International Consortium Summary (1998)

Scientists Meeting In Colorado Report New Advances In Both NF1 And NF2

(Ed. Note: "The NNFF International Consortium For The Molecular Biology Of NF1 and NF2", now in its thirteenth year, is a worldwide collaborative effort of all leading laboratories in NF1 and NF2. It is sponsored and managed by the National Neurofibromatosis Foundation. The Consortium meets annually and is widely hailed for having accelerated the progress in NF research.)

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The NNFF International Consortium For The Molecular Biology of NF1 and NF2 met for four days during June in Aspen, Colorado. The meeting was chaired by Dr. Neal Copeland (National Cancer Institute). It was attended by 103 investigators from the United States and Europe.

The first session was devoted the clinical and molecular genetics of neurofibromatosis and was chaired by Dr. Bruce Korf (Harvard/Boston Children's). The meeting began with an introduction to the Department of Defense/US Army Neurofibromatosis Research Program provided by the program head, Col. Irene Rich. Col. Rich reviewed the history and administration of the program, and announced a new request for proposals for additional funding of neurofibromatosis research. Recently, the Department has approved funding of a pair of natural history studies, one for NF1 and one for NF2. These upcoming studies were described by the principal investigators, Dr. Bruce Korf (Harvard/Boston Children's) for the NF1 study and Dr. William Slattery (House Ear Institute) for the NF2 study. Both studies are intended to collect data on the patterns of growth of neurofibromatosis-related tumors in preparation for eventual clinical trials.

Three talks focused on the molecular genetics of NF1. Dr. Klaus Scheffzek (Max Planck Institute, Germany) described the three-dimensional structure of the GAP protein and the structural basis for its interaction with RAS. Scheffzek has recently been successful in obtaining a crystal form of the RAS-NF1 GAP-related domain and described the structure of the association of the NF1 GAP-related domain with RAS. Through this work, several amino acids that are critical to the binding of Ras were identified. Many of these correspond with known mutations in individuals with NF1; others are candidate sites for mutation. Dr. Ludwine Messaien (University of Gent, Belgium) reported on her experience with screening 38 patients with NF1 for mutations using a multi-step approach. One was found to have a balanced translocation, and none was found to have a large deletion. Additional mutations were found with the protein truncation assay (17 mutations) and by heteroduplex analysis (3 mutations). Interestingly, four mutations were found to reside within exons and yet lead to exon skipping. Overall, mutations were found in 21/38 patients. Several poster presentations also described progress in NF1 mutation analysis.

It has been hypothesized that mutation of both copies of the NF1 gene might underlie the pathogenesis of neurofibromas, although this has been difficult to prove, in part due to admixture of normal and tumor cells in benign neurofibromas. Dr. Margaret Wallace (University of Florida, Gainesville) examined cutaneous and plexiform neurofibromas for loss of heterozygosity of NF1, finding this in 2/15 cutaneous tumors and 5/12 plexiform neurofibromas. Loss of heterozygosity was also found in 3/5 malignant peripheral nerve sheath tumors. Dr. Eduard Serra (University of Barcelona, Spain) presented a poster showing evidence for loss of heterozygosity for NF1 in some neurofibromas. These results provide support for the hypothesis that NF1 functions as a tumor suppressor gene; those tumors not found to have loss of heterozygosity are presumed to have mutations in both NF1 alleles, although these mutations have not been identified.

The second session was chaired by Dr. Andre Bernards (Harvard/MGH) and focused on the cell

biology of NF1. Bernards described the phenotype of Drosophila NF1 knockout mutations. The flies tend to be small and exhibit learning defects. Increasing levels of cAMP rescue the flies from the phenotypic effects of NF1 mutation. Dr. Yi Zhong (Cold Spring Harbor Laboratory) continued the story of the Drosophila NF1 mutants, concentrating on the learning defect. The phenotype is similar to that produced by mutation in the rutabaga locus, involved in the adenyl cyclase pathway; Zhong presented evidence that NF1 in Drosophila may function through that pathway in its effects on learning. Dr. Gihan Tennekoon and Dr. Jeffrey Field (both University of Pennsylvania) presented data on the activity of RAS pathways in Schwann cells. There are multiple pathways through which RAS exerts an effect on transcription. Ha-RAS activity in Schwann cells leads to differentiation, acting through the Raf/Mek/Map kinase pathway. The PI-3 kinase pathway appears to be involved in the inhibition of apoptosis of Schwann cells. Proteins in the Rho family lead to changes in Schwann cell morphology when activated. Another protein involved in Ras signaling is Pak 1. Dominant negative Pak mutants inhibit Ras-dependent transformation of Schwann cells. Dr. Laura Klesse (University of Texas, Southwest) described the role of RAS pathways in neuronal differentiation and survival. The Raf/Mek/Map kinase pathway appears to be involved in neurotrophin-induced differentiation but the PI-3 kinase pathway is required for survival, as for Schwann cells. Dr. Jeffrey DeClue (National Cancer Institute) reported finding activity of the EGF receptor on malignant peripheral nerve sheath cells in culture as well as on benign neurofibromas. Growth of the malignant cells in culture is EGF-dependent. Dr. Wade Clapp (University of Indiana) reported on his study of RAS signaling in hematopoietic progenitor cells. Mouse Nf1 -/- hematopoietic stem cells demonstrate abnormal proliferation in response to cytokines and activation of the Raf/Mek/MAP kinase pathway. It is hypothesized that the NF1 protein functions to modulate this pathway, and its absence leads to abnormal activation. Dr. George Mashour (Georgetown University) reported increased secretion of angiogenic factors from Nf1-/- Schwann cells, including an angiogenic factor Midkine (MK). Interestingly, MK was found to be expressed at increased levels in skin from individuals with NF1, regardless of whether it was adjacent to a neurofibroma. This suggests the possibility of using MK expression as a diagnostic marker, an idea that is being subjected to further test. Dr. Jonathan Epstein (University of Pennsylvania) presented his work on the origin of the cardiac defect in Nf1-/- mice, presenting evidence for abnormal epithelial-mesenchymal transformation. Dr. Camilynn Brannan (University of Florida, Gainesville) presented a poster proposing the alternative explanation that cardiac development is retarded in Nf1-/- mice, leading to a discrepancy of development of the heart and the rest of the animal.

The next session, chaired by Dr. James Gusella (Harvard/MGH), was devoted to the molecular genetics of NF2. Gusella provided a summary of current understanding of the function of merlin and other ERM proteins. Dr. Vijaya Ramesh (Harvard/MGH) reported that merlin co-localizes with actin filaments in cellular projections and membrane ruffles, similar to other ERM proteins. She described a protein NHE-RF, involved in Na+/H+ exchange, that interacts with merlin. Another protein that interacts with ERM proteins, designated EBP50, was described by Dr. David Reczek (Cornell University) and Dr. Stephan Pulst (UCLA/Cedars Sinai) reported that schwannomin (merlin) interacts with bII-spectrin. Dr. Brooke McCartney (Duke University) presented findings with Drosophila NF2 mutants. The phenotype is one of overproliferation of cells. Another gene, expanded, leads to enhancement of the NF2 mutant phenotype when both genes are mutated. Expanded encodes a product with a protein 4.1 domain that co-localizes with merlin. Dr. Gareth Evans (University of Manchester, UK) presented data on NF2 mutation analysis in individuals with unilateral vestibular schwannomas. About 10% of individuals with NF2 presented with unilateral VS and had a significant delay before their contralateral tumor presented, and about 5% of individuals with unilateral VS were found to have germline NF2 mutations.

Dr. Nancy Ratner (University of Cincinnati) moderated the next session, devoted to the cell biology of NF2. She reported that schwannoma cells in culture display increased proliferation and spreading on a matrix compared with normal Schwann cells. The tumor cells also have a disorganized actin cytoskeleton and aberrant membrane ruffling. She presented evidence that the Rho and Rac pathways are activated in schwannoma cells. Dr. Dennis LaJeunesse (Duke University) continued the discussion of Drosophila merlin, describing studies that have revealed a

region in the protein that is critical for its subcellular localization. Several of the speakers described interactions of merlin with itself and other proteins. Dr. Wallace Ip (University of Cincinnati) pointed out that merlin isoform I interacts with the active form of ezrin, whereas isoform II interacts with either active or dormant forms of ezrin. Merlin-merlin interactions are strongest between the two isoforms in either head-tail or tail-tail configurations. Dr. Markku Sainio (University of Helsinki, Finland) mapped regions of merlin required for interaction with ezrin and pointed out that, unlike ezrin, merlin does not require activation to self-associate. Dr. David Gutmann (Washington University, St. Louis) presented evidence that merlin interacts with RhoA, CD44, and focal adhesion kinase, as well as with actin. Dr. Reuben Shaw (MIT) reported that the non-phosphorylated form of merlin may be the active form of the protein and that RhoA is critical for control of this phosphorylation.

The next session focused on animal models of NF1 and NF2, and was chaired by Dr. Tyler Jacks (MIT). Dr. Luis Parada (University of Texas, Southwest) has created a mouse model in which inactivation of NF1 is controlled by Cre-mediated recombination. Sensory neurons with inactivated Nf1 become neurotrophin-independent, consistent with a postulated regulatory role of Nf1 in the response to neurotrophins. Dr. Kristine Vogel (Louisiana State University) has found that Nf1-/- sensory neurons in culture innervate both normal targets and tissues that are not normal targets, in contrast with wild type neurons that only innervate normal targets. Dr. Alcino Silva (Cold Spring Harbor Laboratory) reported on learning in Nf1 mutant mice. Nf1 mutant animals that also harbor an N-Ras mutation show normal learning, and the Nf1 mutant learning phenotype can be rescued by treatment with farnesyl protein transferase inhibitors. These findings support a role of the Ras pathway in the learning deficit in Nf1 mutant mice. Dr. Camilynn Brannan (University of Florida, Gainesville) reported that mice lacking the alternatively spliced exon 23a develop apparently normally. Dr. Radhika Atit (University of Cincinnati) has shown that Nf1+/- mice display abnormal wound healing, with abnormal collagen deposition. NF2 mouse models were described by Dr. Marco Giovannini (CEPH Fondation Jean Dausset, France) and Dr. Andrea McClatchey (Harvard/MGH). Giovannini has used the Cre-lox system to develop an Nf2 mutant that is expressed in developing Schwann cells. A non-frameshift N-terminal deletion produces peripheral nerve tumors and Schwann cell hyperplasia, whereas a C-terminal mutation does not. This has suggested a possible dominant negative role for Nf2 mutations in causing schwannomas. McClatchey reported that Nf2 +/- mice develop osteosarcomas, fibrosarcomas, and hepatocellular carcinomas, in contrast with the human disorder. Nf1 and p53 mutations are cooperative with Nf2 mutations in producing tumors. Malignant peripheral nerve sheath tumors have been seen in Nf2/Nf1 double mutants.

The final session, chaired by Dr. Jackson Gibbs (Merck & Co.) was dedicated to experimental therapeutics. Gibbs provided an update on progress in the development of farnesyl protein transferase inhibitors. He pointed out that these inhibitors are not specific for RAS; among RAS proteins, Ha-RAS is most sensitive, but may be of lesser relevance in malignancies. It is hoped that these drugs will act along with other chemotherapeutic agents. Phase I clinical trials have begun (although not in patients with NF1). Dr. Jon Holmlund (Isis Pharmaceuticals) described the use of antisense oligonucleotides against c-raf and Ha-RAS. Clinical trials have also begun for these agents. Dr. Patricia Molloy (University of Pennsylvania) reported on results of the clinical trial of interferon alpha 2a and cis-retinoic acid in the treatment of plexiform neurofibromas. No objective instances of tumor shrinkage were found, although there were some instances of subjective improvement or improvement in function. She stressed the need for more information on natural history and the need for a control group in assessing clinical outcomes. She also described a study of the use of PET scanning to evaluate astrocytomas in NF1, pointing out that levels of glucose uptake correlated with malignant behavior. Dr. Kevin Shannon (University of California, San Francisco) reported on his work with leukemias in Nf1+/- mice. He presented evidence that GM-CSF stimulation of hemotopoeitic precursors acts via the Ras pathway. The rate of development of leukemia in mutant mice was increased by treatment with the alkylating agent cyclophosphamide.

Overall, a number of important themes emerged from the meeting. First, studies of the mutations

responsible for NF1 and NF2 have been hampered by the wide diversity of mutation types seen. Progress has been made in identifying genotype-phenotype correlations in NF2, but this has been more difficult in NF1, perhaps due to the large size of the gene and the complexity of the NF1 phenotype. Several laboratories have demonstrated the need for a multimodal approach to mutation detection in NF1, which will permit a larger proportion of mutations to be identified. It remains to be seen if this will result in any prediction of specific NF1 complications, and whether a clinically useful diagnostic test can be devised. A second theme concerns the interaction of NF1 and NF2 proteins with other proteins. Both are involved in complex signaling pathways, the components of which are being elucidated. In this instance complexity may be seen as an advantage, since it offers many potential points for pharmacological intervention. A third theme is the need to identify the physiological basis for the clinical phenotypes of NF1 and NF2. For years our knowledge of neurofibromatosis was based on observation of the disorder in people, which stimulated interest in studies in animals and cell culture systems. Now, as information is coming in from these experimental systems there is a need to return to the clinic and sharpen the observations to relate knowledge gained in the laboratory with the clinical behavior of the disorder. This meeting has brought together both clinical and laboratory scientists who are working synergistically to understand the basis for the features of neurofibromatosis and develop therapeutic approaches.