

# **The NNFF International Consortium for the Molecular Biology of NF1, NF2 and Schwannomatosis**

A summary of its meeting,  
May 23-25, 2004, in Aspen, CO



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The National Neurofibromatosis Foundation, Inc. held the 2004 meeting of the *“NNFF International Consortium for the Molecular Biology of NF1, NF2 and Schwannomatosis”* May 23-25 in Aspen CO.

Peter Bellermand, President of the NNFF, welcomed everyone to the meeting, and encouraged an open and lively interaction among the participants. Mr. Richard Horvitz, Esq., Chairman of the Board of Directors of the NNFF, made two special announcements. First, he announced that Mr. Bellermand has informed the Foundation that he will retire on June 30, 2005, and has already turned over the day-to-day management and administrative activities of the Foundation to Mr. John Risner, the interim Executive Director. Mr. Bellermand continues to oversee the Medical and Scientific Programs and the Government Affairs Program of the Foundation until his retirement.

Second, Mr. Horvitz announced that the Foundation has made the decision to change its name, and will become the “Children’s Tumor Foundation” with the byline “Ending Neurofibromatosis Through Research.” This is purely a marketing decision; the Foundation’s focus continues to be solely the neurofibromatoses, NF1, NF2 and Schwannomatosis. The transition to the new name will be complete by the end of the year.

Dr. Shannon praised the work Peter Bellermand has done over the past eighteen years to advance NF research, and upon Dr. MacCollin’s suggestion, the scientists present indicated their appreciation by giving Mr. Bellermand a standing ovation.

### **New Themes Arise At This Year’s Meeting**

Dr. Mia MacCollin (Harvard Medical School/MGH) and Dr. Alcino Silva (University of California, Los Angeles) co-chaired the Consortium Meeting. Mr. Bellermand thanked them both on behalf of the NF Community for a “job very well done” in organizing the meeting.

The focus of the meeting was somewhat different this year from many of the previous meetings in that there was more emphasis on clinical issues. The three main themes were: clinical topics, cognition, and signaling pathways. Some participants commented favorably on the greater emphasis on clinical subjects, while others missed hearing updates on basic research issues reported in 2003. Next year’s co-chairs can take note and bring in a new and different blend of topics for the 2005 Consortium meeting. The changes in meeting organization did not affect attendance at the meeting, which had a record registration of 153 participants (up from 120 in 2003).

The keynote speakers gave excellent presentations, and it was evident that they had given considerable thought to how their work might fit in with NF research. The organizers and the co-chairs of the various sessions put together a program that was well received by the participants.

A number of important advances were reported in NF2 this year, reflecting the fact that more scientists are learning about the biology of the NF2 protein (merlin) and its action in healthy cells. This is helping scientists to understand why things go wrong when the NF2 gene is mutated. With the new information coming in different forms, this year's presentations will be the focus of many telephone calls and emails between scientists sharing information and working to find out how the "puzzle pieces" are fitting together.

There were several exciting stories on bone as it related to symptoms found in NF1. One study demonstrated that there was a difference between bone in NF1 and control samples, even if no skeletal anomalies were obvious. Another study showed that bone precursor cells from different skeletal areas performed differently in a variety of tests concerning their ability to make stronger or healthier bone. Finally, there was a study in a mouse model of NF1 that showed that the of the two major bone cell types responsible for building and remodeling bone, the one that is responsible for breakdown of bone was found in higher numbers and bound more tightly to bone than did similar cells in healthy controls. All of these studies are beginning to lead to a better understanding of the skeletal lesions in individuals with NF1, and scientists can now begin to look for other factors that might explain why not all individuals with NF1 have skeletal symptoms.

There were presentations summarizing the Natural History Studies of NF1 and NF2. For NF1, enrollment is complete, and analysis is beginning but will not be finished until the last MRIs are done in 2006. The Natural History Study for NF2 is in the second phase, and is looking at all NF2 tumors, as well as other symptoms and quality of life in NF2 patients. What has come out of both of these studies is the technology to measure small changes in tumor size for these irregular tumors using 3-D volumetric analysis. This knowledge was taken advantage of by the NNFF, when the Foundation issued a contract in 2003, to develop an imaging archive system that will be able to volumetrically measure tumors, and also to archive them to record differences over time. This work was also presented at the meeting.

Clinical trials were discussed, and while it is too early to predict outcomes, the results do not seem to be as encouraging as we would hope. Hope still lies with interactions between scientists/clinicians and pharmaceutical companies to identify more compounds that might prove to be better suited to fighting NF tumors.

Studies on learning and cognition brought to light the fact that treatment of ADHD in children with NF1 can be treated in the same manner as in children without NF, to achieve similar outcomes. Treatment with FTI (farnesyl transferase inhibitors) is capable of reversing the cognitive problems of NF1 mutant mice, but this drug cannot be given long term to a child without toxic side effects. Treatment with a group of drugs called statins used commonly to lower cholesterol have been shown to have a similar effect on improving cognitive function, without the toxic side effects, and will be pursued as an alternative treatment.

Schwannomatosis was a major topic at this meeting. The NNFF sponsored a Schwannomatosis Consensus Conference last fall, which established that this disorder is separate from NF1 and NF2. The Conference also reported diagnostic criteria for the disorder. While the genetic cause of schwannomatosis is still not known, there has been some interesting discussion as this disorder does not seem to follow the normal Mendelian method of gene inheritance. Pain is the major presenting feature of schwannomatosis, which is a tumor disorder, and there were talks on pain as it relates to all of the NF disorders.

All in all, the presentations were excellent, the sessions dynamic, and we look forward to the “The NNFF International Consortium for the Molecular Biology of NF1, NF2, and Schwannomatosis Meeting” next year on June 5-8 in Aspen, Colorado.

### **Session on Cognition , Mouse Model Workshop & Natural History Study**

Dr. Alcino Silva (UCLA) and Dr. John Gabrieli (Stanford University) co-chaired the first session “Cognition,” bringing together some exciting presentations on learning and behavior associated with NF1, and also highlighting three other areas, pain in NF1, the NF Mouse Models Pathology Workshop, and the NF2 Natural History Study.

Dr. Gabrieli gave the keynote presentation for this session. His work does not focus on NF1, but rather on the ability to use mental resources to solve problems and to achieve challenging goals. He uses functional magnetic resonance imaging (fMRI) to study activity in the brain while the children are doing simple mental tasks. He studies children with ADHD, but suggested that these studies could be used in other disorders that are affected in learning and behavior, such as NF1.

Dr. Robert Lark (Alliant International University) spoke of ADHD in children with NF1. He showed that there were higher reported problems with impulse control and attention span, requirements for diagnosis with ADHD. His group examined 93 children with NF1, and found that 46 (50%) met such diagnostic criteria. These children performed poorly on tests of sustained attention and impulse

control, and on behavior rating tests. Treatment of these children with Ritalin (methylphenidate) for one year improved the performance of these children in both tests. His study also included children with NF1 who have mild mental retardation (MMR) and ADHD. In 9 of 12 children treatment of with Ritalin resulted in improved cognitive and social behaviors. These studies indicate that treatment with medications used to treat ADHD in the general population may result in improvement in the quality of life for children with NF1 and ADHD.

Dr. Alcino Silva spoke about promising new work in his laboratory. He has taken information learned from NF1 mouse models of learning deficits and begun to study the same mechanisms of action in humans. In mice, data suggest that the deficits caused by the NF1 mutations are caused by deficits in the prefrontal cortical function. He has shown previously that decreasing GABA inhibition, with farnesyl transferase inhibitors or GABA drugs rescues the learning deficits in mice. However, treatments with these drugs may not be possible over a lifetime. He has now shown that the statins, drugs that lower cholesterol, inhibit ras, lower MAPK phosphate in the brain, and reverses the spatial learning deficits in NF1 mutant mice. He hopes to develop a study where he can test the ability of lovastatin to reverse spatial and working memory deficits in patients with NF1.

Dr. Yi Zhong (Cold Spring Harbor Laboratory) studies the activity of NF1 in *Drosophila* (fruit flies). In addition to regulating ras GTPase activity, it is required for G-protein stimulation of adenylyl cyclase (AC). His studies have shown that AC can be stimulated by three separate signal transduction pathways, Gsa, Ras, and neurofibromin.

Dr. Cynthia Hingtgen (Indiana University School of Medicine) spoke on issues important to most, if not all, patients with NF1, itching and pain. She is conducting studies to determine what happens to the sensory nerves in patients with NF, what makes them more sensitive to pain. She has shown that NF1 +/- mouse neurons secrete more calcitonin gene-related peptide (CGRP) than normal neurons, indicating already a higher sensitization to pain. Also, NF +/- neurons show increased excitability. Further understanding of these mechanisms of sensitivity may explain the abnormal sensory responses to injury experienced by people with NF1.

Dr. Anat Stemmer-Rachamimov (Harvard Medical School) described the results of an DOD-supported workshop that was attended by an international panel of pathologists. The panelists classified the lesions from published and unpublished NF mouse models, genetically engineered mice (GEM), compared to corresponding lesions in humans. The classification was based on the WHO classification of peripheral nerve tumors, but there were sufficient differences to create a novel system for the mouse tumors. The panel also recommended general guidelines for the work-up and morphological evaluation of the peripheral nerve sheath lesions in GEM models.

Dr. William Slattery (House Ear Institute) described the NF2 Natural History Study, currently in its fifth year. The current study is gathering data on intracranial and spinal tumor burden, ophthalmological testing, auditory testing, physical functioning, and quality of life. Studies by Dr. Michael Lev at MGH have demonstrated that MRI sequences to visualize one type of tumor are not necessarily the best for other tumors. Clinicians will have to consider this before ruling out spinal tumors in patients.

### **Session on “Schmerlin to Schwannomatosis: What’s New on 22 ?”**

This session was co-chaired by Dr. Mia MacCollin (Harvard Medical School/MGH) and Dr. Gareth Evans (University of Manchester/St. Mary’s Hospital, UK).

Dr. Evans spoke about 76 patients with multiple non-cranial schwannomas, not fulfilling the NIH criteria for NF2. In his opinion, 1:80,000 such patients with schwannomas but without vestibular schwannomas (VS) will develop one VS each year. He suggests that patients who develop 2 schwannomas before they are 20 years of age will have NF2, and those with two only after they are over 20 years will have schwannomatosis. Based on a number of studies, Dr. Evans believes that the prevalence of schwannomatosis could be anywhere from 1 in 40 to 1 in 450,000, clearly an unacceptable range which needs to be more closely examined.

Dr. Jan Dumanski (Uppsala University, Sweden) described his recent studies on Schwannomatosis, an NF2-related, but distinct genetic disorder. His lab has used a chromosome 22 genomic microarray to detect gene copy number abnormalities outside of the NF2 locus, in a cohort of sporadic and familial schwannomatosis patients as well as in patients with severe/moderate NF2. He found three overlapping deletions of 40 kb, 800 kb, and 250 kb, in severe NF2, sporadic, and familial schwannomatosis patients, respectively. He is hypothesizing that a single candidate transcript may be responsible for the generation of the schwannomatosis phenotype, as well as the phenotypic variation observed in NF2. He proposes that there is recombination between markers on 22q and the NF2 gene, which varies between the various phenotypes of NF2 and schwannomatosis and suggests a contiguous gene syndrome.

Dr. Ludwine Messiaen (University of Alabama, Birmingham) described her studies of segmental neurofibromatosis. Segmental NF1 is presumed to be a somatic mosaicism, where only a part of the body is affected, and patients present with either multiple CAL-spots, and freckling only, or with multiple isolated neurofibromas without other cutaneous findings, or with CAL-spots, freckling and neurofibromas. She presented two cases of patients with multiple isolated neurofibromas.

Patient 1 had multiple neurofibromas on her face, throat, right arm and both hands. Analysis of lymphocytes indicated mosaicism for the total NF1 gene deletion. Her children were at risk as the mosaicism may have been germline, and could be passed to them. None of the children inherited the NF1 mutation.

The second patient developed neurofibromas in his thirties, in a band from the middle of his back to the middle of his abdomen. He had no CAL-spots, no freckles, and no Lisch nodules. No NF mutations were found in lymphocytes. Genetic analysis of the tumors demonstrated that all tumors arose from different mutations in the NF1 gene, and that there was no microsatellite instability in the tumors. More studies are needed to understand the natural history of this patient.

Dr. Feng-Chun Yang (Indiana University School of Medicine) spoke about the role of mast cells in the growth of neurofibromas in NF1. NF1 -/- Schwann cells are the initiating cells for tumor growth in NF1, but it has been shown that a haploinsufficient tumor environment is also important for tumor formation. Dr. Yang had shown previously that the NF1 -/- Schwann cells secreted kit-L to promote the migration of NF1 +/- mast cells. This study demonstrated that the NF1 +/- mast cells also secreted growth factors that promote Schwann cell proliferation via Rac2 activation.

Dr. Hamid Salamipour (Harvard Medical School/MGH) presented radiographic data on twelve patients with definite or presumptive schwannomatosis. Schwannomatosis tumors were found to be discrete, well defined, rounded or oval lesions distributed along the course of peripheral nerves in the extremities and in the paraspinal nerve roots. He was able to conclude that schwannomas could be distinguished quite easily from plexiform neurofibromas of NF1, though perhaps not as reliably from discrete or nodular lesions seen in some NF1 patients. Dr. Salamipour hopes to be able to develop a rapid and cost effective MRI protocol that will reliably determine the total body burden of tumors in schwannomatosis patients.

Dr. Lisa Smith (University of Indiana School of Medicine) performed a study to classify the types of pain associated with complaints from patients with NF1, both adults and children. For children, the FACES scale was used, for adults, the scale of 1-10. In children, 25% reported having daily pain, but at less than 2.4 on average, and for the most part it did not affect their daily life. In adults, 65% had pain associated with neurofibromas, which was characterized either as sharp, aching or itching, and aggravated by minor trauma or exercise. The study will continue to accrue patients and determine the effects of pain on the quality of life for people with NF1.

Dr. Oliver Hanemann (University of Ulm, Germany) spoke about the changes in the internal structure of the cells in human schwannoma tumors. He has found that intermediate filaments in the cells are clustered around the cell's nucleus,

rather than distributed in the cytoplasm of normal cells. Because of this, the cells cannot assemble a cytoskeleton, the correct internal structure. The cells collapse, resulting in a flattened cell shape and increased migration. Further research is needed to understand the cause of the interruption of the cytoskeleton of the cell.

Dr. Yiping Shen (Harvard Medical School/MGH) spoke about the use of comparative genomic hybridization (CGH) to study genomic changes that occur in meningiomas. Meningiomas are a frequent complication of NF2. There were chromosomal instabilities found in all of the tumors examined, and significantly more in tumors with deletion of chromosome 22 than those without. The work also suggests that loss of chromosome 22 initiates a state of chromosomal segmental instability, resulting in the increased imbalance. Studies will continue using microarrays to determine if certain CGH profiles can predict aggressiveness and clinical behavior of meningiomas.

Dr. Arie Perry (Washington University, St. Louis) spoke about meningoangiomas (MA), a rare seizure-associated lesion encountered either sporadically (75%) or in the setting of NF2 (25%). MA may be associated with a meningioma (MA-M). In this study, there were 9 patients with MA-M and 8 patients with pure MA. Of the 9 MA-M, 7 had gene deletions in the NF2/ 4.1B genes or loss of the respective proteins. In these cases the MA component was neoplastic. The other two cases, along with 6/8 of the pure MA cases did not have the alterations in the NF2/ 4.1B, and were similar in structure to non-neoplastic meningothelial cells. Loss of the genes in the other 2 cases suggested that a small subset of pure MA might be neoplastic in nature.

Dr. Michael Kalamarides (Fondation Jean Dausset, Paris, France) presented his studies on meningiomas, a common tumor type found in the brains of NF2 patients. Several genes have been identified as possible contributors to the formation of meningiomas by their loss, including CDKN2A, p14 (ARF), and CDKN2B. A mouse model has been developed that develops meningiomas when both copies of the NF2 gene are lost. Studies can now be done to look at other genes which may contribute to meningioma development and progression in these mouse models.

### **Session on Clinical Trials in Neurofibromatosis**

This session was co-chaired by Dr. Dusica Babovic-Vuksanovic (Mayo Clinic) and Dr. Brigitte Widemann (National Cancer Institute/NIH).

Dr. Stephen Eck (Pfizer Global Research and Development) was the keynote speaker of the session, and spoke about translating basic research into clinical trials. He stressed that the following are needed to develop a drug for effective treatment (or a cure) of NF:

1. Significant investment of money over 8-10 years.

2. An understanding of the disease process, including aspects that contribute to morbidity and mortality
3. Molecular targets amenable to pharmacologic intervention
4. A platform for designing clinical studies

Dr. Eck used the example of Aspreva, a small company that partners with larger (or other) pharmaceutical companies to discover, research, trial, commercialize & market treatments, orphan drugs & therapeutics to treat rare diseases. But in order to test these drugs for efficacy (usefulness) in a rare disease, there must be a way to measure whether there is clinical benefit. Biomarkers can be used for early studies, such as looking for a decrease in tumor protein activity. But a well-defined clinical endpoint is critical for approval by the FDA. Survival is the gold standard, but prevention of structural damage or increasing time to onset of symptoms might be acceptable standards for a chronic disorder such as NF.

Dr. Scott Plotkin (Harvard Medical School/MGH) described the steps that have to be taken to proceed with a clinical trial. One must understand the basic science well enough to target the underlying mechanism of the NF-related complication. The natural history of the life-long disorder must be known. Preclinical models must exist for initial screening of possible agents. Clinical trial design must focus on quality of life rather than survival, as the meaningful endpoint. Funding opportunities must continue to be available to develop and continue NF-specific trials. IRB approval has to be streamlined, as most NF trials must, by its orphan status, be multi-institutional. Institutional collaborations must be achieved in order to recruit the high numbers of patients required for a successful trial.

Dr. Bruce Korf (University of Alabama, Birmingham) provided a progress report and interim analysis of the Natural History of Plexiform Neurofibromas in NF1. The project has been going for 5 years, with 16 participating centers, a tissue bank, a radiology analysis center and a database center. A total of 274 participants have been recruited to the study, which has been closed to enrollment since October 2003. Interim analysis indicates that a higher proportion of tumors increased in participants < 18 years of age, but there is no difference as a function of tumor site (head and neck vs. trunk and extremity). Final data analysis will not be completed until 2006.

Dr. Michael Baser (Los Angeles) described a number of developing databases describe longitudinal studies of vestibular schwannoma growth rates in patients with NF2. Additional databases are in progress on the development of symptoms and their relationship to the risk of mortality in NF2. The International NF2 database is compiling information from all known databases and from unpublished sources. The database is being used to study genotype/phenotype correlations. A description and a list of NF2 mutations is being distributed to on-line human gene mutation databases.

Dr. Gordon Harris (Harvard University/MGH) described his work on the Neurofibromatosis Digital Image Archive and Metrics Service that was funded as a contract through the NNFF. In a collaborative effort between MGH and Medical Media Systems (West Lebanon, NH) a centralized tumor metrics analysis registry is being developed that can make reliable, semi-automated, quantitative volumetric analyses of serial images of NF1 and NF2 images. In other words, image analyses can be compared over time of the same lesion in the same patient, and presented in graphical format on a password-protected web-based report that can be accessed by the referring physician, and can be incorporated and used as part of the patient's medical history. Once established, this will become a self-supported service financed by reimbursements from medical insurance based on existing CPT codes for image processing.

Dr. Eva Dombi (NCI/NIH) described the ongoing, double-blinded, placebo-controlled, cross-over phase II clinical trial of farnesyl transferase inhibitor R115777 for patients with NF1 and progressive plexiform neurofibromas. Thirty-two patients have been entered into the trial. Thirteen have developed disease progression on the first treatment and have been crossed over to the second treatment. With the trial being blinded and placebo control, there is no way to determine the outcome of the trial at this time.

Dr. Dusica Babovic-Vuksanovic (Mayo Clinic) described a phase I trial of the antifibrotic agent pirfenidone in children with NF1 and plexiform neurofibromas. Pirfenidone has been used for other applications in adults, and a phase II trial in adults with NF1 is nearing completion at the Mayo Clinic. The phase I trial in children was required as toxicity studies had not been done in children. The results of the phase I study determined that the optimal pediatric dose is 500 mg/m<sup>2</sup>/dose, which will be used in the phase II trial. Non-dose limiting toxicities included diarrhea, nausea, vomiting, fatigue, gastritis, and pruritus.

Dr. Brigitte Widemann (NCI/NIH) described the phase II trial of pirfenidone, which will be done in children and young adults, and is scheduled to start this summer. The objectives are to determine whether pirfenidone increases time to progression of tumor growth, determine toxicities over longer periods of time, and assess quality of life of the patients treated with this agent. The control arm of the FTI R115777 study will be used as the historical control for this study. Enrollment will be for patients between 3 and 21 years of age, and will be for approximately 36 patients at 15 participating institutions.

Dr. Gideon Bollag (Plexxikon, Inc.) described work on regulation of the mast cell in the development of neurofibromas. Mast cells respond to a number of compounds, and in neurofibromas the affected Schwann cells over-secrete a stem cell factor. The mast cells respond due to the presence of a c-Kit tyrosine kinase receptor on their surface. Scientists at Plexxikon have developed a series of drugs that inhibit the c-Kit receptor, and have tested these drugs in a series of other mast cell mediated diseases, including multiple sclerosis. One compound

shows promise in a number of models, and is a candidate for evaluating in preclinical studies, including testing in NF1 models.

### **Session On Signaling Pathways In NF**

This session was led by Dr. Nancy Ratner (University of Cincinnati) and Dr. Margaret McLaughlin (MIT Center for Cancer Research).

Dr. Xi He (Harvard Medical School/Children's Hospital) was the keynote speaker for this session. He spoke about Wnt signaling in development and disease. Wnt is a gene that is essential for development of the midbrain and for cerebellar morphogenesis. When disrupted, Wnt causes abnormal embryogenesis and various diseases. He described the combination of techniques, molecular, biochemical, and embryological, that have been used to understand how the Wnt signaling pathway works in vertebrate development and in human cultured cells. His studies parallel the types of studies that are being used to understand the signaling pathways used by both the NF1 and NF2 genes to understand their roles in early development, and the changes that occur when the genes are disrupted.

Ms. Nitasha Manchanda (Harvard University/MGH) described studies on the NF2 protein merlin and the related ERM proteins ezrin, radixin and moesin, and a new role that has been identified for these proteins, in addition to their known role as links between the cells plasma membrane and the actin cytoskeleton. Rho GTPases can also act via the WASP (Wiskott-Aldrich Syndrome Protein) family of proteins to regulate an actin nucleator, the ARP2/3 complex. Studies reported here indicate that the MERM proteins can also interact with neural-WASP proteins to cause actin reorganization and to inhibit actin polymerization. This shows that the MERM proteins function in a more active role as regulators of actin dynamics, rather than just acting as a link to the plasma membrane.

Dr. Helen Morrison (University of Karlsruhe, Germany) presented work on how CD44, merlin and ezrin are involved in a molecular switch that signals either cellular growth or growth inhibition. She showed that activated merlin interfered with several signaling pathways by blocking the activation and function of Ras. This interference was specific to Ras. Dominant negative mutants of ezrin mimic the action of activated merlin. Her data suggested that these proteins assemble components in the cell that are essential for signal transfer for the Ras-to-MEK pathway.

Dr. Daniel Scoles (Cedars-Sinai Medical Center/UCLA) described studies of proteins that interact with the NF2 protein. Earlier studies had identified a protein, hepatocyte growth factor tyrosine kinase substrate (HRS) that interacts with the NF2 protein. Overexpression of HRS resulted in decreased Epidermal Growth Factor Receptor (EGFR) degradation, but not EGFR trafficking to

endosomes. He is now using this model system to determine a role for the NF2 protein in HRS-mediated inhibition of EGFR signaling.

Mr. Taru Muranen (University of Helsinki, Finland) works on understanding the interaction between merlin and microtubules. Merlin associates with microtubules during mitosis at mitotic spindles and during cytokinesis at the midbody. His group has identified two tubulin-binding sites in merlin, one located at the N-terminal FERM domain, and the other in the C-terminus. Both sites must be present for the binding to occur correctly. This study suggests a role for merlin in the control of spindle organization.

Dr. Margaret McLaughlin (MIT) is studying the role of merlin during mouse embryonic development. NF2<sup>-/-</sup> mice die early in gestation, due to an extraembryonic defect. In a mouse model of NF2, where NF2 gene is fully deleted in the developing central nervous system, and in a mosaic fashion through the rest of the body, the mice exhibit a spectrum of neural tube defects. In addition, tissue fusion does not occur at a number of other sites in the embryo. This phenotype is similar to four known mouse planar cell polarity (PCP) mutants and to several mouse actin-binding protein mutants. Establishment of PCP involves signaling through the noncanonical Wnt pathway to effectors such as actin binding proteins that mediate remodeling of the actin cytoskeleton. Rac also participated in the noncanonical Wnt pathway, and the authors propose that merlin may also participate in the noncanonical Wnt pathway, possibly as a negative regulator of Rac.

Dr. James Winkler (Array BioPharma) described the development of a selective MEK inhibitor, ARRY-142886. MEK is a key player in the Ras/Raf/MEK/MAPK pathway that has been implicated in tumor growth. MEK is downstream of both Ras and Raf, and activated ERK1/2 through phosphorylation of key tyrosine and threonine residues. ARRY-142886 is active against MEK at a very low concentration, and does not inhibit serine or threonine kinases. ARRY-14886 has been shown to be effective against a large number of tumor cell lines, and against xenograft models of tumors. Tumor regression was observed, and ARRY-14886 was also effective in reducing tumor size of re-established tumors, demonstrating sensitivity after a treatment free period. Pre-clinical studies have been completed, and this compound is being tested in phase I clinical trials in cancer patients. When asked, Dr. Winkler felt that it might be possible to test this drug in NF mouse models, and this possibility will be explored.

Dr. Klaus Scheffzek (University of Heidelberg, Germany) spoke about his laboratory's interest in studying the crystal structure of other areas of the NF1 protein, in an attempt to determine other functions of the NF1 gene. The only known function of the NF1 protein at this time is the small area that codes for a Ras specific GTPase activating protein (or Ras-GAP), an area referred to as the GAP-related domain or GRD. Dr. Scheffzek's group has solved the crystal structure of a 31 kDA segment of NF1 adjacent to the GRD. The analysis has

identified an unexpected phospholipid-binding domain that was not predicted based on sequence or other bioinformatics methods. Dr. Scheffzek will continue to study these smaller segments of the NF1 protein to identify other areas that might have functional significance in the NF1 disorder.

Dr. Karen Cichowski (Harvard University/BWH) presented work from her laboratory looking at the control of the level of neurofibromin in the cell and how it is controlled by other cellular activity. Normally, neurofibromin is rapidly degraded in response to a number of growth factors. It requires protein kinase C and sequences adjacent to the GAP-related domain. Neurofibromin sequences are rapidly re-elevated after growth factor treatment, and this newly synthesized product is phosphorylated. The phosphorylation occurs precisely as Ras is being inactivated, suggesting that phosphorylation may enhance neurofibromin's function to terminate Ras activity. Dr. Cichowski and colleagues have shown that activation of the Ras/Raf/MEK pathway is both necessary and sufficient for neurofibromin phosphorylation. They have also identified a novel neurofibromin-interacting protein only under conditions in which neurofibromin is phosphorylated. Studies are continuing to learn more about this aspect of neurofibromin and its function.

Dr. Michael Stern (Rice University) studies factors that affect the growth of glial cells in *Drosophila* peripheral nerves, cells that are analogous to Schwann cells in mammals. Active Ras expression in these cells increase growth of these cells. He found that an NF1 mutation suppressed this effect of Ras, suggesting that NF1 in *Drosophila* promotes, rather than inhibits cell growth in glial cells. The second activity of NF1 that has been identified in *Drosophila*, that of activation of protein kinase A, may have a role in glial cells. Dr. Stern showed that PI3 Kinase, but not Raf pathways, strongly increased the glial growth, implying that PI3 kinase is another important mediator of growth-promoting effects of Ras in peripheral nerves.

Dr. Nancy Ratner (University of Cincinnati) has observed that overexpression of epidermal growth factor receptor (EGFR) has been seen in some neurofibroma, MPNST cells. She hypothesized that overexpression of EGFR, in addition to the NF1 mutation, is necessary for the formation of NF1 tumors. A transgenic mouse line which overexpresses human EGFR in Schwann cells was studied for effects on nerves and cells. The nerves had a 3-10-fold increase in cross-sectional area, aberrant Schwann cell processes, disruption of unmyelinated fiber bundles, increased collagen deposition in the nerve matrix, accumulation of mast cells, and thickened myelin sheaths. Blocking human EGFR with Erbitux, an antibody against human, but not mouse EGFR, was performed at various times from birth to 3 months of age. She found that treatment in a short window beginning at birth resulted in normal nerve for as long as 3 months. She suggests that treatment of young animals may have the potential to block tumorigenesis by halting a crucial step in a cascade of events that leads to complex changes in neurofibroma formation.

## **Session On Clinical Aspects and Mutational Analysis of Neurofibromatosis**

This session was chaired by Dr. Brad Welling (Ohio State University).

Dr. David Stevenson (University of Utah) spoke about his work on the skeletal manifestations of NF1. Common skeletal defects include tibial dysplasia and pseudoarthrosis, scoliosis, and sphenoid wing dysplasia. In addition there are a number of less common symptoms. Dr. Stevenson and his group studied a group of 40 individuals with NF1 using two imaging techniques, to examine overall bone density and bone structure, compared to individuals who did not have NF1. They found evidence that all NF1 individuals have a different bone architecture, bone density, and bone strength compared to individuals without NF. This suggests that NF1 is a constitutional disorder of bone, that should be monitored prospectively, and he suggested that individuals with NF1 might benefit from osteoporosis drugs.

Dr. Juha Peltonen (University of Oulu, Finland) described studies of congenital pseudoarthrosis, looking at mesenchymal stem cells (MSC) from the site of the pseudoarthrosis in 2 cases of NF1 and comparing them to MSC from healthy controls and MSC from the iliac spine of an NF1 patient. Healthy MSCs are able to differentiate into bone forming cells. Bones from the three sources were analyzed using biochemical markers that showed the ability of the cells to differentiate. Dr. Peltonen's group found that MSCs from the pseudoarthrosis sites was significantly lower in these markers than in healthy MSCs and even in MSCs from NF1 iliac spine. He described one of his studies involving 5 patients with NF1 who were facing amputation for pseudoarthrosis. As a last step before amputation, Dr. Peltonen had transplanted MSCs from healthy iliac crest cells of the same patient into the pseudoarthrosis area. The study was only 3 weeks old, but so far, the patients have no pain, nor acute effects. There was, however, no evidence of ossification yet, so he does not know yet if it will help.

Dr. Wade Clapp (Indiana University) presented studies on the lytic bone lesions associated with skeletal defects in NF1. There are two main cells associated with bone remodeling, the osteoblast and the osteoclast. Osteoblasts contribute to bone formation. Osteoclasts bind to bone and are responsible for bone resorption, which leads to changes in bone size and shape during growth and helps maintain healthy bone in adulthood. Dr. Clapp studied the osteoclast progenitor cells between NF1 and healthy control mice. He found that there was an increase in the number of large, multinucleated osteoclasts in NF+/- mice. In addition, the cells had increased adhesion to bone slices, and there were increased number and size of resorption pits in the NF1+/- bone compared to healthy controls. Studies have previously shown that bone in these NF+/- mice had reduced bone stiffness. These studies suggest that neurofibromin may have a role in regulating cells that contribute to skeletal formation, resulting in skeletal abnormalities in individuals with NF1.

Dr. Thomas De Raedt (University of Leuven, Belgium) spoke about gastrointestinal neurofibromatosis (GNF). In GNF, neurofibromas are limited to the intestine, are adult onset with incomplete penetrance, variable expression, autosomal inheritance, and absent of other features of NF1 or NF2. In a familial case of GNF, a mutation was found in a region of the PDGFRa gene. This is one of the genes that has been found to be mutated in sporadic gastrointestinal stromal tumors (GIST). The gastrointestinal neurofibromas have a distinctive pathology, different from the GIST tumors, although the activating genes are similar. GISTs have been shown to be responsive to Gleevec, and this suggests a possible treatment for patients with GNF, which has yet to be tried.

Dr. Howard Feit (Henry Ford Hospital, Detroit) described the challenges of running an "Adult" NF Clinic. The clinic, in which both adults and children are seen, includes several doctors working together, including a pediatrician and geneticist, an adult neurologist, with resources available for pediatric ophthalmology, neurosurgery (peripheral nerve), general surgery with an interest in sarcoma surgery. The focus of care of adults is dictated by the natural history of the disease in each patient. The primary issues are benign neurofibromas which are becoming symptomatic because of size or location, and the 10% risk of the development of a malignant nerve sheath tumor. Patients must be educated as to the need for prompt evaluation of the symptom of unremitting pain, the cardinal symptom for a malignant tumor. Lack of medical insurance is a major obstacle in getting appropriate care for adult patients with NF.

Dr. Karlyne Reilly (NCI/NIH) is using an NF1 mouse model which also has a mutation in p53, to identify other genes that may act as modifiers affecting tumorigenesis. Some of the modifier loci have been identified by crossing different strains of mice, which have variations in genes that might affect the expression of tumors in a mouse with a known tumor mutation. She has found a susceptibility to astrocytomas on chromosome 11, close to the NF1 and p53 gene. She has shown susceptibility to sarcomas to chromosome 15 and chromosome 19 in the mouse. Dr. Reilly and colleagues are working to identify these modifier genes and to determine the roles and interactions they play in tumorigenesis.

Dr. Margaret Wallace (University of Florida, Gainesville) presented data on germline and some somatic mutations from patients with NF1. She presented patients with similar phenotypes, and described the different types of mutations contained within the group. Groups include patients with MPNST, with plexiform neurofibromas but not malignancies, and other specific categories. She categorized by sporadic vs. familial, gender, age of onset of symptoms, etc. These studies continue to bring more information into the clinical field which may help with predicting patient outcomes for patients newly diagnosed with NF1.

Dr. Ophelia Maertens (University of Ghent, Belgium) spoke of a comprehensive somatic mutation analysis strategy for the NF1 gene on Schwann cells grown from dermal neurofibromas. Because the germline mutation has not been useful in most cases in predicting the outcome or patient risk, she took the path of trying to determine if the somatic mutation might be contributing information about the tumor burden for individuals with NF1. In 26 tumors from 5 NF1 patients, she identified 20 somatic mutations. Eighteen were subtle mutations of different types, and only two showed loss of heterozygosity of the NF1 region. She plans to continue this work to determine if there is a pattern of somatic mutations that will predict tumor burden for individuals with NF1.

## Poster Session Presentations

In addition to the oral presentations, there were 44 poster presentations. The table below gives the presenting authors name, the affiliation, and the title of the poster.

Alfthan (University of Helsinki, Finland)	Protein Kinase A Phosphorylates Merlin At Serine 518 Independently of P21-Activated Kinase and Promotes Merlin-Ezrin Heterodimerization
Anlar (Hacettepe University, Turkey)	Investigation of the Intelligence Profile of Children Diagnosed With NF1
Ayter (Hacettepe University, Turkey)	Phenotypic Variations in NF1: Examples of Two NF1 Families With Exon 4b Mutations
Bernards (Harvard Medical School/MGH)	Identification of Genetic Modifiers of Neurofibroma Burden in NF1
Boyanapalli (University of Michigan)	Interaction Between Neurofibromin and Caveolin-1, Two Tumor Suppressor Gene Products
Buckley (Uppsala University, Sweden)	Gene Copy Number Profiling of Meningioma Using A Chromosome 1 `Genomic Microarray
Chang (The Ohio State University)	Development Expression of the NF2 Gene and Genetic Analysis of Merlin Function
Cui (UCLA)	A Heterozygous Null Mutation of the NF1 Gene Causes Increased GABA Release From Hippocampal Interneurons
Gursel (NCI/NIH)	Epidermal Growth Factor Receptor (EGFR) Signaling in Mouse Model of Nervous System Tumors Associated with Neurofibromatosis Type 1
Diebold (University of Ulm, Germany)	Sensitive Detection of Deletions of One or Few Exons in the NF2 Gene by Multiplexed Gene Dosage PCR
Donarum (Barrow Neurological Institute)	Investigation into the Cognitive Deficits Associated with NF1: The Role of Dendritically Localized mRNA
Friedrich (University Hospital Hamburg-Eppendorf, Germany)	Subtotal and Total Resection of Superficial Plexiform Neurofibromas on Face and Neck
Goutebroze, (INSERM, France)	Overexpression of Mutated Tumor Suppressor Schwannomin/Merlin in Schwann Cells Alters the Organization of Nodal and Paranodal Regions in Peripheral Myelinated Fibers
Hughes, J. (Cornell University)	A Role for Merlin in Membrane Trafficking?
Hughes, S. (Duke University)	Analysis of a Functional Interaction Between Moesin, Merlin and the Putative Drosophila EBP50

Ismat (University of Pennsylvania/ Children's Hospital of Philadelphia)	NF1, Ras, and NFATc1 in Cardiovascular Development
Kluwe (University Hospital Hamburg-Eppendorf)	Screening 528 Unselected NF1 Patients for Deletions of the NF1 Gene
Lazaro (Harvard Medical School/MGH and Institut de Recerca Oncologica, Spain)	Design of a Phakomatosis Resequencing Array
Lee (NICHD/NIH0)	Transcriptional Profiling in MPNST-Derived Schwann Cells
Lepont (University of Cincinnati Medical Center)	Point Mutation in the NF2 Gene of HEI-193 Schwannoma Cells Results in Exclusive Expression of Merlin Isoform III
Li (UCLA)	Reversing the Learning and Attention Deficits in a Mouse Model for NF1
Lorenz (The Ohio State University)	Vestibular Schwannomas Xenografted in SCID Mice and Expression of Cyclin D1, D3, and Telomerase
Mantripragada (Indiana University School of Medicine)	High Resolution array-CGH analysis of a Candidate Schwannomatosis Locus on Chromosome 22
Mensink (Mayo College of Medicine)	Connective Tissue Dysplasia in Two New Patients with NF1 Microdeletion: Further Expansion of Phenotype
Messerli (Harvard Medical School/MGH)	Treatment of Schwannomas with an Oncolytic HSV-1 Recombinant Virus in Murine Models of NF2
Messiaen (University of Alabama, Birmingham)	Comprehensive Mutation Analysis in Over 450 NF1 Patients Fulfilling the NIH Diagnostic Criteria and in Over 100 Patients Presenting with Only 1 Criterion
Miller (University of Cincinnati School of Medicine)	Gene Expression Profiling in Schwann Cells and MPNST Cells: Toward A Molecular Model of Tumor Progression
Morrison (University of Michigan)	Neural Crest Stem Cells Undergo Multilineage Differentiation in Developing Peripheral Nerves To Generate Endoneurial Fibroblasts in Addition to Schwann Cells
Mosher (University of Michigan)	Intrinsic Differences Between Spatially Distinct Populations of Neural Crest Stem Cells
Nunes (Harvard Medical School/MGH)	Inactivation Patterns of NF2 and DAL1 in Sporadic Meningiomas Using LOH and CGH Analysis
Oguzkan (Hacettepe University, Turkey)	A Large Deletion in the NF1 Gene Associated with Neurofibromatosis – Noonan Syndrome

Pandita (The Hospital for Sick Children, Toronto, Canada)	Construction of A High Resolution Genetic Alteration Map of Transformed Schwann Cells in Peripheral Nerve Sheath Tumors (PNST)
Peltonen, S (Turku University, Finland)	Tight Junctions in Peripheral Nerve and Neurofibroma
Pros (Institut de Rederca Oncologica, Spain)	Semiquantitative Analysis of Transcripts Produced by Mutations Affecting NF1 Splicing
Rizvi (University of Cincinnati)	Loss of Neurofibromin Leads to Abnormal cAMP & Ras Signaling in Progenitors Derived From Developing Mouse Brain
Schmale University of Miami)	A Unique Virus-Like Agent Associated with Neurofibroma Formation in A Fish
Scoles (Cedars-Sinai Medical Center/UCLA School of Medicine)	The NF2 Tumor Suppressor Schwannomin Interacts with the Eukaryotic Initiation Factor 3 (eIF3) Subunit p110 and Inhibits In Vitro Protein Translation
Slattery (House Ear Institute)	Quality of Life Changes in NF2 Patients
Stickney (University of Cincinnati Medical Center)	Activation of the Tumor Suppressor Merlin Modulates its Interaction With Lipid Rafts
Viskochil (University of Utah)	Genotype Analysis of the NF1 Locus in Pseudoarthrosis Tissue
Walker (Harvard Medical School/MGH)	Genetic Screens for Modifiers of Drosophila NF1
Wang (University of Washington)	Identification and Mapping of NF1 Contiguous Gene Deletions Using Real-Time Competitive PCR
Weinberg (Mt. Sinai School of Medicine, NY)	A New Method for the Rapid Treatment of Multiple Cutaneous and Subcutaneous Lesions of Neurofibromatosis
Welling (The Ohio State University/Children's Hospital)	Long-Term Hearing Rehabilitation in NF2 Patients with Cochlear Implants