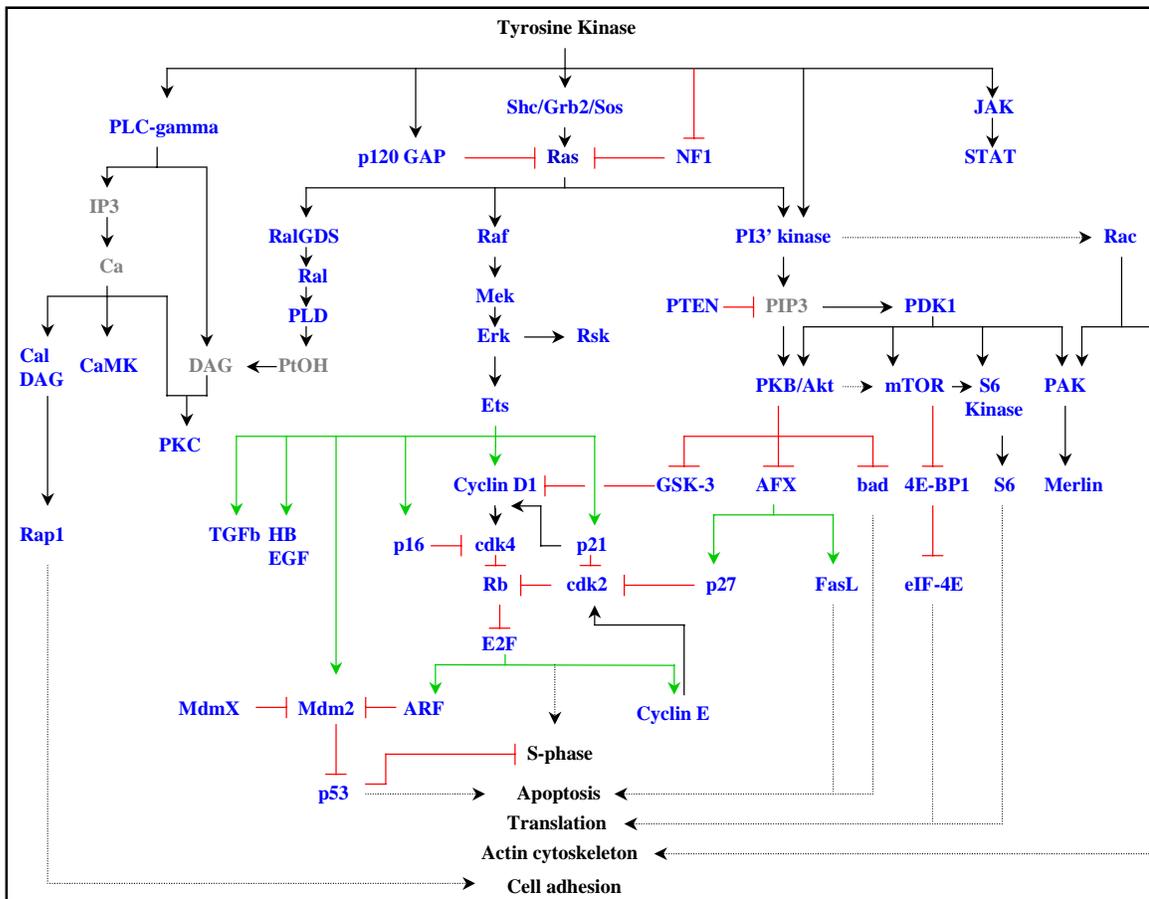


Figure 2. The Ras Pathway, circa 2002



Dr. Jan Friedman (University of British Columbia) spoke about NF1. He spoke about conventional wisdom of the clinical picture of NF1: that NF1 is mainly a cosmetic problem, and that the course of NF1 is not predictable. He dispelled these theories by describing the slightly increased risk for malignancy and the shorter life expectancy for patients with NF1. He also has done studies that show that there is a correlation between certain features of NF1 among families, including pigmentation, tumor burden, and risk for malignancy. Dr. Mia MacCollin (Harvard Med. School/MGH) described NF2, with the major presenting symptoms being hearing loss or vestibular imbalance. Patients tend to have an increased tumor burden, with schwannoma on the 8th cranial nerve being the diagnostic tumor. Current treatment is limited to surgery, although some doctors are in favor of radiosurgery.

Dr. Bruce Korf (Harvard Med. School/Partners) described the progress of the Natural History Study of NF1 Plexiform Neurofibromas. Using sophisticated imaging technology, it is possible to measure the 3-D volume of the tumors, and to follow the progression of these tumors over time. A network of clinical centers around the world has been established, and enrollment is still open, however, most needed are the adult cases with internal plexiform tumors. It is hoped that the NF1 clinical network will be utilized as a central resource for clinical trials and other clinical studies. Dr. William Slattery (House Ear Institute) presented information about the Natural History Study of NF2 Vestibular Schwannomas. The study has been recently expanded and will now cover NF2 tumors in the entire body. The study will include clinical evaluation, mutation analysis, and tumor growth and pathology.

Dr. Bernd Algermissen (Klinikum Neukoelln, Berlin, Germany) described the use of optical scanning to measure the size of cutaneous neurofibromas, which could be used to measure growth of these tumors, but would be limited only to the portion that was visible externally. Dr. Andreas Kurtz (Harvard Med. School/MGH) described mitochondrial DNA mutations associated with the variations in symptoms seen in NF1 patients. Dr. Eric Legius (Univ. of Leuven, Belgium) described LEOPARD syndrome, characterized by café-au-lait (CAL) macules, with lentigines (brown or black nevi) and related to Noonan syndrome. Mutations on PTPN11, a nonreceptor type of protein tyrosine phosphatase, are found in LEOPARD syndrome, as well as in some Noonan syndrome patients. He suggests looking for these mutations in Noonan-NF syndrome and in patients with CAL but no neurofibromas or Lisch nodules.

Model systems for NF have become more sophisticated, and will be useful both for research to better understand NF and for preclinical studies evaluating potential therapies. Dr. Kevin Shannon (UCSF) reported on the DOD NFRP-funded NF Mouse Models Consortium (NFMMC), which is the only non-NCI funded component of the NCI's Mouse Models of Human Cancer Consortium (MMHCC). To participate in the MMHCC, investigators agree to submit mouse models to the mouse models repository for use by other research groups. To date, the NFMMC has produced models which develop tumors found in NF1 and NF2, and has enhanced the NF1 leukemia model. In addition, these mice have provided new data on the biochemical action of the NF proteins.

Mutant mice can be created by deletion of the NF genes, however, mice with 2 deleted copies of the NF1 or NF2 gene do not develop normally and die before birth. Chimeric mice can be made by mixing mutant and normal cells at an early stage of development, and these mice contain NF mutations in only a percentage of every tissue. Conditional NF mice (called flox mice) have been developed in which the NF gene is altered by addition of deletion sites that are activated by a special enzyme (cre). By crossing these flox mice with special strains of mice that express cre only in certain tissues, the NF genes can be deleted locally, resulting in tumors that are specific to that tissue.

Dr. Tyler Jacks (MIT Center for Cancer Research) described mouse models of NF1. Mice that have mutations in one copy each of the NF1 gene and the p53 gene develop malignant peripheral nerve sheath tumors (MPNST) and astrocytomas. He has also determined that the background strain of the mice is important in determining tumor incidence. Dr. Aaron Gitler (Univ. of Pennsylvania) has studied the cardiac abnormalities associated with the NF1 null phenotype. Using tissue-specific cre/NF1^{flox} crosses, he has defined a role of NF1 in endothelial cells, but not neural crest cells, in heart development. Dr. Richard Chao (UCSF) used NF1 mice with one mutation (NF+/-) to study the effects of radiation and chemotherapy on the risk of developing treatment-induced malignancy. He found treatment-related sarcomas exclusively in NF+/- mice as compared to wild type mice. Exposure to cyclophosphamide alone or in combination with radiation increased the risk of sarcoma, one-third of those mice treated with both. Loss of the wild type NF1 gene was common in the malignant tumors.

Dr. Luis Parada (UT Southwestern Medical Center) described conditional NF1 mutant mice that developed benign plexiform neurofibromas by 3 months and begin progression to malignancy by 6 months. Malignant tumors show a loss of both NF1 and p53 genes. Also, specific expression in glial cells resulted in optic gliomas, were capable of progression to high-grade astrocytomas and glioblastomas. Dr. David Gutmann (Washington Univ. St. Louis) described conditional NF1 mice that lose the NF1 gene in astrocytes. Although there were increased numbers of growing astrocytes, the mice did not develop astrocytomas. There may be other conditions in the brains of these mice that control the formation of these tumors. Studies are being done to look at genetic and environmental factors that may play a role in astrocytoma formation. Dr. Nancy Ratner (Univ. of Cincinnati) described mouse models of neurofibroma formation. Mice overexpressing epidermal growth factor receptor (EGFR) in Schwann cells showed markedly altered nerve ultrastructure. When these mice were crossed to NF1+/- mice, nerve hyperplasia occurred. Using microarray analysis, the overexpression of brain lipid binding protein was also shown to be overexpressed 30-fold in NF1 cells compared to WT. This overexpression was shown to follow EGFR expression, resulting in axon-glia interactions. BLBP was detected in 12/14 tumor cell lines derived from NF1/p53 mutant mice and in 3/5 human MPNST cell lines. All cell lines expressed EGFR. More details were available in posters presented by others in the lab.

Dr. Marco Giovannini (INSERM, Paris, France) described NF2 mouse models. Conditional loss of NF2 in Schwann cells resulted in Schwann cell overgrowth and schwannoma formation. If NF1 or p53 genes were also lost, a variety of tumor types appeared, including neurofibromas, both dermal and plexiform, and MPNST. Dr. Michael Kalamarides (INSERM, Paris, France) described studies using the NF2 mice. By selective mutation of NF2 in the lining of the brain or spinal cord, mice developed meningiomas, providing a powerful tool for studying tumor progression and evaluation of therapies.

In addition to mouse models, studies are also being done in *Drosophila Melanogaster* (fruit fly). Dr. Andre Bernards (Harvard Med. School/MGH Cancer Center) studies NF1 mutations in the fruit fly. NF1 mutant flies are reduced in size and have defects in learning, in neuropeptide signaling, and in a circadian rest-activity rhythm. Of these, only the latter is associated with increased Ras-MAPK signaling. The other changes have been shown to respond to increased signaling through the cAMP-Protein Kinase A pathway. In addition, loss of a fruit fly gene called *dunce* will rescue the size defect in the NF1 mutant flies. Dr. Frances Hannan (Cold Spring Harbor Laboratory) described her studies on the NF1-regulated adenylyl cyclase pathway in fruit flies. The human NF1 gene is able to rescue the size and learning defects in *Drosophila* NF1 mutant flies. She showed that the even hNF1 mutated inside and outside of the GRD region were able to at least partially rescue the cAMP-dependent phenotypes. Dr. Amita Sehgal (Univ. of Pennsylvania) described the role for the NF1 gene in regulating circadian rhythms, not affecting daily oscillations of clock genes *period* and *timeless*, but affecting adult locomotor activity. The effects are mediated by the Ras/MAPK signaling pathway, and can be rescued by loss-of-function mutations in that pathway.

Dr. Olga Nikiforova (Duke Univ.) studies moesin, a fruit fly protein related to the ERM proteins, and part of the Protein 4.1 superfamily of proteins that also include merlin, the NF2 protein, and play a role in cell adhesion, division, shape and motility. Loss of moesin disrupts cell actin filament organization. Genetic studies indicate that moesin is a negative regulator of the rho signaling pathway. Dr. Monique Arpin (Institut Curie, Paris, France) described the role of PIP2 and phosphorylation in the activation of ezrin, another ERM protein. Ezrin mutations that do not bind PIP2 are not associated with the cell membrane or the actin cytoskeleton, and are not phosphorylated on threonine 567. A mutation at this T567 results in an active form of ezrin, which allows for cell growth and perturbs cell adhesion. Dr. Peter Herrlich (Karsruhe Research Center, Germany) compared the activities of merlin and ezrin in regulating cell growth. He showed that merlin, a negative growth regulator, and ezrin, a positive growth regulator, interact with CD44, a transmembrane glycoprotein.

Dr. Laura Klesse (UT Southwestern Univ) used cell lines derived from the NF1/p53 mutant mice to study the intracellular signaling components involved in tumorigenicity. Inhibition of ras prevented growth and tumorigenesis for all cell lines tested. Inhibiting mek was capable of inhibition of tumorigenicity on only a subset of the cell lines. Expression of p53 tumor suppressor gene also had the effect of inhibiting tumorigenicity

in only a subset of the cell lines. These studies show that tumorigenesis can be inhibited by stopping cell growth, rather than by inducing cell apoptosis (cell death).

Dr. Kristine Vogel (UT Health Sciences Center) used NF1^{-/-} mutant mice to assess the motility properties of neural crest cells. The cells extended axons into dorsal root ganglia and trigeminal neurons, as is appropriate, but also extended axons into heart explants, which was never seen with WT neural crest cells. NF1/p53 mutant mice were used to study how the motility of neural crest-derived sarcoma cells was affected by treating with transforming growth factor 1. Treatment for 3-4 days significantly reduced the invasiveness of the sarcoma cells. Dr. David Ingram (Indiana Univ) described experiments investigating the role of mast cells in NF1 tumor formation. Mast cells from NF1^{+/-} mice bind more strongly to integrins than the WT cells, and demonstrate increased migration on fibronectin. There is also a two-fold increase in PI3 kinase and Rac2 activity compared to WT cells.

Dr. Karen Cichowski (Harvard Med School/Brigham and Women's Hospital) studied the effect of growth factors on neurofibromin regulation of ras signaling pathways. She found that the proteasome plays a role by degrading neurofibromin in response to a number of different growth factors. Neurofibromin is rapidly degraded, but its level is re-elevated within 30 minutes of growth factor treatment. Sequences adjacent to the GAP-related domain (GRD) are required for the degradation.

Kelly Morgan (Univ of Minnesota) described NF1^{-/-} fetal liver cells that exhibit hypersensitivity to the growth promoting effects of granulocyte-macrophage colony stimulating factor (GM-CSF), which is associated with increased and prolonged Ras activation. Reintroduction of the NF1-GRD restores Ras regulation and normal growth control to NF1^{-/-} cells. Dr. Wendy See (UCSF) spoke about how the regulation of signaling pathways plays a role in the development of juvenile myelomonocytic leukemia (JMML) in NF1 patients. Fetal liver cells from mice that are NF1^{-/-}, but not wild type (WT) have features of human JMML cells, including formation of myeloid colonies in the absence of GM-CSF, elevated levels of Ras-GTP, and aberrant activation of the Raf/MEK/ERK and PI3K/Akt cascades. WT cells undergo apoptosis (programmed cell death) 4 hours after removal of the GM-CSF, however, NF^{-/-} cells are resistant to apoptosis. Inhibitors of the PI3K signaling pathway (LY294002) restored apoptotic activity to NF^{-/-} cells, but MEK inhibitor PD184352 did not induce apoptosis at a dose that restored ERK to normal levels. These and other data suggest that PI3kinase/PKB pathway is crucial for growth advantage and survival of the NF^{-/-} cells.

Dr. Susanne Thomson (Univ. of Florida) described the use of cDNA microarrays to identify genes that are expressed differentially in NF1 tumor cells compared to normal Schwann cells. Thus far, she has found 11 differentially expressed genes.

Dr. Marcello Curto (Harvard Med. School/MGH Cancer Center) and Dr. John Stickney (Univ. of Cincinnati) both described the localization of the NF2 protein (merlin) to the detergent Triton X-100 insoluble portions of the cell membrane, including the lipid rafts and caveolae. This suggests a role for merlin in growth suppression due to structural

interactions with cytoskeletal elements. Dr. Cristina Fernandez-Valle (Univ. of Central Florida) described the interaction of merlin and paxillin, two proteins that co-localize to the cell membrane in Schwann cells. Binding to paxillin is required for membrane localization of merlin.

Dr. Andrea McClatchey (Harvard Med. School/MGH) used NF2-deficient mouse embryo fibroblasts (MEF) to study growth properties. While normal MEF grow only until they contact other cells (contact inhibition) and fail to grow in the absence of growth factors, NF2 MEF exhibit growth advantages and loss of contact inhibition. Further studies will help to identify other alterations that may be related to tumor formation. Dr. Juergen-Theodor Fraenzer (House Ear Institute) described the overexpression of the NF2 protein merlin and inhibition of cell proliferation in a schwannoma cell line (HEI193). Merlin-expressing cells also were able to prevent activation of the MAPK and PI3K signaling pathways upon stimulation by PDGF. PDGF was also internalized and degraded more rapidly in merlin overexpressing cells.

Dr. Long-Sheng Chang (Ohio State Univ.) described the cloning and expression of a full-length NF2 gene. The gene consists of 17 exons, 6067 nucleotide bases. Eight alternatively spliced NF2 isoforms were isolated from cells that are not of Schwann cell origin. In vestibular schwannomas, a distinct pattern of alternatively spliced NF2 transcripts was found. The NF2 promoter was mapped by site-directed mutagenesis, and a core promoter was identified extending 400 bp from the major transcription initiation site. Dr. Joseph Kissil (MIT) described the role of phosphorylation in merlin. Merlin is inactivated by phosphorylation. He identified p21-activated kinase 2 as the merlin kinase. Dr. Thorsten Wiederhold (Harvard Med. School/MGH) described the identification of a novel interactor for merlin, using a yeast two-hybrid strategy. This protein associates with the actin cytoskeleton and may provide a link between merlin and the Grb2 mediated signaling pathways. Dr. Reshma Rangwala (Univ of Cincinnati) described interactions of CD44 and erbB2/erbB3 and merlin in primary Schwann cells. Hyaluronate is a major component of extracellular matrices, and binding to CD44 controls the phosphorylation of erbB2, and can also induce dephosphorylation of merlin.

In addition to the oral presentations described above, there were about 75 poster presentations, which were available for discussion throughout the meeting.

Clinical treatment remains a key area for discussion. There were two posters with information on surgical interventions in young NF1 patients (Dr. Tena Rosser, Children's National Medical Center). The most common surgery was for plexiform neurofibromas, followed by orthopedic procedures for scoliosis and pseudoarthrosis. Dr. G. Tirino (Second Univ of Naples, Italy) described clinical features of optic pathway gliomas in children with NF1. Complications include visual loss and precocious puberty. Reduction of tumor size by chemotherapy did not improve clinical symptoms, and tumor growth did not necessarily involve visual impairment.

A number of posters focused on development or preclinical testing of therapies. Several drugs have been identified and are being tested in animal models or in human

tumor cell cultures. These include pirfenidone, which is being tested in clinical trials for plexiform neurofibromas, and in cell models of malignant gliomas (Dr. Dusica Babovic, Mayo Clinic). Exisulind, an apoptosis-inducing drug, is being tested in cell culture models of MPNST (Dr. Victor Mautner, Univ Hospital Eppendorf and Klinikum Ochsenzoll, Germany). Oncolytic Herpes Simplex Virus, which targets and kills neurological tumor cells, is being tested in cell culture models of MPNST (Dr. Andreas Kurtz, Harvard Medical School/MGH). Dr. Brigitte Widemann (NCI) presented work being done to develop an automated system to measure the size of tumors using 3-D analysis of MR images of plexiform neurofibromas. These imaging studies are part of the Natural History Study of NF1 Plexiform Neurofibromas, and will be needed to assess outcomes of clinical trials for tumors in NF1 and NF2.

Posters addressed the cognitive and behavioral aspects of NF1. Scientists working with Dr. Bartlett Moore (UT MD Anderson Cancer Center) described the use of functional MRI (fMRI) to evaluate the visual spatial processing and phonological processing in NF1 patients. In addition, the same group suggested the use of visual-spatial performance deficits as a diagnostic indicator in children with questionable diagnosis of NF1. There is an increase in psychosocial problems in children with NF1.

Dr. Gene Fisch (Yale Univ) analyzed age-related characteristics in IQ of children with NF1, Fragile X syndrome, and Williams syndrome. He showed that there were no significant age-related declines among children and adolescents with NF1, as was the case for both Fragile X and Williams syndromes. Learning disabilities associated with NF1 also occur in NF1 mouse models. Three posters from Dr. Alcino Silva's laboratory (UCLA) describe biochemical processes involved in learning and memory that are affected by loss of the NF1 gene. Dr. Dietrich Stephan (Children's National Medical Center) presented studies using expression microarrays to identify alterations in gene expression that would explain the cognitive deficits in NF1 mutant mice.

Molecular analysis of NF1 gene mutations continues as scientists look for clues that will help to better understand the disorder and its variability. Repetitive sequences around the NF1 gene on chromosome 17 suggest that there is a predisposition to recombination and deletion in this area (Dr. Thomas De Raedt, University of Leuven, Belgium). The occurrence of this large deletion including the NF1 gene and several adjacent genes consistently results in a more severe form of NF1, with cognitive deficits, larger tumor burden, and an increased risk of developing a MPNST, as is supported by a case report described by Dr. Katsumi Tanito (Univ. of Utah). Other than patients with the large deletions, there is no correlation between the site on the gene or the type of mutation in the NF1 gene and the type or number of neurofibromas or other symptoms of NF1 (Dr. Victor Mautner (University Hospital Hamburg-Eppendorf, Germany). Dr. Sibel Oguzkan (Univ. of Hacettepe, Turkey) described the identification of NF1 mutations in 10 Turkish families. Dr. Ayter Sukriye (Univ of Hacettepe, Turkey) described the analysis of mutations in the GAP-related domain of the NF1 gene in NF1 patients. Dr. Rosemary Foster (Harvard Med. School/MGH) described a set of identical twins with the same NF1 mutation and very different clinical presentation, with one sister unaffected except for one café-au-lait spot, and the other sister symptoms

including multiple neurofibromas and a plexiform neurofibroma. Dr. Meena Upadhyaya (University of Wales, UK) described a Portuguese family with 3 independent mutations in the NF1 gene in different family members. In two families with spinal neurofibromatosis, even with the symptoms running true between families, the mutations were not the same (Dr. Ludwine Messiaen, University Hospital Ghent, Belgium). Dr. Conxi Lazaro (Hospital Duran i Reynals, Spain) described the experiences in genetic testing and prenatal diagnosis in a clinic in Spain.

Dr. Ina Vandenbroucke (University of Ghent, Belgium) presented data on the identification of nine splice variants of the NF1 gene, with variant transcript levels in different human tissues. Dr. Kristine Vogel (UT Health Sciences Center) described the role of one splice variant, exon23a, in neuronal survival.

Studies suggest aberrant methylation patterns in the NF1 promoter region in some plexiform tumors, affecting the expression of the NF1 gene and suggesting a mechanism for tumorigenesis (Dr. Lauren Fishbein, Univ. of Florida). Methylation also plays a role in NF1 expression during early development in mice (Dr. David Rodenhiser, Univ of Western Ontario, Canada). Dr. Fabien Bonneau (EMBL, Germany) is studying the non-Ras-GAP domains of the NF1 gene to identify other possible functions of this large protein.

Dr. Yuan Zhu (UT Southwestern Medical Center) described studies on formation of neurofibromas in NF1 mice. Using conditional NF1 mice, he was able to generate mice with NF1 loss in Schwann cells. He found that loss of NF1 was sufficient to generate tumors. However, the tumor development was influenced by the surrounding tissue. Tumors formed only when the surrounding tissue contained loss of one copy of the NF1 gene. Dr. Dieter Kaufmann (Univ. Ulm, Germany) demonstrated that cells with one mutant copy of the NF1 gene and one half the WT amount of protein (called haploinsufficiency) results in a variation in melanocyte cell structure.

Microarray analysis is a useful technique for studying differential gene expression in NF1 tumors compared to non-tumor tissue. If certain genes are expressed at higher or lower levels in the tumors, this information can be used to further understand the role of the NF1 gene preventing tumor growth. Dr. Andre Bernards (Harvard Univ/MGH) presented data from microarray experiments using Drosophila NF1 flies that resulted in the identification of about 50 genes whose expression is up or down compared to WT flies.

Dr. Karlyne Reilly (NCI) is using genetic crosses in mice to map modifier genes that may confer resistance to NF1-associated tumors. Dr. H Zhou (Univ of Utah) described studies comparing genetic markers between plexiform neurofibromas and MPNST, to identify markers that would be useful for predicting the formation of MPNSTs at an earlier stage.

Dr. Doan Le (UCSF) described studies where NF1^{flox} mice were treated such that the NF1 gene was deleted from blood cells or blood cell precursors in fetal liver, to create

models of JMML. These mice are being used in preclinical studies of a drug PD184352, an inhibitor of Mek, part of the Ras/Raf/Mek/ERK signaling cascade that is active in NF1-associated cancers. Dr. Min Wu (Univ. of Florida) described the development of mouse models of NF1 tumorigenesis that uses implants of tumor tissues or primary cells to induce tumors in scid/NF1^{+/-} mice. Dr. Michael Schmale (University of Miami) is studying a neurofibromatosis type that occurs in damselfish, and has identified a virus-like agent that can be transmitted between fish.

Dr. Chengkai Dai (Memorial Sloan Kettering Cancer Center) presented studies on human gliomas and signaling pathways. He showed that Akt and Ras pathways are involved in astroglial tumors, and PDGF signaling drives oligodendroglial tumors. Dr. Katharina Wimmer (Univ. Vienna, Austria) presented data supporting the lack of evidence for a role of the NF1 gene in sporadic pilocytic astrocytomas.

Dr. James Yeager (Rice Univ) described the role of the NF1 gene in regulating the growth of the perineurial glial cells in *Drosophila* nerve. Ras activity is necessary and sufficient to promote perineurial glial growth, and in the absence of NF1, there is a thickened perineurial glial phenotype. Reduction of ras activity is sufficient to rescue this phenotype in NF1 mutant flies.

Dr. Lynne Fieber (Univ. of Miami) showed that potassium channel blockers could inhibit growth of NF1 Schwann cells. Dr. Timo Korkiamaki (Univ. of Oulu, Finland) described how mutations in the NF1 gene results in altered calcium mediated cell signaling. Dr. Juha Peltonen (Univ of Oulu, Finland) described the interplay between the cell contacts, cytoskeleton, and calcium mediated signaling in NF1 deficient cells. Dr. Fatima Rangwala (Univ of Cincinnati) showed that NF1-deficient Schwann cells have enhanced migration, and this can be inhibited by a dominant negative R-ras.

NF2 in children is being more easily diagnosed with better imaging techniques and molecular diagnostics, however, surgical treatment results in less favorable outcomes in children compared to adults (Dr. Fabio Nunes, Harvard/MGH and Univ Colorado School of Medicine). NF2 patients may suffer from a peripheral neuropathy (Dr. Clemens Hanemann, Univ. Ulm, Germany). Analysis of peripheral nerves in NF2 patients found multiple tumorlets along the length of peripheral nerves, which may cause compression of adjacent nerves.

Analysis of mutations associated with presenile cataracts and spinal tumors in patients with NF2 suggest that there are no specific mutations associated with either of these symptoms (Dr. Michael Baser). There is evidence that demonstrates the occurrence of mosaicism in 17 sporadic (nonfamilial) patients. In these patients, loss of the NF2 gene can be detected in tumor tissue, but not in blood cells (Dr. Lan Kluwe, Uni-Krankenhaus, Hamburg, Germany).

Comparative genomic hybridization/microarray analysis (CGH) is a method for identifying mutations in genetic disorders. Several groups (Dr. Gareth Evans, St. Mary's and Christie Hospital, Manchester, UK; Dr. Kiran Mantripragada, Rudbeck Laboratory,

Uppsala, Sweden) have used CGH/array analysis for NF2 patients, to identify possible deletion mutations in the NF2 gene or in genes that interact with or modify the NF2 gene action. It is thought that these genes may play a role in the variability of features found in NF2 patients. Several genes have been identified in this way and are being analyzed for possible roles in the development of symptoms in NF2 patients. Dr. Sarah Hughes (Duke Univ.) described studies in *Drosophila* that used a genetic screen to identify modifiers of the *dmerlin* gene. Dr. Sushmita Maitra (Duke Univ) described studies concerning the role of the EGF receptor in the regulation of merlin in *Drosophila*.

Dr. Michiko Niwa-Kawakita (INSERM, Paris, France) has generated NF2 mutant mouse strains that are altered such that only one of the two major isoforms of the NF2 protein is expressed. These mice are viable and physically normal. Either of the isoforms alone can also rescue the embryonic lethality of the NF2^{-/-} mutant mice. Dr. Kristen Johnson (MIT Center for Cancer Research) described the introduction of the N-terminal domain of NF2 into NIH3T3 cells and into nude mice. In both cases there was transformation, and tumor formation was robust in the nude mice. Introduction of WT NF2 into human schwannoma primary cells reduced proliferation and increased apoptosis, supporting the role of the NF2 protein as a tumor suppressor (Dr. Clemens Hanemann, Univ. Ulm, Germany). Dr. Dominique Lallemand (MGH Cancer Center) described studies on how the conformation of the merlin protein is regulated by phosphorylation and interactions with growth factors and cell contact.

Dr. Mikaela Gronholm (University of Helsinki, Finland) studied the localization of ezrin and merlin in rat hippocampal cells and found that ezrin is associated with cell extensions while merlin was in the cell body in astrocytes, and the opposite was found in neurons. Studies on DAL-1, a new member of the Band 4.1/ERM family of proteins that may act as a tumor suppressor independent of merlin were described by several groups (Dr. Robin Kuns, Univ of Cincinnati; Dr. Victoria Robb, Washington Univ., St. Louis).