6-11-2012

Antioxidant Rescue of Nf1/Ras-Induced Myelin and Vasculature Dysfunction

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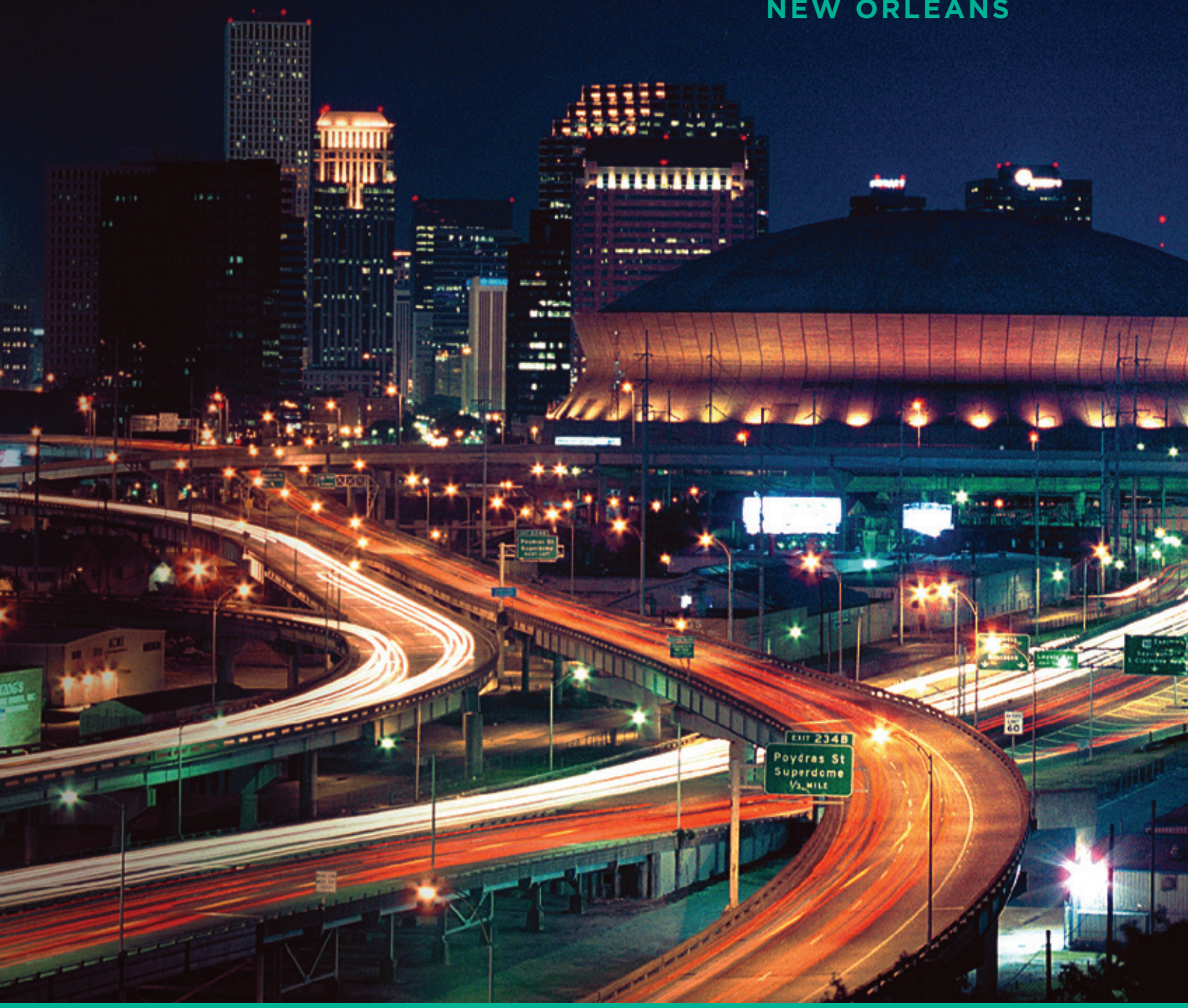
PROGRAM BOOK

Children's Tumor Foundation

CONFERENCE 2012

JUNE 9-12 · HILTON NEW ORLEANS HOTEL
NEW ORLEANS



CHILDREN'S TUMOR FOUNDATION
95 PINE STREET, 16TH FLOOR, NEW YORK NY 10005 | WWW.CTF.ORG | 212.344.6633

Children's Tumor Foundation

CONFERENCE 2012

Dear NF Conference Attendees:

On behalf of the Children's Tumor Foundation, welcome to the 2012 NF Conference. We are very pleased that you could join us in beautiful, historic New Orleans for this important event. It is an honor to have over 300 doctors, scientists, clinicians, and researchers in attendance to present the latest developments in neurofibromatosis (NF) research and clinical care.

This year marks the first time that the NF Conference, a convention of the foremost experts in neurofibromatosis, and the NF Forum, a patient and family symposium at which attendees learn more about the disorder and develop supportive, personal relationships, will be held in conjunction. This will provide an unprecedented opportunity for the Forum to hold family-targeted sessions, benefiting from the presence of many international physicians, researchers, and clinicians, sharing the latest in medical advancements.

Five years ago we developed a strategic plan that addressed the vital need to accelerate the pace of NF research, to find effective treatments for neurofibromatosis, and to improve the lives of those living with the disorder. In order to accomplish this, we identified specific goals to target our resources: fund preclinical testing of drug candidates; establish a nationwide network of NF clinics; establish a tissue repository; identify new NF biomarkers; and support pilot clinical trials.

Much of what we set out to accomplish has been successfully addressed: there are now 26 ongoing NF-specific clinical trials; we are now funding preclinical testing of 50 candidate drugs through our Drug Discovery Initiative and NF Preclinical Consortium; the NF Clinic Network now has 44 members, and growing, and is seeing a record number of patients; we are funding three pilot clinical trials and improving trial design through our Clinical Research Awards; and we are launching the NF Patient Registry, which is part of a larger effort by the National Institutes of Health to create a rare disease registry. CTF will collaborate with the scientific and clinical community to identify drug candidates, make connections to industry, and liaise where necessary to further diminish the time it takes treatments to leave the laboratory and arrive at the clinic.

As we look forward to the next five years, our ambitious new strategic plan will commit $30 million to build upon the successes of the past five years, focusing on the implementation of translational research that will lead to the identification of even more rational clinical candidates. Our mission has not changed, and our goal remains the same - to find effective treatments that will significantly improve the lives of those with NF.

Thank you again for attending and enjoy the conference!

John Risner
President

Annette Bakker
Chief Scientific Officer

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The Friedrich von Recklinghausen Award: Neurofibromatosis Tradition and Progress

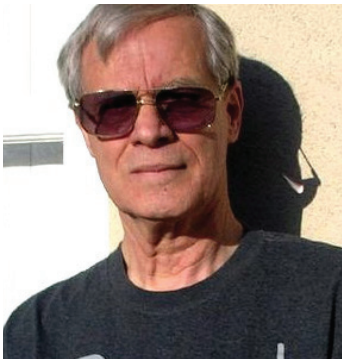
The Children's Tumor Foundation's Friedrich von Recklinghausen Award is given to individuals in the professional neurofibromatosis community who have made significant contributions to neurofibromatosis research or clinical care. Named after Friedrich Daniel von Recklinghausen (1833-1910) the German physician who first described 'von Recklinghausen's disease' – what we now know as neurofibromatosis type 1.

2012 Friedrich von Recklinghausen Award Recipient

We are delighted to announce that the recipient of the 2012 Friedrich von Recklinghausen Award is David H. Gutmann, MD, PhD (Washington University). Dr. Gutmann has a long-standing and preeminent role in neurofibromatosis research and clinical care, and has long been involved with the Children's Tumor Foundation.

Dr. Gutmann was nominated for the Award by his colleagues in the neurofibromatosis research community and will be presented with the Award at the 2012 NF Conference on the evening of June 8th at the Welcome Dinner and Reception.

First awarded by the Foundation in 1989, the initial recipients of the Friedrich von Recklinghausen Award included CTF's Chair of Medical Affairs, Dr. Bruce Korf (University of Alabama), Dr. John Carey (University of Utah) and current National Institutes of Health Director, Dr. Francis Collins. CTF did not issue the Friedrich von Recklinghausen Award for a number of years, but revived the tradition in 2008. The following are the most recent recipients of the Award, identified through a public nomination process:



2008
Vincent 'Vic' Riccardi, M.D.,
The Neurofibromatosis Institute

2009
Luis Parada, Ph.D.,
University of Texas Southwestern

2010
Nancy Ratner, Ph.D.,
Cincinnati Children's Hospital Medical Center

FOUNDATION STAFF

Children's Tumor Foundation

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**at NF Conference*

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Children's Tumor Foundation National Programs

NF Walk Program (www.nfwalk.org)

The Children's Tumor Foundation NF Walk Program was established in 2009 as a national fundraising effort to support neurofibromatosis research, raise awareness, and provide support for individuals with NF and their families. A key feature of the Walk program is that it is a community-based event organized by local volunteers, which offers the opportunity for individuals, their families, friends, neighbors, and organizations to join together, through a truly enjoyable event, in the fight against NF. **Every Step Makes a Difference.**

NF Endurance Program (www.nfendurance.org)

NF Endurance offers the opportunity for individuals to participate in marathons, triathlons, bike races, and other high endurance sporting events to raise money for research, promote awareness, and provide a network of caring support for those living with NF and their families. **NF Endurance: iNFinite possibilities.**

Racing4Research (www.racing4research.org)

Racing4Research (R4R) utilizes competitive, professional auto racing as a vehicle to increase awareness of neurofibromatosis and raise funds for research through corporate sponsorship, personal donations, and individual fundraising by NF Heroes: children and adults from around the country who live with neurofibromatosis. The program offers children and families living with the disorder a uniquely empowering weekend, and has raised over $2 million dollars since its inception five years ago. **Fuel the Cure.**

Cupid's Undie Run (www.cupidsundierun.com)

Putting the hilarity back in charity, Cupid's Undie Run is an entirely volunteer-organized fundraiser that exists to benefit the Children's Tumor Foundation. It began in Washington D.C. in 2010 and has grown from a $10,000 event with 400 participants to 3,000 runners in six cities who raised nearly $400,000 in 2012. Cupid's is dedicated to fundraising for the Children's Tumor Foundation through fun, but "PG-13" means. "**Fewer Clothes, More Funds!**"

Schedule At-A-Glance

	TIME		EVENT	LOCATION
FRIDAY JUNE 8	2:00 PM	6:00 PM	Registration	Grand Ballroom A (1st Floor)
SATURDAY JUNE 9	8:00 AM	1:00 PM	Registration	Grand Ballroom A (1st Floor)
	9:30 AM	9:40 AM	Welcoming Remarks: *John W. Risner, President*	Grand Ballroom A (1st Floor)
	9:40 AM	9:55 AM	CTF Updates: Annette Bakker, Ph.D., *Chief Scientific Officer*	Grand Ballroom A (1st Floor)
	10:05 AM	11:00 AM	**KEYNOTE 1**: Luis Parada, Ph.D., *University of Texas Southwestern*	Grand Ballroom A (1st Floor)
	11:00 AM	1:00 PM	**SESSION 1: Deciphering the Signaling Pathways of NF2**	Grand Ballroom A (1st Floor)
	1:00 PM	2:30 PM	Lunch (On your own)	
	2:30 PM	4:00 PM	**SESSION 2: Genetics of Schwannomatosis: An Update**	Grand Ballroom A (1st Floor)
	4:00 PM	6:00 PM	**SESSION 3A Concurrent: Optic Pathway Tumors**	Grand Ballroom A (1st Floor)
	4:00 PM	6:00 PM	**SESSION 3B Concurrent: Massively Parallel Sequencing – Massively Parallel Results**	Grand Salon A (1st Floor)
	6:00 PM	7:00 PM	Poster Session & Reception with NF Family Forum	Hilton Exhibition Center A (2nd Floor)
	7:00 PM	9:00 PM	NF Conference/Forum Awards Dinner	Napoleon Ballroom (3rd Floor)
SUNDAY JUNE 10	7:00 AM	9:45 AM	NF Historic Walking Tour: With NF Family Forum Participants	Hilton Hotel Entrance
	10:05 AM	11:00 AM	**KEYNOTE 2**: Rhona Mirsky, Ph.D., *University College London, United Kingdom*	Grand Ballroom A (1st Floor)
	11:00 AM	1:10 PM	**SESSION 4: Deciphering the Signaling Pathways of NF2**	Grand Ballroom A (1st Floor)
	1:10 PM	3:00 PM	**POSTER SESSION I (Clinical)**: Lunch (box lunch to be served)	Hilton Exhibition Center A (2nd Floor)
	3:00 PM	6:00 PM	**SESSION 5: Therapeutic Frontiers 1: NF1 Mouse Models & Preclinical Study Development**	Grand Ballroom A (1st Floor)
	6:00 PM	6:30 PM	Break	Grand Salon B (1st Floor)
	6:30 PM	8:00 PM	**SESSION 6A Concurrent Abstract Platform Presentations: Clinical**	Grand Salon A (1st Floor)
	6:30 PM	8:00 PM	**SESSION 6B Concurrent: Abstract Platform Presentations: Basic Research**	Grand Ballroom A (1st Floor)
MONDAY JUNE 11	8:00 AM	8:45 AM	**KEYNOTE 3**: Silvia Cappello, Ph.D., *Helmholtz Zentrum München & Inst. for Stem Cell Research, Germany*	Grand Ballroom A (1st Floor)
	8:45 AM	11:35 AM	**SESSION 7A Concurrent: NF and the Brain**	Grand Salon A (1st Floor)
	8:45 AM	11:30 AM	**SESSION 7B Concurrent: Therapeutic Frontiers 2: NF2 Mouse Models and Preclinical Study Development**	Grand Ballroom A (1st Floor)
	11:30 AM	12:00 PM	Break (Box Lunch to be Provided)	Grand Salon B (1st Floor)
	12:00 PM	1:00 PM	**KEYNOTE 4**: René Bernards, Ph.D., *Netherlands Cancer Institute, The Netherlands*	Grand Ballroom A (1st Floor)
	1:00 PM	2:30 PM	**SESSION 8: Management of NF: Panel Discussion** – (Box Lunch Provided)	Grand Ballroom A (1st Floor)
	2:30 PM	5:00 PM	**SESSION 9: NF2 and NF1 Clinical**	Grand Ballroom A (1st Floor)
	5:00 PM	5:30 PM	Break	Grand Salon B (1st Floor)
	5:30 PM	7:30 PM	**SESSION 10A Concurrent: Brainstorming – Speeding up Interventions for NF2**	Grand Ballroom A (1st Floor)
	5:30 PM	7:10 PM	**SESSION 10B Concurrent: Vascular Anomalies in NF**	Grand Salon A (1st Floor)
	7:10 PM	8:30 PM	Dinner Break (on your own)	
	8:30 PM	10:00 PM	**POSTER SESSION II (Basic Research)**	Hilton Exhibition Center A (2nd Floor)
TUESDAY JUNE 12	8:00 AM	8:45 AM	**KEYNOTE 5**: Jan Tuckermann, Ph.D., *University of Ulm & Leibniz Institute for Age Research, Germany*	Grand Ballroom A (1st Floor)
	8:45 AM	11:15 AM	**SESSION 11A Concurrent: NF1 Bone Abnormalities**	Grand Salon A (1st Floor)
	8:45 AM	11:15 PM	**SESSION 11B Concurrent: Cell Biology and Pathobiology of Schwannomatosis and Pain**	Grand Ballroom A (1st Floor)
	11:15 PM	1:00 PM	Lunch (on your own)	
	1:00 PM	3:30 PM	**SESSION 12 : Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS)of Schwannomatosis and Pain**	Grand Ballroom A (1st Floor)
	3:30 PM	4:00 PM	Break	Grand Salon B (1st Floor)
	4:00 PM	6:30 PM	**SESSION 13: Therapeutic Frontiers 3: Preclinical Studies Guiding Development of Novel Agents**	Grand Ballroom A (1st Floor)
	8:00 PM	10:30 PM	Closing Reception at Jazz Club	The Bombay Club

Important Notes to Chairs, Speakers & Poster Presenters

Important Notes to Speakers, Chairs & Poster Presenters

NOTE TO SPEAKERS

- Bring your slides to the meeting on a flash drive. DO NOT bring your own laptop. You will be notified by Foundation staff at registration as to when to bring your slides to the audiovisual staff.
- Be available by the podium 30 minutes before the start of the session in which you are speaking to understand audiovisual setup and make sure your slideshow is running smoothly.
- Check your time allotment on the agenda. Be prepared to complete your talk in the time-frame given on the agenda.
- If you run over time, you may be 'cut off' briefly summarize what you see as the key 'take home' points of the session.

NOTE TO SESSION CHAIRS

- Please stand by the podium 30 minutes before start of session you are chairing to ensure speakers have arrived, go through audiovisual setup, etc.
- It is your responsibility to convene your session PROMPTLY per the schedule.
- Introduce speakers by name and affiliation; and whether they are keynotes, invited speakers or selected abstract speakers. If they are CTF awardees (indicated on the agenda), please mention so in the introduction.
- Introduce the keynote speaker in more detail, by current affiliation, career, etc. (Their biosketch can be found on their abstract page.)
- It is your responsibility to keep your speakers ON TIME. Visual prompts (clock, lights) will be given; you are also encouraged to give a 3-minute warning.
- When fielding questions from the audience, have the audience member identify him/herself, and ensure they speak into a microphone.
- At the close of the session, please briefly summarize what you see as the key 'take home' points of the session.

PREPARING A SUMMARY OF YOUR SESSION

The meeting co-chairs will be assembling a report from the Conference that can translate into a publication after the meeting. Session co-chair(s) are requested to collaborate on providing a **one to two page summary of your session**. This should be succinct but sufficiently comprehensive to be meaningful. You are encouraged to liaise with your session speakers in putting this together. If there are critical references you want to mention please include the citation for the reference.

NOTE TO POSTER PRESENTERS

- Posters will be on display throughout the Conference from Friday, June 8th to Tuesday, June 12th
- Even number (Clinical) poster presenters will need to stand by their posters on Sunday (June 10th) 1:10 – 3:00 PM.
- Odd number (Basic Research) poster presenters will need to stand by their posters on Monday (June 11th) 8:30 – 10:00 PM.
- Pushpins will be provided!
- Posters can be set-up on Friday, June 10th; it should be on display for the entire duration of the Conference

Questions?

Please contact a Foundation staff member!

AGENDA

Friday · June 8, 2012

2:00 PM	**6:00 PM**	Registration	Grand Ballroom A Pre-Function (1st Floor)

Saturday · June 9, 2012

8:00 AM	**1:00 PM**	Registration	Grand Ballroom A Pre-Function (1st Floor)
9:30 AM	**9:40 PM**	John W. Risner: Welcoming Remarks *CTF President*	Grand Ballroom A (1st Floor)
9:40 AM	**9:55 PM**	Annette Bakker, Ph.D.: CTF Updates *Chief Scientific Officer*	
9:55 AM	**10:05 AM**	2012 NF Conference Chairs: Welcome Opening	
KEYNOTE PRESENTATION 1:		New Lessons from Old Models	
10:05 AM	**11:00 AM**	**Luis Parada, Ph.D.**, *University of Texas Southwestern*	
SESSION 1:		Deciphering the Signaling Pathways of NF2 (1)	Grand Ballroom A (1st Floor)
Chairs: **Cristina Fernandez-Valle, Ph.D.**, *University of Central Florida* & **Minja Pehrsson, Ph.D.**, *Biomedicum Helsinki, Finland*			
11:00 AM	**11:25 AM**	Pilot NF2 High-Throughput Screening Using Merlin-null Schwann Cells **Cristina Fernandez-Valle, Ph.D.**, *University of Central Florida*	
11:25 AM	**11:50 AM**	Merlin's Tumor Suppressor Function **Wei Li, Ph.D.**, *Memorial Sloan-Kettering Cancer Center*	
11:50 AM	**12:15 PM**	Integrin and Axl signalling in human schwannoma **Sylwia Ammoun, Ph.D.**, *University of Plymouth, United Kingdom*	
12:15 PM	**12:40 PM**	Merlin-p120 RasGAP complex mediates contact inhibition of growth **Susanne Schirmer, Ph.D.**, *Leibniz Institute for Age Research, Germany*	
12:40 PM	**1:00 PM**	The role of Merlin in Microtubule Based Vesicular Transport **Robert Hennigan, Ph.D.**, *Cincinnati Children's Hospital*	
1:00 PM	**2:30 PM**	Lunch (on your own)	Grand Salon B (1st Floor)
SESSION 2:		Genetics of Schwannomatosis: An Update	Grand Ballroom A (1st Floor)
Chairs: **D. Gareth Evans, M.D.**, *St. Mary's Hospital/University of Manchester, United Kingdom* & **Allan Belzberg, M.D.**, *Johns Hopkins University*			
2:30 PM	**2:45 PM**	An update on the frequency and spectrum of SMARCB1 mutations in schwannomatosis **Miriam Smith, Ph.D.**, *University of Manchester*	
2:45 PM	**3:00 PM**	SMARCB1 mutational spectrum in schwannomatosis **Laura Papi, M.D., Ph.D.**, *University of Florence, Italy*	
3:00 PM	**3:15 PM**	Evaluation of DNA methylation and subchromosomal structural rearrangements in schwannomatosis cases with no first hit mutations in the SMARCB1 gene **Arkadiusz Piotrowski, Ph.D.**, *University of Alabama*, Current CTF YIA Recipient	
3:15 PM	**3:45 PM**	The clinical and molecular heterogeneity of schwannomatosis **Gareth Evans, M.D.**, *University of Manchester, United Kingdom*	

Saturday · June 9, 2012

SESSION 3: CONCURRENT SESSIONS

SESSION 3A Concurrent: Optic Pathway Tumors

Grand Ballroom A (1st Floor)

Chairs: **Michael Fisher, M.D.**, *Children's Hospital of Philadelphia* & **Joshua Rubin, M.D., Ph.D.**, *Washington University School of Medicine*

4:00 PM	**4:20 PM**	Clinical and biological implications of non NF1 RAS/MAPK alterations in pediatric low grade gliomas **Uri Tabori, M.D.**, *University of Toronto*
4:20 PM	**4:40 PM**	Mouse models of NF1-associated optic glioma **David Gutmann, M.D., Ph.D.**, *Washington University*
4:40 PM	**5:00 PM**	Genetic Modifiers of Glioma Risk in NF1 **Josh Rubin, M.D., Ph.D.**, *Washington University*
5:00 PM	**5:20 PM**	Clinical Trials for Optic Pathway Gliomas **Giorgio Perilongo, M.D.**, *University Hospital of Padua, Italy*
5:20 PM	**5:40 PM**	Visual Outcomes Following Chemotherapy for NF1-Associated Optic Pathway Glioma **Michael Fisher, M.D.**, *Children's Hospital of Philadelphia*
5:40 PM	**6:00 PM**	Hand-Held Optical Coherence Tomography During Sedation Detects Visual Acuity and Visual Field Loss in Young Children with Optic Pathway Gliomas **Rob Avery, D.O., MSCE** (Abstract Platform Presentation), *Children's National Medical Center*

SESSION 3B Concurrent: Massively Parallel Sequencing - Massively Parallel Results

Grand Salon A (1st Floor)

Chairs: **Douglas Stewart, M.D.**, *National Cancer Institute* & **Eric Legius, M.D., Ph.D.**, *Catholic University of Leuven, Belgium*

4:00 PM	**4:30 PM**	From Tissue to Treatment in a Single Trial: Early Lessons from Molecularly Informed Trials in Pediatric Oncology **Melinda Merchant, M.D., Ph.D.**, *National Cancer Institute*
4:30 PM	**4:50 PM**	Next-Generation Sequencing of NF1 Tumors **Douglas Stewart, M.D.**, *National Cancer Institute*
4:50 PM	**5:10 PM**	How to find the genetic cause of a rare form of neurofibromatosis? **Eric Legius, M.D., Ph.D.**, *Catholic University of Leuven, Belgium*
5:10 PM	**5:30 PM**	Benefits and pitfalls of next generation sequencing in the diagnosis of single gene disorders **Gareth Evans, M.D.**, *University of Manchester, United Kingdom*

6:00 PM **7:00 PM** Poster Session & Reception with NF Family Forum — Hilton Exhibition Center A (2nd Floor)

NF Conference / Forum Awards Dinner

Napoleon Ballroom (3rd Floor)

7:00 PM - 9:00 PM

AGENDA

Sunday · June 10, 2012

NF Historic Walking Tour: With NF Family Forum Participants

7:00 - 9:45 AM

Hilton Hotel Front Entrance

KEYNOTE PRESENTATION 2: The Schwann cell lineage: potential and plasticity — Grand Ballroom A (1st Floor)

10:05 AM – 11:00 AM **Rhona Mirsky, Ph.D.**, *University College London, United Kingdom*

SESSION 4: Deciphering the Signaling Pathways of NF2 (2) — Grand Ballroom A (1st Floor)

Chairs: **Joe Kissil, Ph.D.**, *Wistar Institute* & **Susanne Schirmer, Ph.D.**, *Leibniz Institute for Age Research, Germany*

11:00 AM	**11:30 AM**	Novel functions of Merlin at the cell membrane **Joe Kissil, Ph.D.**, *Wistar Institute*
11:30 AM	**11:55 AM**	Screening for new Hippo tumour suppressor pathway regulators **Nicolas Tapon, Ph.D.**, *London Research Institute*
11:55 AM	**12:20 PM**	Regulation of the Hippo pathway by cell polarity **Georg Halder, M.D.**, *MD Anderson Cancer Center*
12:20 PM	**12:45 PM**	NF2 and Hippo signaling in normal growth and tumorigenesis **DJ Pan, Ph.D.**, *Johns Hopkins University*
12:45 PM	**1:10 PM**	mTORC1 and mTORC2 signaling in human meningeal and Schwann cells with NF2 loss **Vijaya Ramesh, Ph.D.**, *Harvard Medical School/Massachusetts General Hospital*

1:10 PM – 3:00 PM Poster session I (Clinical): Lunch (box lunch to be served) — Hilton Exhibition Center A (2nd Floor)

SESSION 5: Therapeutic Frontiers 1: NF1 Mouse Models & Preclinical Study Development — Grand Ballroom A (1st Floor)

Chairs: **Nancy Ratner, Ph.D.**, *Cincinnati Children's Hospital Medical Center* & **D. Wade Clapp, M.D., Ph.D.**, *Indiana University*

3:00 PM	**3:30 PM**	Efforts toward curing NF1 and Ras-Driven Nervous System Pathology **Nancy Ratner, Ph.D.**, *Cincinnati Children's Hospital Medical Center*
3:30 PM	**4:00 PM**	Sex-specific modifiers of NF1 malignancies and implications for therapy **Karlyne Reilly, Ph.D.**, *National Cancer Institute*
4:00 PM	**4:30 PM**	Modelling NF1 in the mouse **Allison Lloyd, Ph.D.**, *University College London, United Kingdom*
4:30 PM	**5:00 PM**	Identification of therapeutic windows for NF1-associated diseases in mouse models **Yuan Zhu, Ph.D.**, *University of Michigan*
5:00 PM	**5:30 PM**	A central role for PTEN and ERbB2 in a novel genetically engineered mouse model of malignant peripheral nerve sheath tumour **Paul Brennan, M.D.**, *Western General Hospital*
5:30 PM	**6:00 PM**	Creation and use of iPS cells from NF1 mice **Jonathan Chernoff, M.D., Ph.D.**, *Fox Chase Cancer Center*

Sunday · June 10, 2012

6:00 PM **6:30 PM** Break — Grand Salon B (1st Floor)

SESSION 6: CONCURRENT ABSTRACT PLATFORM SESSIONS

SESSION 6A Concurrent: Abstract Platform Presentations: Clinical

Grand Salon A (1st Floor)

Chairs: **Robert Hennigan, Ph.D.**, *Cincinnati Children's Hospital Research Center*, **Cristina Fernandez-Valle, Ph.D.**, *University of Central Florida*, **Bruce Korf, M.D., Ph.D.**, *University of Alabaman at Birmingham* and **Kathryn North, M.D.**, *The Children's Hospital at Westmead, Australia*

6:30 PM **6:45 PM** The Baser Criteria for Diagnosis of NF2: Putting it to the test
Amanda Bergner, M.S., CGC, *Johns Hopkins Comprehensive Neurofibromatosis Center*

6:45 PM **7:00 PM** Preliminary results of a phase II trial of Pegylated Interferon-Alfa-2B(PI) in pediatric patients with documented progression of neurofibromatosis type1-related unrespectable plexiform neurofibromatosis (PNF)
Eva Dombi, M.D., *National Institutes of Health*

7:00 PM **7:15 PM** Computerized cognitive training for children with Neurofibromatosis Type 1 (NF1): A pilot study
Kristina Hardy, Ph.D., *The Jennifer and Daniel Gilbert Neurofibromatosis Institute*

7:15 PM **7:30 PM** Natural History of Plexiform Neurofibromas in NF1
Bruce Korf, M.D., Ph.D., *University of Alabaman at Birmingham*

7:30 PM **7:45 PM** Clinical Features of Schwannomatosis A Retrospective Analysis of 87 Patients
Vanessa Merker, *Massachusetts General Hospital / Harvard Medical School*

7:45 PM **8:00 PM** Progression rate of plexiform neurofibromas after surgery – a retrospective study in 52 patients with nerofibromatosis type 1
Rosa Nguyen, M.D., *University Medical Center Hamburg-Eppendorf, Germany*

SESSION 6B Concurrent: Abstract Platform Presentations: Basic Research

Grand Ballroom A (1st Floor)

Chairs: **Andre Bernards, Ph.D.**, *Massachusetts General Hospital / Harvard Medical School* & **Andrea McClatchey, Ph.D.**, *Massachusetts General Hospital / Harvard Medical School*

6:30 PM **6:45 PM** Anti-Tumor Activity of MAPK and mTOR Inhibitors in a Novel Mouse Model of NF1-Mutant Soft-Tissue Sarcoma
Rebecca Dodd, Ph.D., *Duke University*

6:45 PM **7:00 PM** Merlin regulates expression levels and downstream signaling of multiple transmembrane receptors including $p75^{NTR}$
Augusta Fernando, Ph.D., *University of Iowa*

7:00 PM **7:15 PM** Merlin targets the Cdh1/fzr inhibitor Rae1 to regulate mitosis and establish organ size homeostasis
Maryam Jahanshahi, *Mount Sinai School of Medicine*, Current CTF YIA Recipient

7:15 PM **7:30 PM** Aurora A as a therapeutic target against MPNST
Pooja Mohan, *University of British Columbia (UBC), Canada*

7:30 PM **7:45 PM** A Genetic Modifier Screen Implicates Specific Tyrosine Kinase and Neuropeptide Signaling Pathways in the Drosophila Neurofibromatosis-1 Growth Deficiency
James Walker, Ph.D., *Massachusetts General Hospital / Harvard Medical School*

7:45 PM **8:00 PM** Co-targeting the PI3K/AKT/mTOR and MAPK Pathways in a Novel Mouse Model of Malignant Peripheral Nerve Sheath Tumors
Adrienne Watson, *University of Minnesota*, Current CTF YIA Recipient

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KEYNOTE PRESENTATION 3: A new mouse model of 'double-cortex' – the role of RhoA in cortical development — Grand Ballroom A (1st Floor)

8:00 AM **8:45 AM** **Silvia Cappello, Ph.D.**, *Helmholtz Zentrum München & Inst. for Stem Cell Research, Germany*

SESSION 7: CONCURRENT SESSIONS

SESSION 7A Concurrent: NF and the Brain — Grand Salon A (1st Floor)

Chairs: **Maria Acosta, M.D.**, *Children's National Medical Center* & **David Gutmann, M.D., Ph.D.**, *Washington University School of Medicine*

8:45 AM **9:05 AM** Learning and memory in children with NF1
Kathryn North, M.D., Ph.D., *Children's Hospital Westmead, Australia*

9:05 AM **9:35 AM** Reduced HCN channel function is underlying GABA-ergic dysfunction in NF1 mice
Ype Elgersma, Ph.D., *Erasmus University Medical Center, The Netherlands*

9:35 AM **9:55 AM** Social Cognition in NF1: Dissecting the phenotype
Maria Acosta, M.D., *Children's National Medical Center*

9:55 AM **10:25 AM** The search for brain biomarkers of NF1-related cognitive deficits: intrinsic functional connectivity
F. Xavier Castellanos, M.D., *Nathan Kline Institute*

10:25 AM **10:55 AM** Defining the Molecular and Cellular Etiology for NF1 Attention Deficits in Mice
David Gutmann, M.D., Ph.D., *Washington University*

SESSION 7B Concurrent: Therapeutic Frontiers 2: NF2 Mouse Models & Preclinical Study Development — Grand Ballroom A (1st Floor)

Chairs: **Marco Giovannini, M.D., Ph.D.**, *House Research Institute* & **Sylvia Ammoun, Ph.D.**, *Peninsula College of Medicine and Dentistry, United Kingdom*

8:45 AM **9:15 AM** Translating NF2 preclinical studies into clinical trials
Marco Giovannini, M.D., Ph.D., *House Research Institute*

9:15 AM **9:45 AM** Function of Merlin/ERM proteins in organizing the cell cortex
Andrea McClatchey, Ph.D., *Harvard Medical School*

9:45 AM **10:10 AM** The role of merlin isoform 2 in neurofibromatosis type 2-associated polyneuropathy
Alexander Schulz, *Leibniz Institute for Age Research*

10:10 AM **10:35 AM** A role for Sox10 in the phenotype of Merlin null schwannoma cells
David Parkinson, Ph.D., *Massachusetts General Hospital*

10:35 AM **11:00 AM** Targeting PDGF-R pathway in NF2 from bench to bedside
Oliver Hanemann, M.D., Ph.D., *University of Plymouth, United Kingdom*

11:00 AM **11:25 AM** Targeting metabolic stress as a therapeutic approach against NF2
Toshifumi Tomoda, M.D., Ph.D., *City of Hope*

11:30 AM **12:00 PM** Break (Box lunch to be provided) — Grand Salon B (1st Floor)

KEYNOTE PRESENTATION 4: Functional Genetic Approaches to Guide the Choice of Therapy in Cancer — Grand Ballroom A (1st Floor)

12:00 PM **1:00 PM** **René Bernards, Ph.D.**, *Netherlands Cancer Institute, The Netherlands*

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SESSION 8: Management of NF: Panel Discussion - (Box Lunch Provided) Grand Ballroom A (1st Floor)

Chairs: **Sue Huson, M.D.**, *St. Mary's Hospital/University of Manchester, United Kingdom* & **Ros Ferner, M.D.**, *Guy's and St. Thomas' NHS Foundation Trust, United Kingdom*

1:00 PM **2:30 PM** Patient Management/ UK Clinical network: Tiered care and centralized care
Sue Huson: NF2 · Rosalie Ferner: NF1

SESSION 9: NF2 and NF1 Clinical Grand Ballroom A (1st Floor)

Chairs: **Oliver Hanemann, M.D, Ph.D.**, *Peninsula College of Medicine & Dentistry, United Kingdom* & **Scott Plotkin, M.D., Ph.D.**, *Massachusetts General Hospital / Harvard Medical School*

2:30 PM **2:55 PM** Clinical Progression in NF2
Ashok Asthagiri, M.D., *National Institutes of Health*

2:55 PM **3:20 PM** Molecular Pathology of Schwannomas - Updates
Anat Stemmer-Rachamimov, M.D., *Harvard Medical School*

3:20 PM **3:35 PM** NF2 Clinical Trials with Molecular Targeted Agents
Matthias Karajannis, M.D., *NYU Langone Medical Center*

3:35 PM **3:50 PM** AKT inhibitors in NF2-related Vestibular Schwannomas and Meningiomas
Brad Welling, M.D., Ph.D., *Ohio State University*

3:50 PM **4:05 PM** Guiding the patient with NF2
Jaishri Blakeley, M.D., *Johns Hopkins University*

4:05 PM **4:20 PM** NF: Progress in Clinical Trials and Future Studies
Roger Packer Ph.D., *Children's National Medical Center*

4:20 PM **4:35 PM** Clinical Trial Update on Molecular Targeting Plexiform Neurofibromas at Indiana University
Kent Robertson, M.D., Ph.D., *Riley Hospital for Children*

5:00 PM **5:30 PM** Break (Box lunch to be provided) Grand Salon B (1st Floor)

SESSION 10: CONCURRENT SESSIONS

SESSION 10A Concurrent: Brainstorming – Speeding up Interventions for NF2 Grand Ballroom A (1st Floor)

Moderator: **Anthony Bretscher, Ph.D.**, *Cornell University*

Panelists: **Alison Lloyd, Ph.D.**, *University College London, United Kingdom,* **Larry Sherman, Ph.D.**, *Oregon Health and Science University,* **Victor Mautner, M.D.**, *University Clinic Hamburg-Eppendorf, Germany,* **Jaishri Blakeley, M.D.**, *Johns Hopkins University,* and **Anthony Bretscher, Ph.D.**, *Cornell University.*

5:30 PM **6:00 PM** Merlin controls growth in its open state and phosphorylation converts it to a less-active more-closed state
Anthony Bretscher, Ph.D., *Cornell University*

6:00 PM **6:30 PM** Oncolytic herpes simplex virus vectors to treat neural tumors arising in NF
Samuel Rabkin, Ph.D., *Massachusetts General Hospital / Harvard Medical School*

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SESSION 10B Concurrent: Vascular Anomalies in NF — Grand Salon A (1st Floor)

Chairs: **Nicole Ullrich, M.D., Ph.D.**, *Children's Hospital Boston* & **David Ingram, M.D.**, *Indiana University School of Medicine*

5:30 PM	**5:55 PM**	Insights into the pathogenesis of NF1 vascular disease utlizing mouse models **David Ingram, M.D.**, *Riley Hospital for Children*
5:55 PM	**6:20 PM**	**Jan Friedman, M.D., Ph.D.**, *Child & Family Research Institute*
6:20 PM	**6:45 PM**	Clinical features and management of cerebrovascular disease in NF1 **Nicole Ullrich, M.D., Ph.D.**, *Children's Hospital Boston*
6:45 PM	**7:00 PM**	Moyamoya in Patients with NF1 - Surgical Management and Advances in Biomarker Analysis **Ed Smith, M.D.**, *Children's Hospital Boston / Harvard Medical School*
7:10 PM	**8:30 PM**	Dinner Break (on your own)
8:30 PM	**10:00 PM**	Poster Session II (Basic Research) — Hilton Exhibition Center (2nd Floor)

Tuesday · June 12, 2012

KEYNOTE PRESENTATION 5 Control of bone formation and transformation – New Players emerging — Grand Ballroom A (1st Floor)

8:00 AM	**8:45 AM**	**Jan Tuckermann, Ph.D.**, *University of Ulm & Leibniz Institute for Age Research, Germany*

SESSION 11 CONCURRENT SESSIONS

SESSION 11A Concurrent: NF1 Bone Abnormalities — Grand Salon A (1st Floor)

Chairs: **Elizabeth Schorry, M.D.**, *Cincinnati Children's Hospital* & **David Stevenson, M.D.**, *University of Utah*

8:45 AM	**8:55 AM**	Introduction Clinical Bone phenotype **Betty Schorry, M.D.**, *Cincinnati Children's Hospital*
8:55 AM	**9:20 AM**	Toward an integrated mechanism explaining the NF1 skeletal dysplasiae **Florent Elefteriou, Ph.D.**, *Vanderbilt University*
9:20 AM	**9:45 AM**	Tibial Dysplasia and Pseudarthrosis: Clinical and Translational Aspects **David Little, M.D., Ph.D.**, *Children's Hospital Westmead*
9:45 AM	**10:00 AM**	Defective bone matrix mineralization, not only osteoblast differentiation, may contribute to NF1 pseudoarthrosis (Abstract Platform Presentation) **Jean De La Croix Ndong, Ph.D.**, *Vanderbilt University*
10:00 AM	**10:15 AM**	Loss of neurofibromin increases micro- and macroporosity in cortical bone resulting in diminished mechanical resistance (Abstract Platform Presentation) **Mateusz Kolanczyk, Ph.D.**, *Institut for Medical Genetics*
10:15 AM	**10:30 AM**	The role of osteoclasts in NF1 pseudarthrosis (Abstract Platform Presentation) **Steven Rhodes**, *Indiana University*

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10:30 AM **10:45 AM** Hyperactive transforming growth factor-beta1 signaling and pseudarthrosis in neurofibromatosis type 1 (Abstract Platform Presentation)
Feng-Chun Yang,M.D., Ph.D., *Indiana University*

10:45 AM **11:00 AM** Conditional double inactivation of NF1 in skeletal muscle leads to a severe muscle myopathy with abnormal metabolic function (Abstract Platform Presentation)
Aaron Schindler, Ph.D., *University of Sydney, Australia*

11:00 AM **11:15 AM** Skull defects in patients with neurofibromatosis type 1 (Abstract Platform Presentation)
Daniel Arrington, M.D., *Harvard Medical School / Children's Hospital Boston*

SESSION 11B Concurrent: Cell Biology and Pathobiology of Schwannomatosis and Pain

Grand Ballroom A (1st Floor)

Chairs: **Vijaya Ramesh, Ph.D.**, *Massachusetts General Hospital / Harvard Medical School*, **Larry Sherman, Ph.D.**, *Oregon Health and Science University* & **Jaishri Blakeley, M.D.**, *Johns Hopkins University*

8:45 AM **9:25 AM** Pain phenotypes in mice with Schwann-cell targeted Snf5/Ini1 mutations
Larry Sherman, Ph.D., *Oregon Health and Science University*

9:25 AM **9:50 AM** Mouse model of Snf5-initiated peripheral nerve tumorigenesis
Jeremie Vitte, Ph.D., *House Research Institute*

9:50 PM **10:15 PM** Transcriptional Regulation of inflammatory genes by INI1/hSNF5
Ganjam Kalpana, Ph.D., *Albert Einstein College of Medicine*

Surgery, Therapeutic Opportunities and Non-Operative Approaches

10:15 PM **10:30 PM** The Management of Neuropathic Pain
Sanjog Pangarkar, M.D., *UCLA*

10:30 PM **10:45 PM** Surgical management of Schwannomtosis: good thing or bad thing?
Allan Belzberg, M.D., *Johns Hopkins University*

10:45 PM **11:15 PM** Integrating Whole Body MRI into the management of schwannomatosis patients
Scott Plotkin, M.D., Ph.D., *Massachusetts General Hospital / Harvard Medical School*

11:15 PM **1:00 PM** Lunch (on your own) Grand Salon B (1st Floor)

SESSION 12: Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS)

Grand Ballroom A (1st Floor)

Chairs: **Scott Plotkin, M.D., Ph.D.**, *Massachusetts General Hospital / Harvard Medical School*, & **Eva Dombi, M.D.**, *National Cancer Institute*

1:00 PM **1:30 PM** REiNS International Collaboration: Building Consensus for NF Clinical Trials
Scott Plotkin, M.D., Ph.D., *Massachustetts General Hospital*

1:30 PM **1:50 PM** Using MRI to Identify Active Drugs Against NF-Related Tumors
Eva Dombi, M.D., *National Cancer Institute*

1:50 PM **2:10 PM** Patient Reported Outcomes for NF Clinical Trials: Benefits, Challenges, and Selection of Appropriate Measures
Pam Wolters, Ph.D., *National Cancer Institute*

2:10 PM **2:30 PM** Hearing as a Functional Outcome: Recommended Use in NF2 Clinical Trials
Chris Halpin, Ph.D., *Massachusetts Eye and Ear Infirmary*

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2:30 PM **2:50 PM** Functional Outcome Measures for Clinical Trials for NF1-Associated Optic Pathway Glioma: A Recommendation from REiNS
Michael Fisher, M.D., *Children's Hospital of Philadelphia*

2:50 PM **3:30 PM** Discussion

Grand Salon B (1st Floor)

3:30 PM **4:00 PM** Break

SESSION 13: Therapeutic Frontiers 3: Preclinical Studies Guiding Development of Novel Agents — Grand Ballroom A (1st Floor)

Chairs: **Frank McCormick, Ph.D.**, *University of California, San Francisco* & **Karen Cichowski, Ph.D.**, *Brigham and Women's Hospital / Harvard Medical School*

4:00 PM **4:30 PM** Regulation of Neurofibromin through association with SPRED1
Frank McCormick, Ph.D., *University of California, San Francisco*

4:30 PM **5:00 PM** Exploring the Therapeutic Potential of Selective Kinase Inhibition
Gideon Bollag, Ph.D., *Plexxikon*

5:00 PM **5:30 PM** Oxygen, Scotch Tape and New Brain Tumor Immunotherapy Strategies at the University of Minnesota
Chris Moertel, M.D., *University of Minnesota*

5:30 PM **6:00 PM** Insights from preclinical testing in mouse JMML models
Benjamin Braun, M.D., Ph.D., *University of California, San Francisco*

6:00 PM **6:30 PM** Translating discoveries into the clinic: A discussion of new potential therapies for MPNSTs and the broader translational efforts of the Preclinical Consortium
Karen Cichowski, Ph.D., *Brigham and Women's Hospital / Harvard Medical School*

MEETING ADJOURN

Closing Reception at Jazz Club

The Bombay Club

(7-10 minute walk from the Hilton Hotel)

8:00 PM - 10:30 PM

Please see map and directions at the back of the book.

NF Conference Chairs

Helen Morrison, Ph.D., *Leibniz Institute for Age Research, Germany*

Helen Morrison Ph.D., is a group leader at the Leibniz Institute for Age Research located in Jena Germany (Fritz Lipmann Institute). Her lab is generally interested in the regulation and function of Ras and Ras like small GTPases, with a special emphasis on Ras activity control in proliferation and in the development and maintenance of the central and peripheral nervous system (CNS and PNS). A molecular biologist by training, Dr. Morrison has a longstanding interest in the molecular basis of neurofibromatosis type 2 (NF2) with the aim of identifying therapeutic targets for NF2.

The research focus on NF2 tumor suppressor gene product, merlin as well the closely related members of a family of actin-binding proteins, ezrin, radixin and moesin (ERM) has yielded significant new insights into aspects of normal and cancer cell biology. In 2001 she reported, that merlin mediates contact inhibition of growth through signals from the extracellular matrix. These findings published in Genes Dev were the first indication that during cell-cell contact active merlin is targeted to transmembrane proteins forming a molecular switch that specifies proliferation arrest. Later work published in Nature and Cancer Research identified that Merlin and ERM act as counterplayers in Ras activity control. Her lab also dissected novel components in the ERM protein/merlin signalling network that included the phosphatase MYPT-1-PP1δ and its inhibitor CPI-17. This previously undescribed tumour suppressor cascade containing MYPT-1-PP1δ and its substrate merlin can be hindered in two ways, not only mutation of the NF2 gene encoding merlin but also upregulation of the newly discovered oncoprotein CPI-17.

Born in the UK, Dr. Morrison earned her Ph.D. in Life Sciences in 2001 from the University of Karlsruhe, which is part of the German University Excellence initiative. She spent one year at the University of Cincinnati, Institute of Cell Biology before returning to Germany as a post doc at the Helmholtz Centre for Infectious Research in Braunschweig Germany. In 2003, she returned to Karlsruhe as a post doc before joining the Leibniz Institute in Jena. In 2001, she was awarded the Elsa and Walter Hermann PhD prize and in 2006 she was awarded the Thuringia research prize for her work in basic research.

Brigitte C. Widemann, M.D., *National Cancer Institute*

After obtaining her M.D. from the medical school of the University of Cologne, Germany, in 1986, Dr. Widemann became board certified in pediatrics in 1992 and served the NCI in fellowship training until 1995. Since then, she has been a member of the Pediatric Oncology Branch and, since 1999, a tenure track investigator.

Anticancer drug discovery and development are moving towards a more rational and targeted approach. The application of new molecularly targeted agents to the treatment of childhood cancers and neurofibromatosis type 1 (NF1) is a research objective of the Pharmacology and Experimental Therapeutics Section (PETS). In addition to studying the pharmacology, pharmacokinetics, pharmacodynamics, and toxicities of these novel agents, it is also a goal of the PETS to evaluate novel clinical trial designs and trial endpoints, which may be more applicable for molecularly targeted agents. The clinical development of farnesyltransferase inhibitors (FTI), which inhibit the posttranslational farnesylation required for the activity of wild-type and mutant ras proteins for patients with NF1 and refractory leukemias serves as an example for this approach. Pharmacodynamic endpoints that assess the effect of FTI on the target enzyme and farnesylation of cellular proteins are important endpoints of these trials. The clinical development of antimetabolites, such as raltitrexed, and agents that modulate the effects of antimetabolites, such as the recombinant bacterial enzyme, carboxypeptidase-G2 (CPDG2), is another research focus. CPDG2 hydrolyzes methotrexate (MTX) to inactive metabolites. CPDG2 provides an alternative route of elimination for MTX for patients with high-dose MTX- induced renal dysfunction, and plasma MTX concentrations declined by >95 percent within minutes in all patients. The intrathecal administration of CPDG2 has also been successfully used as a rescue agent in patients who received accidental overdoses of intrathecal MTX. A new drug application for the use of CPDG2 in HDMTX-induced renal dysfunction will be filed based on our data.

Keynote Speakers

KEYNOTE 1: Luis Parada, Ph.D., *University of Texas Southwestern*

Luis Parada, Ph.D. will provide the opening keynote presentation on the first day of the Conference. He is an outstanding speaker and well known to the NF community. He was intentionally asked to open the conference, knowing that his research topics have a direct impact on NF research. He is also one of the most popular speakers of the conference and has the capacity to attract a large and broad based basic and clinical audience. His laboratory focuses on the regulatory pathways that control nervous system development and the consequences of inappropriate development, including behavior disorders, as well as cancer. His work has had a major influence on the NF community. His generation of Nf1 null mice as well as the generation of conditional knockouts of Nf1 has become an important model for the NF-1 disease. Additionally, Dr. Parada studied mice with mutations in Nf1, p53, and Pten that develop brain tumors that molecularly resemble human glioma identifying that these tumors arise from neural stem/progenitor cells residing within a neurogenic niche of the brain, namely the subventricular zone. These relevant mouse models of human glioma and NF1 are powerful tools for investigating the initiation and progression of tumors and provide a useful biological system for testing possible therapies. He is a Professor at the Southwestern Medical Center. He is chair of Developmental Biology and was elected to the National Academy of Science (NAS) in 2011. He holds the Diana and Richard C. Strauss Distinguished Chair in Developmental Biology and the Southwestern Ball Distinguished Chair in Basic Neuroscience Research. He also serves as Director of the Kent Waldrep Center for Basic Research on Nerve Growth and Regeneration. Dr. Parada completed his doctorate in biology at the Massachusetts Institute of Technology in 1985 and served postdoctoral fellowships at the Whitehead Institute in Cambridge, Mass., and at the Pasteur Institute in Paris. Before joining the UT Southwestern Faculty in 1994, he was head of the Molecular Embryology Section in the Mammalian Genetics Laboratory of the National Cancer Institute. Apart from National Academy of Science membership, Dr. Parada has received a number of honors, including the Friederich Von Recklinghausen Award in 2009, the American Academy of Arts and Sciences in 2007, and as a fellow of the American Association for the Advancement of Sciences in 2008. He was also named an American Cancer Society basic research professor in 2003.

KEYNOTE 2: Rhona Mirsky, Ph.D., *University College London, United Kingdom*

Rhona Mirsky, PhD will provide a keynote presentation on Day 2 of the Conference. Dr. Mirsky is Professor Emeritus and Senior Research Associate at University College London, UK. Her laboratory, which she runs jointly with Kristjan R Jessen, addresses key issues in Schwann cell biology. Earlier work from the laboratory focussed on early Schwann cell development, the biology of the Schwann cell precursor and control of myelination. More recently the laboratory has been interested in the response of Schwann cells to injury and genetic disease, including the process of demyelination, and generation of a specialised repair cell that controls axon regrowth and nerve repair. Dr. Mirsky is an excellent speaker, and has an outstanding track record in her research area. She was targeted as a keynote speaker because of her broad experience and ground breaking research in Schwann cell biology, and because her research focus has a direct impact on NF research. Her laboratory was the first to identify the Schwann cell precursor, providing the missing developmental link between neural crest cells and Schwann cells. The group also set up a conceptual framework for developmental studies of Schwann cells and has provided many original findings for key signaling events that are essential for normal nerve structure and function. More recently they have found key factors in controlling Schwann cell responses to injury and axonal regrowth. Dr. Mirsky obtained her doctorate in Chemistry from the University of Cambridge, UK. After a post-doctoral period in the USA, from 1975-1981 she was a member of the Medical Research Council Neuroimmunology Project led by Martin Raff at University College London. In 1981 she moved to the Department of Anatomy and Developmental Biology, University College London as a lecturer. Between 1990-2004 she was Professor of Developmental Neurobiology in the Department of Cell and Developmental Biology, University College London. She is on the editorial board of two journals and has held several advisory positions for the Medical Research Council, UK (MRC) and was on the Medical Advisory Board of the UK Neurofibromatosis Association from 1989-2003. She is also a member of the Fellow of the Academy of Medical Sciences.

Keynote Speakers (cont.)

KEYNOTE 3: Silvia Cappello, Ph.D., *Helmholtz Zentrum München German Research, Germany*

Silvia Cappello is currently a postdoctoral scientist with Magdalena Götz at the Helmholtz Center in Munich in the Institute of Stem Cell Research. Silvias major interest is neural stem and progenitor cell biology during mammalian brain development. She has already made major contributions to the understanding of the molecular pathways controlling neurogenesis and neuronal migration during brain development. In particular published in both Nature Neuroscience and Neuron she has shown that modulators of the actin and microtubule cytoskeleton e.g. small GTPases Cdc42 and RhoA are crucial players. She received her doctorate in 2006 from the University of Padua. During her PhD she worked first at the University of Bologna with Dr. Marco Canossa and studied mechanisms of neurotrophin regulation during synaptic plasticity. She then moved to Munich and worked at the Max Plank Institute of Neurobiology and Helmholtz Center and focusing on the Rho family of GTPases in brain development. Silvia was awarded the highly competitive EMBO long term postdoctoral fellowship in 2007 to work in the lab of Dr. Richard Vallee at Columbia University where she dissected the role of NudC, a Lis1 regulator, in interkinetic nuclear migration and neuronal migration. In 2009 she moved back to Munich, focussing on RhoA activity and function in neurogenesis and neuronal migration during normal brain development in addition to her particular interest in neuronal migration disorders, like for instance Lissencephaly type I and II and double-cortex syndrome.

KEYNOTE 4: René Bernards, Ph.D., *Netherlands Cancer Institute, the Netherlands*

René Bernards, PhD. will provide a keynote presentation on Day 3 of the Conference. Dr.Bernards is head of the division Molecular Carcinogenesis at the Netherlands Cancer Institute and co-founder, Agendias. In the last 10 years, his laboratory has worked on the development of new tools to identify novel genes that act in cancer-relevant pathways. He uses high-throughput loss-of-function RNA interference genetic screens and gain-of-function genetic screens with retroviral cDNA expression libraries to identify novel components of cancer-relevant pathways and genes that modulate cellular responses to cancer drugs. In July of 2003 he also co-founded "Agendia", a genomics-based diagnostic company that offers the first microarray-based diagnostic test for the clinical management of breast cancer. His interests and experience in cancer biology and the application of innovative screening technologies make his work relevant to the NF community interested in identifying additional novel candidate molecules or signaling pathways in disease development. The impact of Dr Bernards work can be exemplified by his contribution to the novel clinical treatment of neuroblastoma. Using a large-scale RNAi genetic screen, he identified a crosstalk between NF1 and retinoic acid-induced differentiation in neuroblastoma. He not only identified NF1 mutations in neuroblastoma cell lines and in primary tumors he could show that inhibition of the NF1 signalling pathway restores responsiveness to RA, suggesting a therapeutic strategy to overcome RA resistance in NF1-deficient neuroblastomas. Dr. Bernards received his docorate in 1984 from the University of Leiden. For his postdoctoral training he joined the Whitehead Institute in Cambridge, USA in the laboratory of Robert Weinberg. He studied the function of both oncogenes and tumor suppressor genes. In 1988 he joined the Massachusetts General Hospital Cancer Center as an assistant professor where he continued this work. In 1992 he was appointed senior staff scientist at the Netherlands Cancer Institute. In 1994 he became part time professor of molecular carcinogenesis at Utrecht University in the Netherlands. He has received several awards for his research, including the Pezcoller Foundation-FECS Recognition for Contribution to Oncology, the Ernst W. Bertner Award for Cancer Research from the M.D. Anderson Cancer Center, the ESMO Lifetime Achievement Award in Translational Research in Breast Cancer and the Spinoza award from the Netherlands Organization for Scientific Research. He is a member of the Royal Netherlands Academy of Arts and Sciences.

KEYNOTE 5: Jan Tuckermann, Ph.D., *Leibniz Institute for Age Research, Germany*

Jan Tuckermann will provide a keynote lecture about mechanisms of bone integrity and their hormonal control. Dr. Tuckermann studied Biology performed his graduate studies in the labs of Peter Herrlich (Karlsruhe, Germany) and Peter Angel (Heidelberg, Germany) and his postdoc with Günther Schütz. He then worked as a group leader in at the Fritz LIpmann Institute (Jena, Germany) and was recently appointed as a full professor to head the Institute of General Zoology and Endocrinology at the University of Ulm (Germany). Dr. Tuckermann made major contributions to the molecular mechanisms of corticosteroids in beneficial and side effects of steroid therapy. With the help of conditional and function selective knockout mice for the glucocorticoid receptor (GR) he identified the critical cell types for anti-inflammatory activities and side effects of glucocorticoids in different inflammatory disease models. A major focus of his work contents the effects of glucocorticoids on bone integrity, since glucocorticoid-induced osteoporosis is the most secondary osteoporosis. Tuckermann and colleagues identified the osteoblast, the bone forming cell, as the major mediator to confer glucocorticoid-induced bone loss.

Select Platform Presentations

Skull defects in patients with neurofibromatosis type 1

SESSION 11A: June 12 | 11:00 AM – 11:15 AM

Daniel K. Arrington
Children's Hospital Boston/Harvard Medical School

Background: Skull defects, including sphenoid dysplasia and calvarial defects, are rare but distinct findings in patients with neurofibromatosis type 1 (NF1). The underlying pathophysiology is unclear. The goal of this study was to identify the clinical characteristics and natural history of skull defects in NF1.

Methods: An electronic search engine of the medical records was used to identify patients with NF1 and calvarial bony anomalies. All clinical, radiographic, pathology information and operative reports were reviewed. Relationship between bony anomalies and significant clinical associations was evaluated. This study received Institutional Review Board approval.

Results: Twenty-one patients were identified. Average age at NF1 diagnosis was 4.2 years. Average age at skull defect diagnosis was 8.6 years (sphenoid wing dysplasia = 10.4 years, calvarial defect = 11.6 years). The majority of defects were associated with either a plexiform neurofibroma or dural ectasia: Sphenoid wing dysplasia was associated with a neurofibroma or dural ectasia in 73.3% and 80.0% of cases, respectively. Calvarial defects were associated with a neurofibroma or dural ectasia in 61.5% and 38.5%, respectively. Absence of either an associated neurofibroma or ectasia was seen in none of the sphenoid wing dysplasias and 23.1% of calvarial defects. Seven patients had both types of skull defects present simultaneously. Serial imaging studies were reviewed for an average follow up time of 7.7 years (0.4-20.0 years). Of these, radiographic progression was found in 46.2% of calvarial defects and 33.3% of sphenoid wing dysplasias. Two patients underwent surgical repair of a skull defect and one required repeat procedures.

Conclusions: Several case reports suggest that skull defects are not developmental dysplasias but rather occur later in life and worsen over time. This cohort supports the concept of disease progression in a significant proportion of patients. The majority of skull defects were also associated with an adjacent structural lesion such as a plexiform neurofibroma or dural ectasia. Potential mechanisms by which these secondary lesions contribute to pathogenesis of the bony defect may include changes in the bony microenvironment. A better understanding of the pathophysiology of skull defects will help guide detection, treatment and improve outcome and may contribute to our understanding of the pathogenesis of bony lesions in NF1.

Full author list: Daniel Arrington, Analise Peleggi, Amy Danehy, Mark R. Proctor, Mira Irons, Nicole J. Ullrich -- Children's Hospital Boston, Boston, MA

Hand-Held Optical Coherence Tomography During Sedation Detects Visual Acuity and Visual Field Loss in Young Children with Optic Pathway Gliomas

SESSION 3A: June 9 | 5:40 PM – 6:00 PM

Robert A. Avery, D.O., MSCE
The Gilbert Family Neurofibromatosis Institute, Children's National Medical Center

Detecting visual acuity (VA) and visual field (VF) loss in young children with optic pathway gliomas (OPGs) can be challenging. Retinal nerve fiber layer (RNFL) thickness as measured by optical coherence tomography (OCT) has been considered a biomarker of VA/VF in older children with OPGs. Similar to VA/VF testing, younger children have difficulty cooperating with OCT. We investigated whether RNFL measures using hand-held OCT (HH-OCT) in younger children under sedation could detect VA/VF loss.

A cross-sectional sample of children with OPGs from a single-institution were enrolled in a longitudinal HH-OCT study. Patients were included in this analysis if they required sedation to complete their MRI, had successful HH-OCT imaging while sedated and were cooperative for VA testing. Average RNFL values were compared to VA and VF outcomes.

Twenty-three children with OPGs (17 NF1-related, 6 sporadic) were included for a total of 46 study eyes. Mean age was 5.7 years (range, 2.1-12.6). 10 eyes had abnormal VA, 15 eyes had abnormal VF and another 8 eyes had both abnormal VA/VF. OPGs were isolated to the optic nerve (n=9 eyes), involved the optic chiasm (n=16 eyes) or involved the optic tracts (n=14 eyes). RNFL thickness was decreased in the abnormal VA (65 $\pm$ 26 microns), abnormal VF (68 $\pm$ 13 microns) and abnormal VA/VF (55 $\pm$ 11 microns) groups compared to the normal VA/VF group (95 $\pm$ 11 microns) ($F = 26.3$, $p < 0.0001$). RNFL thickness had a strong relationship to VA ($R = 0.37$, $p < 0.0001$).

RNFL thickness is significantly decreased in young children with VA and or VF loss from their OPGs. RNFL thickness measures acquired with HH-OCT during sedation could be used as a biomarker of vision in children who cannot cooperate for VA/VF testing.

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Funding Source: CTF Clinical Research Award (R.A.A.); The Gilbert Family Neurofibromatosis Institute

Select Platform Presentations

The Baser Criteria for Diagnosis of NF2: Putting it to the Test

SESSION 6A: June 10 | 6:30 PM – 6:45 PM

Amanda Bergner, M.S., CGC
Johns Hopkins Comprehensive Neurofibromatosis Center

About half of patients with NF2 have *de novo* mutations. These patients may not meet established diagnostic criteria at the time of presentation, leading to delayed identification. A new set of clinical criteria for NF2 was proposed in 2011, the Baser criteria, and is thought to allow for earlier identification than existent models.

The Johns Hopkins Comprehensive Neurofibromatosis Center (JHCNC) follows a large cohort of NF2 patients. We applied the Baser criteria to our population to determine whether it improved diagnostic sensitivity.

68 patients presenting to our clinic for NF2 evaluation were identified from the JHCNC database. 58 met clinical criteria for NF2 based on the existent Manchester model; 10 did not meet criteria and were labeled as “possible NF2”. The 58 who met criteria were further divided into “unequivocal nonmosaic NF2” and “definite NF2” based on guidelines from the Baser study methods section. Clinical records were reviewed for each patient and a Baser score was calculated. Additional data collected included reproductive history, timing of diagnosis, and referring service.

The percentage of patients found to have NF2 by each criteria are listed below:

	Baser	NNFF	Manchester	1991 NIH	1987 NIH
Unequivocal Nonmosiac NF2	100%	100%	100%	100%	100%
Definite NF2 (n=19)	63%	100%	100%	100%	100%
Possible NF2 (n=10)	40%	10%	10%	0%	0%

88% (60/68) of our patients presented with no family history of NF2 and 24% (16/68) presented without bilateral VS. No patients with unequivocal nonmosaic NF2 (n=39) had children prior to receiving a diagnosis of NF2 while 72% (21/29) of the remaining patients had no established diagnosis of NF2 during reproductive years.

We found the Baser criteria most helpful for the “possible NF2” patients by clarifying who should be considered for genetic testing and closer screening. Important differences between our population and the Baser study include: 1) limited longitudinal data; 2) low frequency of genetic testing completed for our patients; 3) lack of integrated healthcare and a robust NF2 registry in the US; 4) fractionated healthcare due to inadequate insurance coverage, leading to delayed access to medical care.

Patients with possible NF2 are often referred to the JHCNC after being followed in another clinic. Educating colleagues in other departments about this tool and establishing a Baser score that would trigger a referral to the JHCNC would lead to more timely diagnosis, allowing for effective reproductive counseling and sequencing of therapeutics.

Full List Authors: Aaron Chance; Jaishri Blakeley, M.D.; Shannon Langmead, CRNP; Allan Belzberg, M.D., FRCSC; Amanda Bergner, MS CGC;. All authors are at the Johns Hopkins Comprehensive Neurofibromatosis Center.

Anti-Tumor Activity of MAPK and mTOR Inhibitors in a Novel Mouse Model of NF1-Mutant Soft-Tissue Sarcoma

SESSION 6B: June 10 | 6:30 PM – 6:45 PM

Rebecca Dodd, Ph.D.
Duke University Medical Center

Patients with Neurofibromatosis Type 1 (NF1) are at increased risk for developing soft-tissue sarcomas, including malignant peripheral nerve sheath tumors (MPNST, 8-13% lifetime risk) and myogenic sarcomas such as rhabdomyosarcoma (RMS, 3-6% lifetime risk). Despite the prevalence of sarcomas in NF1 patients, *the role of NF1 in the development of soft-tissue sarcomas, particularly myogenic tumors, is a neglected area of NF1 research*. Using Cre-loxP technology, we have developed a novel temporally and spatially restricted mouse model soft-tissue sarcoma in NF1 $^{flox/flox}$; Ink4a/Arf $^{flox/flox}$ mice. Following injection of an adenovirus containing Cre recombinase, these mice develop either MPNST tumors (sciatic nerve injection) or high-grade myogenic sarcomas (intramuscular injection). These tumors reflect the histological properties and spectrum of sarcomas found in neurofibromatosis patients. Sciatic nerve injections result in tumors with a fascicular pattern of tightly packed spindle cells surrounding the nerve. These tumors demonstrate mast cell infiltration and focal positivity for S100-beta. In contrast, intramuscular injections generate a spectrum of high-grade myogenic sarcomas, including rhabdomyosarcoma (RMS) and Undifferentiated Pleomorphic Sarcoma (UPS). These tumors express the myogenic marker MyoD1 and have a high Ki67 proliferative index.

We have used the models of MPNST and RMS/UPS described above as preclinical platforms for identifying novel therapies. Immunohistochemical analysis for pERK and pS6 reveals that MAPK and mTOR signaling are elevated in tumors from NF1 $^{flox/flox}$; Ink4a/Arf $^{flox/flox}$ mice. In vitro toxicology assays demonstrate that cell lines from these tumors are sensitive to mTOR inhibitors (rapamycin) and MEK inhibitors (U120, PD98059, PD0325901). Treatment of orthotopic allografts with either rapamycin or MEK inhibitor PD0325901 slowed tumor growth, with combination therapy (rapamycin with PD0325901) producing the greatest benefit. Next, we examined single-agent PD0325901 treatment in our primary mouse model of myogenic NF1 mutant sarcoma. As predicted, treatment with the MEK inhibitor delayed tumor growth in many of the tumors (PR= 83%). TUNEL staining demonstrates that PD0325091 treatment did not increase apoptosis, suggesting this MEK inhibitor works through cytostatic and not cytotoxic mechanisms. These data identify the MEK inhibitor PD0325901 as a novel and promising therapeutic for sarcomas in neurofibromatosis patients.

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Preliminary Results of a Phase II Trial of Pegylated Interferon-Alfa-2B (PI) in Pediatric Patients with Documented Progression of Neurofibromatosis Type 1 – Related Unresectable Plexiform Neurofibromas (PNF)

SESSION 6A: June 10 | 6:45 PM – 7:00 PM

Eva Dombi, M.D.
Pediatric Oncology Branch, National Cancer Institute Bldg

Background: There is no effective medical therapy for PNFs. A Phase I trial of PI in patients with PNFs determined the maximum tolerated dose (MTD) and documented radiographic and clinical responses. Experimental Design: PI was administered at the MTD of 1 mcg/kg/week subcutaneously to patients with documented radiographic progression of a PNF over the year prior to enrollment. PI was continued until progression (≥20% increase in PNF volume). PI would be considered active if it doubled the time to progression (TTP) using Kaplan-Meier analysis compared to the TTP on the placebo arm of a NCI randomized trial.

Results: 30 patients (median age 7.1 yrs, range 1.6-17.6 yrs) were enrolled. PI was well tolerated. Four patients required dose reductions for persistent constitutional symptoms and one patient came off study for recurrent grade 3 transaminitis. Median PFS for the 29 eligible patients is 22.6 months vs. 10.6 for the NCI placebo arm (P=0.023, by two-tailed log-rank test). The slope of tumor growth on PI slowed significantly compared to the slope prior to starting PI (p=0.040 two-tailed Wilcoxon signed rank test). Two patients had a radiographic response (≥ 20% decrease in PNF volume) and 8 had minor volume decrease (range 11-16%). Twelve patients continue on study (median 18 months, range 12-42 months). 17 patients came off study, 12 for progressive disease (median 12 months, range 8-30 months).

Conclusions: Pegylated interferon results in more than doubling of the TTP compared to the NCI placebo arm, and decreases PNF volumes in a subset of patients.

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Merlin regulates expression levels and downstream signaling of multiple transmembrane receptors including p75^{NTR}

SESSION 6B: June 10 | 6:45 PM – 7:00 PM

Augusta Fernando, Ph.D.
University of Iowa

Merlin regulates the expression level and trafficking of transmembrane receptor tyrosine kinases including ErbB family members and platelet derived growth factor receptor (PDGFR). We also find that vestibular schwannoma (VS) cells, which lack functional merlin, express high levels of p75^{NTR}, a member of the TNF family of transmembrane receptors implicated in Schwann cell (SC) death following nerve injury. Here we implicate merlin as a key regulator of p75^{NTR} expression in normal SCs and in VS cells. We show that merlin is inactivated by phosphorylation following nerve injury and this correlates with the increase in p75^{NTR} expression. Further, p75^{NTR} and ErbB2 levels are elevated in the uninjured sciatic nerves from P0Schdel36-121 mice, which lack functional merlin expression in SCs, compared to wild-type controls, suggesting that merlin suppresses p75^{NTR} expression in normal SCs. Interestingly VS cells and SCs from P0Schdel36-121 mice do not undergo p75^{NTR}-mediated apoptosis, despite expressing high levels of the receptor. In fact, p75^{NTR} activation protects VS cells from some forms of cell death. VS cells express sortilin, a p75^{NTR} co-receptor necessary for induction of apoptosis indicating that this failure to undergo p75NTR-mediated apoptosis is not due to the lack of the co-receptor. We are now in the process of examining the differences in downstream signaling events, including c-Jun N-terminal kinase (JNK) phosphorylation and NF-kappaB activation, that differentiate the apoptotic response of normal SCs and the pro-survival response of SC and VS cells lacking functional merlin.

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Funding Sources: NIH NIDCD-R01DC009801, NIH NIDCD-P30 DC 010362

Computerized cognitive training for children with Neurofibromatosis Type 1 (NF1): A pilot study

SESSION 6A: June 10 | 7:00 PM – 7:15 PM

Kristina K. Hardy, Ph.D.
The Jennifer and Daniel Gilbert Neurofibromatosis Institute, Children's National Medical Center/George Washington University School of Medicine

Children with NF1 have a high incidence of executive dysfunction; but few interventions have been empirically evaluated. We aimed to assess the feasibility and preliminary efficacy of a home-based, computerized cognitive training program for children with NF1 and working memory deficits.

This prospective, single-arm trial (target n = 30) employed a pre-post design to evaluate changes in performance-based measures of attention and working memory and parent-completed ratings of executive functioning. Children meeting our eligibility criteria completed training with Cogmed®, a home-based, computerized working memory training program that includes phone-based coaching support over 9 weeks (25 sessions). Primary outcomes included compliance statistics (tracked automatically by the program) as well as change in attention and working memory scores from baseline to post-intervention. Given the limitations associated with our single-arm approach, we employed a neurocognitive assessment battery consisting of measures with available alternate forms and/or with few practice effects.

Ten children (40% male; Mean age = 10.6, Range = 8-15) have completed the study since June 2011. Treatment compliance is high with all participants completing at least 92% of training sessions with no adverse events. Moreover, parents reported that their children enjoyed the intervention, and the majority of parents (90%) indicated they were somewhat or very satisfied with their child's participation. Training improvement in our sample is consistent with standards based on samples of children with developmental Attention Deficit Hyperactivity Disorder (ADHD), indicating that our participants are exhibiting task-specific increases over the course of the intervention. Participants also exhibited post-treatment improvement in attention and executive function on a number of performance-based measures, but not on the parent-rated questionnaire of executive dysfunction. Thus, preliminary data suggests that home-based computerized training is feasible for children with NF1; a larger, randomized clinical trial appears warranted.

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Funding Source: The Jennifer and Daniel Gilbert Neurofibromatosis Institute

Merlin targets the Cdh1/fzr inhibitor Rae1 to regulate mitosis and establish organ size homeostasis

SESSION 6B: June 10 | 7:00 PM – 7:15 PM

Maryam Jahanshahi
Mount Sinai School of Medicine

Neurofibromatosis 2 (NF2) is an autosomal dominant familial syndrome which is caused by loss of function mutations in the NF2/Merlin gene, predisposing carriers to develop tumors of the central nervous system. While it has been demonstrated that Merlin regulates many mitogenic pathways, the precise mechanism by which Merlin restricts cell growth and proliferation, and how that control is abrogated in these tumors, remains unclear. Studies in *Drosophila* have demonstrated that the highly conserved Hippo Tumor Suppressor pathway acts downstream of Merlin to restrain cell proliferation and promote apoptosis.

It is well established that loss of core pathway components (the Serine-Threonine Kinases, Hpo and Wts as well as Sav and Mats) and the crucial transcriptional regulator Yki promotes cell division, cell death resistance, and tumor-like overgrowth in both Drosophila and vertebrates. Dysregulation of these core components, as well as Yki, is pleiotropic, affecting proliferation, survival and growth. In contrast, the anti-apoptotic DIAP1, one of the few known post-translational substrates of the pathway, plays a specific role in apoptosis. Crucial targets responsible for the distinct functions of restricting growth and restricting cell proliferation, targets with distinct roles in the G1-S transition or mitosis, and specific effectors responsible for coordinating organ size and proliferation remain largely unknown. These substrates may represent novel NF2 therapeutic targets.

Using the *Drosophila In Vitro* Expression Cloning (DIVEC) approach, we performed a genome-wide kinase substrate screen to identify Hpo and Wts substrates using a phosphorylation induced gel-shift assay. In this work we characterize the Cdh1-inhibitor Rae1 as a key target downstream of Merlin that regulates cell division. Exogenous Rae1 increases both cell proliferation and organ size. Rae1 is required *in vivo* for S-phase entry and mitotic progression and is phosphorylated and degraded upon activation of Merlin. We propose a model that signaling through Merlin promotes Cdh1-Anaphase Promoting Complex/Cyclosome activity by relieving its Rae1-mediated inhibition. Importantly, Rae1 reduction compromises survival of Merlin-deficient tissue indicating synthetic lethality and a requirement for Rae1 reminiscent of oncogene/non-oncogene "addiction". The "Rae1 addiction" of tissue upon loss of Merlin further implicates Rae1 in tumorigenesis and suggests that Rae1 may represent a therapeutic target for cancers in which Hippo signaling is dysregulated.

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Funding: Children's Tumor Foundation

Maryam Jahanshahi is currently funded by the Children's Tumor Foundation Young Investigator Award Program

Loss of neurofibromin increases micro- and macroporosity in cortical bone resulting in diminished mechanical resistance

SESSION 11A: June 12 | 10:00 AM – 10:15 AM

Mateusz Kolanczyk, Ph.D.
Max Planck Institute for Molecular Genetics and Institute for Medical Genetics, Berlin, Germany

Skeletal manifestations such as osteoporosis, dystrophic scoliosis or tibial dysplasia are commonly in patients with neurofibromatosis type 1 (NF1). An important role of neurofibromin in the regulation of musculoskeletal system development has recently been established. To further explore the origin of NF1 bone dysplasia we now performed detailed analysis of the cortical bone porosities in the Nf1Prx1 mice (a model of NF1 tibial dysplasia) and in the NF1 patients, using high-resolution imaging techniques. One of our aims was to explore how NF1 loss of function affects osteocytes, the mechanosensory cells of the bone. The overall morphology of the humerus from Nf1Prx1 mice appeared severely disordered. Especially at the muscle to bone insertion sites we observed large amounts of fibrocartilaginous tissue. Within the diaphysis we detected large non-mineralized regions of bone tissue that are associated with blood vessels. Thus, the macroporosity was 5-fold increased in Nf1Prx1 mice as compared to controls. Microporosity, which is mainly determined by the size of osteocyte lacunae, was increased. While Nf1Prx1 cortical bone contained a normal number of osteocyte lacunae, the average lacuna volume was increased, yielding higher relative osteocyte volume per bone volume (3.4 % in the mutants vs. 2.0 % in controls). The osteocyte phenotype is likely cell autonomous as increased osteocyte lacuna volume was also detected in the Nf1Col1 mice. Similarly, a quantitative volumetric analysis of cortical bone samples from NF1 patients demonstrated increased osteocyte lacuna size. Mechanical testing of bone slices from Nf1Prx1 mice showed significant reduction of elastic behaviour and ultimate strength. These findings suggest that neurofibromin is required for normal osteocyte function, and facilitates bone homeostasis. Thus, our collective data reveal a significant impact of neurofibromin on cortical porosity establishing a further aspect of NF1 bone dysplasia.

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Natural History of Plexiform Neurofibromas in NF1

SESSION 6A: June 10 | 7:15 PM – 7:30 PM

Bruce R. Korf, M.D., Ph.D.
University of Alabama at Birmingham

Background: Better understanding of the natural history of plexiform neurofibromas (PN) will advance the development of effective therapies for these tumors. Previous studies have suggested that plexiform neurofibromas tend to grow most rapidly in young children. Objective: To define PN growth rates in patients not receiving tumor therapy. Methods: Children and adults with NF1 and a measurable PN who were enrolled on a multicenter NF1 natural history study underwent MRI evaluation using a standardized MRI protocol. PN volumes were determined using a semi-automated body lesion detection program (MEDx v3.44). PN were defined as superficial, deep, or mixed. Linear regression analysis was performed to assess the slope of PN growth for each patient. The Pearson Correlation Coefficient was used to describe the relationship between age, rate of PN growth and PN type. One-way ANOVA and the pooled t test were used to compare PN growth rate in 3 age groups (0-10, 11-18, and ≥19 years) and PN types. Results: The NF1 Natural History study enrolled 261 subjects between 12/26/1997 and 8/25/2003. At least two MRIs amenable to 3D analysis were available for 131 subjects (male 71, female 60), and 370 MRIs were analyzed. Forty-seven patients had deep, 29 had superficial and 55 had mixed PN. The median age at the first MRI was 13.3 years (range 2.5-55.5); the median tumor volume was 187 ml (range 3.0-21,803). The median observation period was 24.1 months (range 6.4-76.9). The median percentage change in PN volume per year was 6.94% (range negative 5.76 to 58.9). Older patients had slower increases in PN volume compared to younger patients ($p=0.001$). Sixteen of 45 patients (36%) 0-10 years old had PN growth >20% per year, while only 5 of 86 patients (6%) >10 years old had similar increase. PN growth rate did not differ significantly in the 3 PN types ($p=0.7665$) or by sex ($p = 0.64$). Conclusion: Using a large cohort of NF1 patients not selected for morbidity and not receiving PN treatment, we demonstrated that PN growth rates vary among patients and that younger patients have faster growing PNs. The majority of patients who would qualify for ongoing clinical trials requiring disease progression for eligibility (≥20% PN volume increase within a year) were in the 0- 10 years age group.

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Schwannomatosis is a neurogenetic disorder characterized by multiple, non-vestibular, non-cutaneous schwannomas. As more cases are identified, the reported phenotype continues to expand and evolve. In this report, we describe the spectrum of clinical findings in a large cohort of 87 patients meeting criteria for schwannomatosis.

Clinical Features of Schwannomatosis: A Retrospective Analysis of 87 Patients

SESSION 6A: June 10 | 7:30 PM – 7:45 PM

Vanessa Merker
Massachusetts General Hospital/ Harvard Medical School

We retrospectively reviewed the clinical records of patients seen at our institution between 1995 and 2011 who fulfilled either research or clinical criteria for schwannomatosis. Clinical, radiographic, and pathologic data were extracted with attention to symptom onset, location of tumors, surgical and medical treatments, and diagnosis of comorbidities.

Eighty-seven patients met criteria for the study. The most common presentation was pain unassociated with a mass (46%). Peripheral schwannomas were present in 77/87 patients (89%), spinal schwannomas in 49/66 (74%), and intracranial meningiomas in 4/77 (5%). Three patients were initially diagnosed with a MPNST; however, following pathologic review, the diagnoses were revised in all 3 cases. Chronic pain was the most common symptom (68%) and usually persisted despite aggressive surgical and medical management. Other common diagnoses included headaches, depression, and anxiety.

Peripheral and spinal schwannomas are common in schwannomatosis patients. These findings support a proactive surveillance plan in which neurological symptoms and signs are actively investigated by MRI to identify tumors. Severe, chronic pain is common, and often associated with anxiety and depression. Early referral to specialty care is warranted.

Additional Authors: Sonia Esparza B.A.; Miriam J. Smith Ph.D.; Anat Stemmer-Rachamimov M.D.; and Scott R. Plotkin M.D., Ph.D., all of Massachusetts General Hospital.

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Aurora Kinase A as a rational therapeutic target against malignant peripheral nerve sheath tumours

SESSION 6B: June 10 | 7:15 PM – 7:30 PM

Pooja Mohan
University of British Columbia (UBC), Canada

Malignant peripheral nerve sheath tumours (MPNST) are rare, hereditary cancers associated with mutations in the *neurofibromin 1* gene. These are early-onset, aggressive tumours that require novel therapies. Published analysis of copy number variation identified hemizygous loss of the *Receptor for Hyaluronan Mediated Motility (RHAMM)* gene in half of the examined high-grade MPNST, but not in benign neurofibromas or low grade tumours (1). We have described RHAMM as a molecular brake for the mitotic kinase Aurora A (2). Now, we propose that the loss of RHAMM in high grade MPNST may oncogene-addict tumours to Aurora A activity and sensitize them to aurora kinase inhibitors (AKI).

We have profiled three MPNST cell-lines for the expression and activity of Aurora A as well as their responses to three AKI. The most proliferative cell-lines, S462 and 2884, express equivalent levels of Aurora A but differ in the expression of regulators for kinase activity, like the Targeting Protein for XKlp2 (TPX2) (the accelerator) and RHAMM (the brake). Relative to 2884, S462 cells express significantly more TPX2 and significantly less RHAMM, which was quantified at the protein, message, and genomic levels with immunoblot, quantitative PCR, and comparative genomic hybridization. Consistently, S462 cells show elevated Aurora A activity, as inferred by the abundance of two substrates, pHistone H3 (Ser10) and pRHAMM (Thr703). All three AKIs reduced kinase activity in a dose-dependent manner, and cellular responses to AKI, such as apoptosis, endoreduplication and cellular senescence, correlating to the activity of the kinase in these cell-lines. The S462 cell line has a population of tumour-initiating cells (TICs) which can be enriched through culture as neurospheres (3). Compared to the adherent cells, S462 TICs have elevated Aurora A activity and their propagation and self-renewal *in vitro* is arrested by AKI. We are now examining AKI in MPNST animal models with preliminary analysis showing elevated pRHAMM in these tumours.

Cure, or control, of rare tumours may be more heavily dependent upon rational-designed treatment strategies, given the expense associated with clinical trials and the reduced size of the affected population. These therapies will likely require biomarkers that predict disease response. Here, we describe encouraging preclinical results for MPNST treated with AKI, and find that the response of cell-lines to these drugs may be predicted by the levels of pRHAMM. Furthermore, populations enriched for TICs are sensitive to AKI, which implies these drugs may also be effective against MPNST relapses.

References: [1] Mantripragada K et al. Clin Can Res 2008; (14):1015 [2] Maxwell CA et al. Plos One 2011; (9):e1001199 [3] Spyra M et al. Plos One 2011 (6):e21099

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Granting Agency: Sick Kids Foundation

Defective bone matrix mineralization, not only osteoblast differentiation, may contribute to NF1 pseudoarthrosis

SESSION 11A: June 12 | 9:45 AM – 10:00 AM

Jean de la Croix Ndong, Ph.D.
Vanderbilt University

Neurofibromatosis is an autosomal dominant disease caused by mutations in the *NF1* gene. The incidence of NF1 is about 1:3000, and approximately 1-5% of NF1 patients present with pseudoarthrosis (PA), i.e. non-bone union following fracture. Despite substantial advances in the past years, the etiology NF1 PA remains unclear, and most NF1 patients with PA still require multiple and invasive surgeries. Our goal in this study was to characterize the maturation of *Nf1*$^{-/-}$ osteoprecursor cells using bone marrow stromal stem cells (BMSCs) isolated form WT and *Nf1*$_{col2}$$^{-/-}$ mice or from Nf1$^{flox/flox}$ mice following in vitro cre-adenovirus infection.

Compared to WT BMSCs, *Nf1*$^{-/-}$ BMSCs formed a significantly reduced number of alkaline phosphatase-positive (cfu-AP) colonies and mineralized alizarin red-positive (cfu-Ob) colonies following 10 and 20 days of differentiation, respectively, which was suggestive of impaired differentiation and/or mineralization. Accordingly, the expression of osteoblast differentiation marker genes such as *Col1*, *Ocn*, *Bsp*, *Runx2*, *Osx* and *Tnsalp* was markedly decreased in *Nf1*$^{-/-}$ BMSCs, whereas the expression of *Rankl*, a stimulator of osteoclast formation, was increased. Surprisingly, BMP2 treatment was able to correct the differentiation defect of *Nf1*$^{-/-}$ BMSCs, as measured by increased number of cfu-AP and correction of gene expression marker genes, but was unable to correct the defective mineral deposition of *Nf1*$^{-/-}$ cells. These data indicate that *Nf1*$^{-/-}$ osteoblasts are characterized by a differentiation and a mineralization defect.

To characterize this mineralization defect, genes controlling mineralization were measured. The expression of *Ank*, a transporter of extracellular inorganic pyrophosphate (ePPi), which is a potent inhibitor of hydroxyapatite formation and propagation, and *Opn*, an inhibitor of mineral nucleation, were significantly increased in *Nf1*$^{-/-}$ BMSCs. In contrast, the expression of *Tnsalp*, whose activity is to cleave PPi, was strongly reduced in *Nf1*$^{-/-}$ BMSCs. Accordingly, the level of ePPi was constitutively increased in the conditioned medium of *Nf1*$^{-/-}$ BMSCs. Importantly, co-treatment of *Nf1*$^{-/-}$ BMSCs with rBMP2 and a recombinant form of human TNSALP (Asfotase) rescued both differentiation and matrix deposition in *Nf1*$^{-/-}$ BMSCs, and in vivo administration of Asfotase alpha to *Nf1*$_{col2}$$^{-/-}$ mice improved their vertebral bone volume.

Taken together, our data provide a proof of concept that pharmacological enhancements to treat NF1 PA will require both stimulation of osteoblast differentiation and inhibition of PPi accumulation to allow proper bone healing.

Additional Authors: Philippe Crine, Florent Elefteriou

Progression rate of plexiform neurofibromas after surgery – a retrospective study in 52 patients with nerofibromatosis type 1

SESSION 6A: June 10 | 7:45 PM – 8:00 PM

Rosa Nguyen, M.D.
University of Maryland

To analyze tumor growth rate and identify prognostic features for tumor progression in post-operative plexiform neurofibromas (PNFs) of patients with neurofibromatosis type 1 (NF1).

We retrospectively analyzed clinical and MRI data of post-operative NF1 patients with PNFs that were seen at the University Hospital Hamburg-Eppendorf between 1999 and 2010. Post-operative tumor volume change per time was calculated. Linear regression models were applied with age as a continuous and categorial variable (≤/> 21 years) to identify tumor characteristics associated with tumor growth rate.

Fifty-two patients (median age 22.5 years, range 2.7 to 67.6 years) with 57 PNFs were identified. Initial median tumor volume was 66.3 mL (SD 2231; range 0 to 13740). Surgical indications included cosmetic disfigurement (n=18), functional deficits (n=13) and pain (n=10). Surgical complications included hematoma (n=4), delayed wound healing (n=2), necrosis and residual symptoms (each n=1). Eleven patients (21%, 9 children vs. 2 adults) with residual tumor had repeated surgery due to tumor progression. In a fully adjusted regression model with age as a continuous variable, only tumor type was significantly associated with tumor progression; specifically diffuse PNFs progressed faster than nodular ones ($p < 0.005$). With age as a categorical variable, those 21 and younger had highest progression rate ($p<0.01$). In models stratified according to site, this age effect was maintained in abdominal and lower extremity tumors. In PNFs of the upper extremities, this age effect was reversed. Among head and neck tumors, those that were deep tumors in younger than 21 year olds had higher tumor progression rates than superficial PNFs in 21 year olds and over ($p=0.02$). Among those with lower extremity PNFs, superficial tumors progressed more rapidly than those that were deep ($p <0.01$). Thirteen PNFs (10 nodular vs. 3 diffuse ones) in 11 patients (3 children vs. 8 adults), of which 5 were removed electively, were visually completely resected and did not relapse during observation (mean 3.7 years, 1.0-7.5).

Age, tumor type, location, and depth may predict progression of partially resected PNFs. Considering these factors may help to decide when and where to perform surgery. Patients may benefit from elective surgery of small and completely removable PNFs.

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Skeletal manifestations cumulatively affect ~70% of neurofibromatosis type 1 (NF1) patients. Tibial pseudarthrosis, the chronic non-union of a spontaneous tibial fracture, is a debilitating skeletal malady affecting children with NF1 early in life. These non-healing fractures typically respond poorly to surgical intervention and often require amputation.

The role of osteoclasts in neurofibromatosis type 1 pseudarthrosis

SESSION 11A: June 12 | 10:15 AM – 10:30 AM

Steven D. Rhodes
Indiana University

Previous studies have implicated *Nf1* deficient osteoblasts (OBLs) in the recalcitrant bone repair process. Although osteoclast (OCL) bioactivity increases during fracture repair, the role of OCLs in the pathogenesis of NF1 pseudarthrosis remains unknown. Over recent years, studies have confirmed that OCLs from NF1 patients and mice exhibit multiple gain-in-functions, including enhanced proliferation, survival, and bone resorptive capacity. We recently established that transplantation of $Nf1^{+/-}$ bone marrow (BM) cells to $Nf1^{f/f}$*;Col2.3Cre* mice [carrying $Nf1^{-/-}$ OBLs and wild-type (WT) background] can induce osteoporosis and severe non-union fracture healing as compared to transplantation of WT BM cells, suggesting that in concert with $Nf1^{-/-}$ OBLs, $Nf1^{+/-}$ BM cells play a critical role in pathogenesis of these osseous defects.

Based on these data, we hypothesized that $Nf1^{+/-}$ OCLs and their progenitors (macrophages) are the key hematopoietic cell lineage contributing to the osteoporosis and impaired fracture healing in mice harboring $Nf1^{-/-}$ OBLs and $Nf1^{+/-}$ BM cells. To test this hypothesis, we generated $Nf1^{f/+}$*;LysMCre* mice permitting conditional inactivation of a single *Nf1* allele in macrophages (pre-OCLs) and OCLs. Here we report that $Nf1^{f/+}$*;LysMCre* mice exhibit increased frequency of OCL progenitors (CFU-M), osteoclastogenesis, actin ring formation, and bone resorption *in vitro*. To examine the role of $Nf1^{+/-}$ OCLs on fracture healing *in vivo*, lethally irradiated $Nf1^{f/f}$*;Col2.3Cre* mice were transplanted with BM cells from WT or $Nf1^{f/+}$*;LysMCre* mice. A fixed tibial fracture was subsequently induced after stable reconstitution. Compared to WT BM cells, $Nf1^{f/+}$*;LysMCre* BM cell reconstituted $Nf1^{f/f}$*;Col2.3Cre* mice exhibited significantly reduced callus bone volume fraction (BV/TV) as determined by microcomputed tomography (micro-CT), revealing a substantial deficit in fracture repair. Collectively, these results suggest that in the context of *Nf1* deficient osteoblasts, $Nf1^{+/-}$ macrophages and OCLs are the culprit hematopoietic cell lineage responsible for the pathogenesis of *NF1* non-union fracture. As such, therapy targeting both the osteoclast and osteoblast lineages may be necessary to maximally augment bone healing in *NF1* pseudarthrosis.

Steven D. Rhodes; Yongzheng He, M.D., Ph.D.; Karl W. Staser, Ph.D.; Keshav Menon; Feng-Chun Yang, M.D., Ph.D., Indiana University

Support: Children's Tumor Foundation

Steven Rhodes is currently funded by the Children's Tumor Foundation Young Investigator Award Program

Conditional double inactivation of NF1 in skeletal muscle leads to a severe muscle myopathy with abnormal metabolic function

SESSION 11A: June 12 | 10:45 AM – 11:00 AM

Aaron Schindeler, Ph.D.
The Children's Hospital at Westmead, University of Sydney, Sydney Australia

Children with Neurofibromatosis type 1 (NF1) have been indicated by anecdotal reports and small clinical studies to show weakness, poor muscle tone, and/or reduced co-ordination. While these traits have often been attributed to an underlying neurological basis, there is emerging evidence that muscle function may be impaired in some children with NF1. To explore this further, we have performed muscle analyses in two models of NF1 deficiency in muscle.

The *Nf1*$^{+/-}$ mouse has one defective copy of the NF1 gene and presents with a mild phenotype with only some of the clinical features of NF1. These mice showed no difference in overall weight or lean tissue mass, and grip strength tests indicated no substantive muscle weakness. In a model of botox-induced limb disuse, both *Nf1*$^{+/-}$ mice and wild type controls showed similar muscle degeneration/regeneration and bone loss and restoration.

To establish whether NF1 was dispensable in muscle, a MyoD-cre transgene was introduced to the *Nf1*$^{flox/flox}$ strain to generate *Nf1*$_{muscle}^{-/-}$ mice. These mice showed a failure to thrive and neonatal lethality. Early data indicates that muscle fiber size/fiber type proportions were normal at postnatal day 3 and slightly reduced at day 6. Notably, electron microscopy indicated a significant increase in intra-myofiber fat globules, and this was confirmed by Oil Red O staining.

These data demonstrate that muscle expression of NF1 is critical for viability in mice and that *Nf1*^{null} muscle shows abnormal metabolic function. We are currently performing detailed studies on the enzymes involved with respiration and lipid storage in an effort to elucidate the underlying mechanism.

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A Genetic Modifier Screen Implicates Specific Tyrosine Kinase and Neuropeptide Signaling Pathways in the Drosophila Neurofibromatosis-1 Growth Deficiency

SESSION 6B: June 10 | 7:30 PM – 7:45 PM

James A. Walker, Ph.D.
Massachusetts General Hospital/Harvard Medical School

Amongst previously described Drosophila NF1 (*dNf1*) loss of function phenotypes, reduced growth and learning/memory defects resemble common human NF1 symptoms. Interestingly, these and other *dNf1* phenotypes are relatively insensitive to manipulations that affect Ras signaling strength, but suppressed by increasing signaling through the cyclic-AMP (cAMP)-dependent Protein Kinase A (PKA) pathway, or are phenocopied by activating this pathway. However, the molecular links between *dNf1* and cAMP/PKA signaling remain poorly understood. To identify rate-limiting components of *dNf1* pathways in Drosophila, we have conducted a genetic screen for dominant modifiers of the cAMP/PKA-sensitive *dNf1* organismal growth defect using a set of precisely defined chromosomal deletions. Candidate modifier genes were tested using mutant alleles or by short hairpin RNA (shRNA)-mediated suppression. Validating the screen, identified suppressors include the previously implicated neuronal *dAlk* tyrosine kinase and its activating ligand *jelly belly* (*jeb*), among other proteins involved in Ras signal transduction. Other modifiers encode several proteins with synaptic functions, including the calcium sensor frequenin-2, nicotinic acetylcholine receptor subunits, the cAMP-coupled CCKLR-17D1 drosulfakinin receptor, recently implicated as a regulator of synaptic architecture and signaling, as well as other proteins involved in cAMP/PKA signaling. Remarkably, given that overall growth is a function of insulin controlled growth rate, and juvenile hormone and 20-hydroxyecdysone determined growth duration, no genes involved in either pathway are among the identified *dNf1* modifiers. Instead, we hypothesize that a cell autonomous increase in dAlk/Ras/ERK signaling leading to a neuropeptide/cAMP signaling defect may be ultimately responsible for the *dNf1* growth deficiency. Results from gene expression and metabolomic profiling studies of *dNf1* mutants which complement the genetic screen data will also be presented.

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Hyperactive transforming growth factor-beta1 signaling and pseudarthrosis in neurofibromatosis type 1

SESSION 11A: June 12 | 10:30 AM – 10:45 AM

Feng-Chun Yang, M.D., Ph.D.
Indiana University

Up to ninety percent of tibial pseudarthrosis cases are associated with Neurofibromatosis type 1 (NF1). Although tibial pseudarthrosis is a rare pediatric orthopaedic condition, it progresses to spontaneous fracture and subsequent fibrous nonunion. Treatment of tibial pseudarthrosis has been notoriously difficult, ultimately requiring amputation of the affected limb in many cases. Still, the molecular etiology of these pathological defects remains unclear.

Transforming growth factor-beta1 (TGF-beta1) is known to be a critical factor regulating the spatiotemporal coupling of bone resorption and bone formation. Intriguingly, haploinsufficiency of Nf1 in myeloid cells has been shown to result in hypersecretion of TGF-beta1 *in vitro*. Given the fact that disruption of TGF-beta signaling gradients is associated with a spectrum of osseous defects in both patients and mouse models, we sought to investigate the impact of TGF-beta1 signaling in NF1 associated skeletal manifestations.

Here, we show that compared to WT mice, TGF-beta1 serum levels are 5.7 fold higher in *Nf1*$^{flox/-}$*;Col2.3Cre* mice, which recapitulate multiple NF1 skeletal defects including, short stature, osteoporosis, and mechanically induced nonunion fracture. We further found that Nf1 deficient osteoblasts, the principal source of TGF-beta1 in bone, overexpressed TGF-beta1 in a gene dosage dependent fashion at both the mRNA and protein levels. Moreover, Nf1 deficient osteoclasts and osteoblasts were hyperresponsive to TGF-beta1 in tissue culture, preferentially augmenting *Nf1*$^{+/-}$ osteoclast bone resorptive activity, while inhibiting *Nf1*$^{-/-}$ osteoblast differentiation. These cellular phenotypes were accompanied with biochemical hyperactivation of the Smad pathway following TGF-beta1 stimulation. As an *in vivo* proof of concept, we found that administration of the pharmacologic TGF-beta type I receptor inhibitor, SD-208, can rescue both osteoporotic and non-union fracture phenotypes in *Nf1*$^{flox/-}$*;Col2.3Cre* mice, restoring bone mass and callus bone volume to WT levels. This study provides direct preclinical evidence for the critical role of Nf1 dependent TGF-beta1 signaling in the pathogenesis of NF1 associated osteoporosis and pseudarthrosis, thus implicating the TGF-beta signaling pathway as a potential therapeutic target in the treatment for NF1 osseous defects.

Steven D. Rhodes; Khalid S. Mohammad, M.D., Ph.D.; Theresa A. Guise, M.D.; Feng-Chun Yang, M.D., Ph.D., Indiana University

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Poster Presentation (odd numbers)

MONDAY, JUNE 11, 2012 (8:30 – 10:00 PM)

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Neurofibromin (NF1) and the Leucine Rich Pentatrico Peptide Repeat-motif Containing (LRPPRC) Protein Interact in RNA Granules and are Important for Peripheral Nerve Development

Vedant Arun
Hospital for Sick Children, Toronto, Canada and University of Toronto, Canada

Neurofibromin acts as a p21-Ras-GAP to directly interact with and inactivate p21-Ras, through its GAP Related Domain (GRD). This accounts for the neoplastic manifestations of NF1, but evidence suggests that non-Ras-GAP functions mediated through interactions with domains outside of the GRD are of importance. We have identified the Leucine Rich Pentatrico Peptide Repeat–motif Containing protein (LRPPRC) and Dynein Heavy Chain 1 (DHC) as novel NF1-Tubulin Binding Domain (TBD) interacting proteins. LRPPRC was of interest as it is mutated in Leigh's Syndrome, French Canadian (LSFC) variant, a cytochrome-oxidase deficiency syndrome characterized by neurodegeneration and psychomotor retardation, thereby having some similarities with non-tumor manifestations of NF1. Using a number of *in vitro*, *in situ* and *in silico* techniques we have identified the binding regions of the two proteins that are necessary and sufficient for the interaction, and have determined the binding affinity to be high (K_d=103nM). Towards elucidating the biological relevance of the interaction, we have established that the NF1-LRPPRC interaction occurs predominantly along microtubules, and complexes with motor proteins in a complex known as RNA granules. Use of conditional knockout triple transgenic mice have shown that Schwann cell-specific loss of Nf1 and Lrpprc results in a peripheral neurodegenerative phenotype characterized by demyelination, axonal degeneration, decreased Sciatic Nerve conduction velocities and poor performance on the RotaRod test. Further studies into the etiopathogenesis of NF-1 and LSFC in context of RNA granule function may help our understanding of the molecular mechanisms that contribute to the developmental phenotypes associated with these two debilitating syndromes.

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Detection of Coronin 1A Expression in NF 1 associated Tumors and the effect of p53 on this expression

Sükriye Ayter, Ph.D.
Hacettepe University, Turkey

Development of neurofibroma is a hallmark of Neurofibromatosis type 1 (NF1) and Schwann cells (SC) are considered as the primary cellular origin of Neurofibromas. Newly identified protein Coronin 1A, belongs to the actin regulating protein family and essential for cytoskeletal organization. Actin remodeling is important for cancer development. An imbalance of F-actin assembly/disassembly may be responsible for the invasive potential of human cancers. Recent studies have shown that the coronin 1A protein level is distinctively increased /decreased in various kinds of cancer, such as melanoma, breast cancer and hepatocellular carcinomas. However, its expression in NF 1 related tumors is unknown.

Therefore we studied the location and the expression level of Coronin 1A and 1B in NF1 related tumors and SC which are predominant cell population of the neurofibromas. As a preliminary work, expression analysis of coronin 1A performed with RT-PCR based studies. Recently it has been also shown that p53 transactivates the expression of Coronin 1B. Coronin 1A which is structurallly similar to Coronin 1B might also be regulated by the p53. To prove this we will use GAP 43 (Growth Associated Protein-43) gene expression levels in order to determine the functionality of p53 by indirect way. From axon regeneration studies it is known that p53 is required fort he expression of GAP 43. Standard immunohistological procedure was performed for coronin 1A, S100 and p53 in 9 sporadic NF tumors. Normal human SC were used as control. Coronin 1A found around the perinuclear area in the cell. Results were correlated with our previous study and Coronin 1A had reduced expression in tumor samples. Detailed results covering different types of tumors and the existence of GAP43 will be presented on poster. There are some data related to Coronin expression in literature which has been done on different tumors but this is the first study with NF1. The aberrant expression of Coronin 1A and its possible relation to p53 could be a useful marker for NF 1 tumors.

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[1] Tedeschi A, Nguyen T, Puttagunta R, Gaub P, Di Giovanni S A p53-CBP/p300 transcription module is required for GAP-43 expression, axon outgrowth, and regeneration., *Cell Death Differ*, 16:543–554, 2008. [2] Wu L, Peng CW, Hou JX, Zhang YH, Chen C, Chen LD, Li Y. Coronin-1C is a novel biomarker for hepatocellular carcinoma invasive progression identified by proteomics analysis and clinical validation. J Exp Clin Cancer Res. 29:17, 2010

The role of cMAF in Neurofibromatosis type 1

Meghan E. Brundage
Cincinnati Children's Hospital Medical Center, University of Cincinnati

Mutation or loss of NF1 results in elevated Ras signaling and the formation of neurofibromas and malignant peripheral nerve sheath tumors (MPNST). The cell of origin for tumorigenesis is thought to be of the Schwann cell lineage, however the progressive downstream changes within Schwann cells or their precursors that cause tumorigenesis and/or proximal consequences of NF1 loss remain unknown. Differential gene expression analyses comparing primary tumor-derived Schwann cells, MPNST cell lines, and NF1 solid tumors identified SOX9, a neural crest transcription factor required for stem cell survival, as a tumor biomarker and survival gene product (Miller et al. EMBO Mol Med, 2009). **Here, we identify cMAF, a transcription factor known to bind to SOX9, in the NF1/SOX9 molecular pathway as a regulator of Schwann cell development and differentiation and as a potential tumor suppressor in MPNST**. cMAF expression was strongly inversely correlated relative to SOX9 in human gene expression analyses of tumors versus normal Schwann cells. This negative correlation was validated *in vitro* as evidenced by high Sox9 expression and low cMaf expression in mouse Schwann cell precursors when compared to mature Schwann cells. cMAF expression or activity was rescued in MPNST cell lines by restoring NF1, inhibiting RAS, MEK, or AP-1 activity, indicating that cMAF is regulated (directly or indirectly) by an NF1/RAS//MAPK/AP-1 pathway. Restoring cMAF expression in MPNST cell lines revealed a tumor suppressive role as characterized by decreased expression of SOX9, increased expression of Schwann cell differentiation markers, and increased cell death. However, we found that cMAF inhibits DEPTOR, an important regulator of the AKT/mTOR pathway. Therefore, the increased TORC1 activity we have seen by restoring cMAF may also have a pro-survival effect. Preliminary xenograft results with cMAF transduced S462TY cells show a trend toward slower tumor growth, however increased TORC1 activity as a result of cMAF inhibition of DEPTOR may decrease efficacy. Dual inhibition of the MAPK and AKT/mTOR pathways in *in vitro* assays have revealed various combinations of chemotherapeutic agents that potently inhibit MPNST survival. **These studies suggest that regulation of cMAF may contribute to tumorigenesis by affecting Schwann cell differentiation, reveal a novel crosstalk pathway in MPNST between the MAPK and AKT/mTOR pathways, and reveal a mechanism by which dual inhibition of these pathways may not only be rational but necessary.**

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AR-42, a Pan-Histone Deacetylase Inhibitor (HDACi), Causes G2 Arrest in Meningioma Cells while Arresting Normal Meningeal Cells at G1 and Potently Inhibits Tumor Growth in a Quantifiable NF2-deficient Benign Meningioma Model

Sarah S. Burns
Nationwide Children's Hospital and The Ohio State University

Meningiomas constitute ~34% of primary intracranial tumors and are associated with an increased risk of mortality in patients with neurofibromatosis type 2 (NF2). Current treatment for these tumors is challenging. To develop a medical therapy, we have established a quantifiable model for *NF2*-deficient meningiomas. We showed that Ben-Men-1 cells harbored a single nucleotide deletion in *NF2* exon 7, leading to a premature termination. Consequently, these cells did not express the merlin protein. Using Ben-Men-1 cells, we investigated the mechanism underlying growth inhibition by AR-42, an HDACi. AR-42 treatment inhibited proliferation of both normal meningeal and Ben-Men-1 cells by increasing expression of CDK inhibitors $p16^{INK4A}$, $p21^{CIP1/WAF1}$, and $p27^{KIP1}$. Also, AR-42 increased proapoptotic Bim expression and decreased anti-apoptotic Bcl_{XL} levels. However, AR-42 induced cell-cycle arrest at G1 in normal meningeal cells while it arrested Ben-Men-1 cells at G2. Consistently, AR-42 treatment substantially decreased the levels of cyclin A and PCNA, two proteins induced in S phase, in meningeal cells while moderately reducing these proteins in Ben-Men-1 cells. To compare the *in vivo* efficacies of AR-42 and AR-12, a PDK1 inhibitor, we generated luciferase-expressing Ben-Men-1-LucB cells and established intracranial Ben-Men-1-LucB xenografts that grew over time. AR-42 and AR-12 treatment reduced tumor size by 80~98% and 34~65%, respectively, after six months. Importantly, AR-42-treated tumors showed minimal regrowth when xenograft-bearing mice were switched to normal diet. In conclusion, an intracranial *NF2*-deficient benign meningioma model has been established and used to demonstrate AR-42 and AR-12 as potential therapies for these tumors. The differential effect of AR-42 on cell-cycle progression of normal meningeal and meningioma cells may have implications for why AR-42 is well-tolerated while it potently inhibits tumor growth.

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Elucidation of Merlin's Biochemical Function through Purification of Specific CRL4^{DCAF1} Substrates

Jonathan Cooper
Memorial Sloan-Kettering Cancer Center

Merlin, the protein encoded by the *NF2* tumor suppressor gene, functions in contact inhibition and tumor suppression by inhibiting potentially multifarious mitogenic signaling pathways. The mechanisms by which Merlin suppresses mitogenic signaling, and therefore the pathways that are paramount in Merlin's normal biological function and tumor suppression, remain elusive. Genetic epistasis analysis and gene expression profiling reveal that Merlin inhibits growth and suppresses tumorigenesis by translocating to the nucleus and inhibiting the Cul4-Roc1$^{DDB1/DCAF1}$ (CRL4^{DCAF1}) E3 ubiquitin ligase. Identification of the physiologic targets of CRL4^{DCAF1} is an important step in identifying Merlin's biochemical function in the nucleus and establishing drug targets for the treatment of Merlin-deficient tumors. There are no known substrates of CRL4^{DCAF1} that are relevant in the context of Merlin-deficient tumorigenesis. Furthermore, identification of E3 ubiquitin ligase targets is a great technical challenge, entreating a more efficient process to identify specific binding partners of the ligase. We have designed a purification system using pathogenically relevant cell lines in combination with quantitative proteomics to identify specific interactors of CRL4^{DCAF1}. We will present methods to identify specific targets of CRL4^{DCAF1} and preliminary results based on our initial screens.

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Role of Retinoic Acid Signaling in subtypes of NF1 associated Peripheral Nerve Sheath Tumors

Susan Fischer
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Since subtypes of neurofibromatosis type 1 (NF1) associated neurofibromas, cutaneous and plexiform ones, show differences in biological behaviour and clinical outcome we primarily aimed to investigate differentially expressed protein patterns. By comparative proteome analysis of human cutaneous and plexiform neurofibroma derived Schwann cells we identified, beneath others, the cellular retinoic acid binding protein (CRABPII) as being significantly higher expressed in cutaneous compared to plexiform neurofibromas.

Our candidate protein, the tumour suppressor CRABPII, is crucial for transport of alltrans retinoic acid (ATRA) into the nucleus and thus for controlling the cellular functions of retinoic acid (RA) such as differentiation, organogenesis and inhibition of neoplastic proliferation. We hypothesize that decreased CRABPII expression in plexiform neurofibroma reduces the anti-proliferative effects and the differentiationpromoting function of RA and thus contributes to infiltrative and diffuse growth as well as to malignant change. Preliminary experiments using NF1 associated primary tumor derived Schwann cells as well as MPNST cell lines showed a significant response to ATRA as demonstrated by a decline of proliferation, induction of differentiation and apoptosis.

We now investigated the role of RA and CRABPII as well as RA signaling pathways in more detail. To determine an RA independent role of CRABPII we performed RNAi mediated knockdown of CRABPII in primary tumor Schwann cells and MPNST cell lines. Additionally, CRABPII/FABP5 (fatty acid binding protein 5) ratio as well as expression of nuclear retinoic acid receptors was examined to understand the differences in therapeutic response to ATRA of the aforementioned cell types. In conclusion, we characterized RA signalling in NF1 associated peripheral nerve sheath tumors, underlining the relevance of using ATRA for therapy of those tumors.

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Development of a Novel Murine Model of Neurofibromatosis Type 2

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Neurofibromatosis type 2 (NF2) is a disease characterized by the development of schwannomas of the cranial, spinal, and peripheral nerves, as well as meningiomas and ependymomas. Bi-allelic inactivation of the NF2 gene is found in over 90% of sporadic schwannomas and over 50% of sporadic meningiomas. The majority of individuals with NF2 experience hearing loss early in life, often in their 20's. The hearing loss in these patients is due to the development of vestibular schwannomas, benign glial cell tumors of cranial nerve VIII (CN VIII) that almost always develop bilaterally. The previous mouse model of NF2 (*P0Cre; Nf2*$^{flox/flox}$) does not accurately recapitulate human NF2 disease, as schwannomas are only seen in 35% of mice, and these mice do not develop intracranial tumors. Additionally, *P0Cre*; *Nf2*$^{flox/flox}$ mice do not develop hearing loss. In preliminary studies, we intercrossed conditional NF2 mutant (*Nf2*$^{flox/flox}$) mice to transgenic PostnCre mice in which a 3.9-kb periostin promoter drives expression of Cre recombinase in neural crest-derived cell lineages in the developing and adult mouse. Using histological analysis, we have observed that these mice universally develop multiple spinal tumors of the dorsal root ganglion (DRG) and peripheral nerves. Additionally, auditory brainstem response (ABR) evoked potential testing has revealed that these mice develop age-dependent hearing loss similar to human patients. At the age of six months, *PostnCre+*; *Nf2*$^{flox/flox}$ mice possess hearing thresholds similar to age-matched controls. However, by 10 months of age many *PostnCre+*; *Nf2*$^{flox/flox}$ mice have hearing thresholds that are considerably higher than age matched controls ($p < .01$). This hearing loss correlates with cranial nerve hyperplasia. Furthermore, preliminary data indicate that *PostnCre+*; *Nf2*$^{flox/flox}$ mice also possess a statistically significant decrease in survival ($p < .05$) as measured by Kaplan-Meier analysis. Collectively, these genetically engineered mice acquire a phenotype that closely recapitulates important features of human NF2 disease and provides opportunities for testing putative therapeutic targets using genetic intercrosses or novel small molecule inhibitors.

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Identification of novel molecular modifiers of NF1 learning disabilities in Drosophila

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Neurofibromatosis 1 (NF1) is a common inherited disorder, affecting 1 in 3,000 worldwide. Among its hallmarks are tumors of the nervous system, short stature and learning disabilities. NF1 is caused by loss-of-function mutations in neurofibromin, a negative regulator of Ras. *Drosophila melanogaster* lacking a conserved NF1 ortholog are reduced in size and display learning defects. Both defects resemble human NF1 symptoms and are restored by increasing cAMP/PKA signaling. However, how loss of NF1 affects the cAMP/PKA pathway remains largely unknown. We recently identified the neuronal *dAlk* tyrosine kinase receptor and its activating secreted ligand *jelly belly* (*jeb*), as rate limiting upstream activators of *dNf1* regulated Ras/ERK signals responsible for both organismal growth and olfactory learning defects. To identify additional rate limiting components of dNf1 pathways, we conducted a genetic screen for dominant modifiers of the *dNf1* growth defect. Identified suppressors confirmed the previously implicated *dAlk* and *jeb*, among other proteins involved in Ras/ERK signal transduction. Other modifiers include members of neuropeptide/cAMP/PKA pathways, components of the synaptic machinery, and proteins involved in vesicular transport. However, whether these candidates can also modify *dNf1* learning defect remains unknown. Hence, we proposed to use the extant modifiers of the *dNf1* size defect to launch a systematic investigation of whether they also modify the learning deficits. Specifically, we are using an arsenal of individual mutants and RNAi lines that modify the *dNf1* size defect and we are currently in the process of testing them using the classical Pavlovian associative olfactory learning model. Based on the fact that many of the identified modifiers of the dNf1 size defect are known to play crucial roles in neuronal function, we anticipate that a substantial number of them will also modify *dNf1* learning defects. We will present and discuss our preliminary results obtained from this ongoing targeted behavioral screen.

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Rac1 controls Schwann cell myelination through NF2/merlin signaling

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During peripheral nervous system development, Schwann cells surround axons and differentiate into Schwann cells myelinating single large axons or non-myelinating Schwann cells ensheathing multiple small axons (Remak bundles). Previous studies implicated both Rac1 in myelination. Through analysis of adult mice, we find that Schwann cell myelination is arrested in Rac1 conditional knockout (Rac1-CKO) mice. Rac1 deletion decreases activation of the effector p21-activated kinase (PAK) and decreases NF2/merlin phosphorylation. NF2/merlin is the protein product of Nf2, a tumor suppressor gene mutated in neurofibromatosis type 2. Based on *in vitro* studies NF2/merlin was proposed to act up and downstream of Rac1. To test the role of NF2/merlin in Rac1 regulated Schwann cell differentiation, we crossbred Rac1-CKO mice with NF2 mutant transgenic mice (NF2-del, P0-SCH-delta-39-121) and studied Schwann cell differentiation in double mutant mice. Myelination and Remak bundle defects in Rac1-CKO mice are rescued by mutation of NF2/merlin. Shorter processes in cultured Rac1-CKO Schwann cells are also rescued by mutation of NF2/merlin. The data demonstrate that NF2/merlin functions downstream of Rac1 signaling in Schwann cell differentiation *in vivo*.

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Decoding palindrome-mediated deletion mechanisms

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Genomic rearrangements represent gross DNA variations—deletions, duplications, insertions and translocations—affecting hundreds of base pairs to mega-bases. Decoding genomic rearrangement mechanisms is important because genomic rearrangements can cause Mendelian and complex traits by dosage effects, gene disruption, gene fusion, position effects, and other molecular mechanisms. So far, non-allelic homologous recombination (NAHR), non-homologous end joining (NHEJ), and fork stalling and template switching (FoSTeS) are believed to be the major mechanisms for genomic rearrangements. Palindromic regions are unstable and susceptible to genomic rearrangement due to DNA replication stalling or endonuclease digestion of DNA secondary structures. Accordingly, palindromic regions may lead to various rearrangements such as deletions, duplications or translocations. Currently, palindrome-mediated translocations and intra-palindrome deletions have been reported mediated by NHEJ and replication slippage, respectively. However, palindrome-mediated deletions and duplications are not yet well studied, so the mechanisms leading to such rearrangements are still unclear. We have identified 5 unrelated neurofibromatosis type 1 (NF1) patients carrying a pathogenic deletion involving exon and intron 31, known to harbor an intronic ~200 bp long palindromic sequence. We hypothesized that in these patients this deletion could be mediated by this palindromic sequence, which was confirmed through precise characterization of the deletion breakpoints. Microhomologies from 1 to 6 bp were found at all breakpoint junctions with the breakpoint affecting the palindrome being the 3' breakpoint and the 5' breakpoint being located within <7.1kb in either intron 29 or 30. Four out of the 5 3' breakpoints clustered in a very small region of 6 bp within the palindrome. Based on these observations, we propose that FoSTeS might be the major mechanism leading to palindrome-mediated deletion. Palindrome secondary structure can stall DNA replication leading the stalled strand to disengage from the original replication fork and invade a nearby replication fork via microhomology. However, as palindromic regions are double strand break (DSB) hotspots, we cannot exclude the possibility that NHEJ is used for DSB repair.

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Mammary tumors developed in mice with *Nf2* knockout in mammary epithelial cells exhibit $CD24^{low}CD44^{high}$ stem cell properties

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Mutations in the *NF2* gene have been detected in human breast cancer. To examine whether *Nf2* plays a role during the development of breast cancer, we conditionally inactivated *Nf2* in mammary epithelial cells at different stages of mammary gland development using Wap1-Cre (designated $Nf2^{Wap1}$ CKO) and Blg-Cre ($Nf2^{Blg}$ CKO). Remarkably, 100% of both $Nf2^{Wap1}$ CKO and $Nf2^{Blg}$ CKO mice developed mammary hyperplasia and mammary tumors following multiple gestation cycles. The mammary tumors from both $Nf2^{Wap1}$ CKO and $Nf2^{Blg}$ CKO mice expressed myoepithelial markers (CK14, SMA, and p63) and pan-keratin AE1-3, and displayed an epithelial-mesenchymal transition (EMT) phenotype. Flow cytometry analysis detected a population of $CD24^{low}CD44^{high}$ cells resembling mesenchymal stem cells (MSCs) in $Nf2^{Wap1}$ CKO and $Nf2^{Blg}$ CKO mammary tumors, and this population of cells efficiently formed mammospheres. Intriguingly, when tumor-derived mammospheres were co-cultured with MCF10A cells, spheres surrounded by cells that displayed characteristics of EMT, as evident by decreased E-cadherin and increased vimentin expression, were observed. Consistently, the supernatant from a tumor-derived mammosphere culture could induce normal mammary epithelial MCF10A cells to undergo EMT. In addition, the tumor-derived mammospheres were more tumorigenic than the original mammary tumor cell culture when inoculated into the fat pad of SCID mice. These results indicate tumorigenic potential of the tumor-derived mammospheres. Also, $Nf2^{Wap1}$ CKO and $Nf2^{Blg}$ CKO mammary tumor cells and spheres showed strong expression of phospho-Jak2 and phospho-Stat3, suggesting that *Nf2* may play a role in regulating Jak2/Stat3 signaling during tumorigenesis. Taken together, our results show that *Nf2* inactivation in mammary epithelial cells results in the formation of mammary tumors exhibiting $CD24^{low}CD44^{high}$ stem cell properties. Further investigation of a link between *Nf2* and human breast cancer is warranted.

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Axl a new Biomarker and Therapeutic Target for NF1

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Using a Receptor Tyrosine Kinase (RTK) array, we have identified 7 RTKs with increased phosphorylation in a panel of four Malignant Peripheral Nerve Sheath Tumor (MPNST) cell lines compared to Normal Human Schwann Cells (NHSC).

We have acquired a novel Multi-Kinase Inhibitor (*denoted MKI; name protected by Material Transfer Agreement*) targeting three of the RTKs found in our array namely: Axl, Met and the plasma derived growth factor receptor (PDGF). MKI reduced cell proliferation in four independent MPNST cell lines with an average IC50 of 0.5 μM. Compared with an IC50 of 5-10μM for specific inhibitors against EGFR, Met or PDGF. Similarly, the numbers of apoptotic cells were significantly increased in a dose dependant manner starting at 0.05μM of MKI. *In vivo*, daily treatment with MKI reduced the tumor growth of already established xenograft tumors with 50%. Altogether this argues for the usage of multi kinase inhibitors in the treatment of MPNSTs.

We went on to characterize the expression of Axl in NF1 related tumors. Axl was found to be an excellent biomarker correlating with tumor burden in NF1 patients. The expression of Axl was higher in MPNST tissues compared to the Sciatic nerve from an NF1 patient, with intermediate levels detected in the majority of dermal neurofibromas. Similar, a soluble fraction of Axl (sAxl) can be detected in human sera. The levels of sAxl was significantly higher in patients with plexiform neurofibromas (pNFA) compared to patients without pNFA ($p<0.01$). Finally, using human specific antibodies we can detect human sAxl in the sera of MPNST xenograft mice. Hence, the tumor cells can secrete sAxl into the sera, further supporting sAxl as a biomarker for NF1 related tumorigenesis.

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Susceptibility to NF1-associated pheochromocytoma is modified in females by *Pheom1* on mouse chromosome 16

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Pheochromocytoma is a rare catecholamine-producing neuroendocrine tumor of the chromaffin cells in the adrenal medulla. These neoplasms can occur sporadically or in conjunction with familial tumor syndromes such as multiple endocrine neoplasia type II (MEN2) or Neurofibromatosis type I (NF1). We previously reported pheochromocytoma in the *NPcis* mouse model for NF1, albeit with limited characterization. Here, we provide more detailed histological characterization of the tumors and demonstrate that inbred *NPcis* females on the C57BL/6J (B6) background were more susceptible to pheochromocytoma than their male siblings. Moreover, B6-*NPcis* females were significantly more prone to adrenal tumors than 129S4/SvJae (129) *NPcis* females revealing strain and sex specificity of the phenotype. Backcross mapping and binary trait linkage analysis of 129x(B6x129)-*NPcis* animals revealed a female specific linkage peak on distal chromosome 16 corresponding to a 32 Mb region we refer to as *Pheochromocytoma modifier 1* (*Pheom1*). Female susceptibility to pheochromocytoma was significantly altered in response to variations in the background genotype of *Pheom1*, whereas males were not affected by *Pheom1* regardless of the genotype. Interestingly, *Pheom1* overlaps the *Ts65Dn* locus which was reported to cause increased susceptibility to adrenal tumors in female *Ts65Dn;NPcis* mice, further supporting our conclusion that *Pheom1* modulates risk to pheochromocytoma. Overall these data indicate that *NPcis* is an appropriate mouse model for pheochromocytoma studies, and that sex and strain specific modifiers in *Pheom1* dictate susceptibility to adrenal medullary tumorigenesis.

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Identification of a new phosphoryiation site on Merlin modulated during contract inhibition of proliferation

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There has been a considerable amount of research in identifying Merlin targets relevant for its tumour suppressor function, however little is known about how Merlin itself is regulated by upstream signaling. Since Merlin is a potent tumour suppressor it is crucial for us to improve our understanding about Merlin's regulation within cells and how loss of those regulatory mechanisms lead to tumourigenesis. During contact inhibition of growth Merlin is activated by dephosphorylation of a C-terminal serine residue 518 (S518) by the myosin phosphatase MYPT-1-PP1delta [Jin et al., 2006]. Using Merlin mutants that mimic either phosphorylated or dephosphorylated state we identified that while the S518 is required it is not sufficient to drive Merlin's growth inhibitory function. These results indicate that there might be other posttranslational modifications that are important for Merlin function. For this reason we started to screen for other posttranslational modifications that are necessary for the activation of Merlin. We have now identified Threonine 272 (T272) as a novel phosphorylation site of Merlin. To determine if phosphorylation at this amino-acid residue has an affect on Merlin's tumor suppressor function, we generated schwannoma cell lines that inducible express full-length Merlin with a mutation of the T272 residue. A substitution that mimics constitutive phosphorylation (T272 D) abrogates the ability of Merlin to interfere with anchorage-independent growth and Ras signaling. Substitution that mimics constitutive dephosphorylation (T272 A) behaves like an active Merlin. In addition to regulation by posttranslational modifications we are interested in the site of activity of Merlin. We have generated a Nmyristoylated Merlin that is constitutively located at the plasma membrane. We can show that expression of lipid-modified Merlin prior to cell-cell contact inhibits Ras activity and cell proliferation indicating that the plasma membrane is at least one site of activity for Merlin in Schwann cells. This localization and inhibition with Ras activity is more than likely a part of its tumor suppressor function.

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Conditional inactivation of *Pten* and overexpression of *EGFR* in Schwann cells results in early high-grade peripheral nerve sheath tumor development

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The mechanisms responsible for genetic evolution from a benign neurofibroma to a malignant peripheral nerve sheath tumor (MPNST) in sporadic or neurofibromatosis type 1 (NF1) syndrome-associated patients remain elusive. It is hypothesized that many genetic changes are required for this transformation process. Currently, Schwann cells and/or their precursor cells are believed to be the primary pathogenic cell source in peripheral nerve sheath tumor (PNST) formation and malignant progression. It is becoming evident that *PTEN* regulation plays an important role in disease progression since *PTEN* transcript is reduced during the progression from plexiform neurofibromas to MPNSTs. Overexpression of *EGFR* has also been shown to be an important driver in low-grade PNST initiation. In order to validate the role of these two genes in PNST tumorigenesis *in vivo*, transgenic mice overexpressing *EGFR* in Schwann cells and/or their precursor cells were bred with mice carrying conditional floxed alleles of the *PTEN* gene. *Desert hedgehog* (*Dhh*) regulatory element driving Cre recombinase transgenic mice (*Dhh*-Cre) were then used to inactivate *PTEN* in Schwann cells and/or their precursor cells. Transgenic mice with *EGFR* overexpression and *PTEN* inactivated have an early postnatal lethality (median survival age of 26-days) and displayed various peripheral nervous system phenotypes. These mice had multiple enlarged dorsal root ganglia, with high incidence of enlarged brachial plexus and trigeminal nerves at various stages of PNST tumorigenesis. Importantly, the peripheral nervous system phenotype displayed in our mouse model recapitulates human MPNSTs histologically. Taken together, our data suggests that reduced *PTEN* expression, together with *EGFR* overexpression, can drive malignant progression of low-grade to high-grade PNSTs. Importantly, our novel mouse model recapitulates sporadic human MPNST and will be useful for testing therapies to prevent or reverse tumor progression.

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Histological Analysis of Angiogenesis in NF2 associated and sporadic meningiomas

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Background: Meningiomas and schwannomas are common tumors in NF2 patients and cause morbidity and mortality in these patients. Bevacizumab has been shown to lead to shrinkage of vestibular schwannomas and hearing improvement in some NF2 patients treated with bevacizumab. On the other hand, meningiomas in these patients have shown limited response to bevacizumab: only 30% of the tumors shrink > 20% in volume and this response was not durable. Tissue analysis of schwannomas shows marked decrease in SEMA3A and SEMA3F expression, inhibitors of the VEGF pathway in schwannomas, suggesting VEGF pathway activation in these tumors. VEGF pathway and angiogenesis are not known in meningiomas and the results would have implications regarding the rationale of using anti-VEGF treatment for meningiomas in NF2.

Methods: We analyzed the expression pattern of components of the VEGF pathway and tumor microvascular density in 26 meningiomas including 13 NF2 associated and 13 sporadic meningiomas.

Results: Tissue analysis showed no correlation between tumor microvascular density and expression of VEGF pathway components in NF2 associated and sporadic meningiomas. SEMA3 expression was retained in both types of meningiomas.

Discussion: In contrast to schwannomas, activation of the VEGF pathway appears not to be the primary driver for angiogenesis in meningiomas and may explain the limited activity of bevacizumab in treatment of NF2-associated meningiomas.

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Characterization of a new phosphorylation site of Merlin

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The neurofibromatosis 2 (NF2) gene product, merlin, belongs to the ezrin–radixin–moesin (ERM) subgroup of the Protein 4.1 family and functions as a tumor suppressor protein and an inhibitor of cells growth. Recent studies have clarified the molecular mechanisms underlying this function. However, less is known concerning how Merlin activity is regulated. However, several phosphorylation events have been documented that modulate Merlin stability, interaction with molecular partners or with the actin cytoskeleton. We postulated that more phosphorylation sites on Merlin may exist that could participate in the regulation of its activity.

Methods and results: Merlin was overexpressed in 293T as a GFP fusion protein and purified by GFP-Trap. Following specific enrichment for phosphoproteins, the immunoprecipitate was then subjected to mass spectrometry analysis for the identification of phospho-peptides. Using this strategy we could confirm the presence of phosphoresidues that were already identified at position 13 (Serine), 315 (Serine) and 518 (Serine). In addition to these sites, we also found a novel phosphorylated threonine that was not previously described previously, in position 581 in the mouse sequence. This phosphorylation has been confirmed in mouse tissues such as spleen and kidney. Interestingly, this site is specific of the isoform I of Merlin, suggesting that it may be involved in some of the functionnal differences that were observed between the two major isoforms.

We have generated various mutants of this new site, alone or in combination with S518 mutants as well as cell lines expressing them. The impact of these mutants on proliferation, migration, actin remodeling will be discussed. We are also investigating the role of T581 in the regulation of cell signaling, more specifically on the Hippo pathway, the binding capacity of the mutant Merlin on several of its components and the interactions between the FERM domain and the C-terminus end of the protein.

Finally, we will discuss our efforts to identify the kinases that phosphorylate Merlin on the threonine 581.

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Merlin Serine-10 Phosphorylation Regulates Schwann Cell Morphology and Alignment on Sensory Axons

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Mutations in the *NF2* gene encoding merlin induce tumors of the nervous system. Merlin activity is regulated by phosphorylation at various sites. Two well-documented residues are: serine-518 in the C-terminal that is phosphorylated by both p-21-activated kinase (PAK) and protein kinase A (PKA), and serine-10 (S10) in the FERM domain that is phosphorylated by PKA. The non-phosphorylated form of merlin-S10 (S10A) was shown to regulate cytoskeletal organization in COS-7 cells and mouse embryonic *Nf2*$^{-/-}$ fibroblasts (Laulajainen et al., 2008). Overexpression of merlin in primary rat Schwann cells (SCs) grown with neurons (SCs/Ns cultures) promotes the alignment of SCs with axons by stabilizing the bipolar morphology (Thaxton et al., 2011). Overexpression of WT merlin modulates SC morphology and increases the number of SCs with unusually long processes (Thaxton et al., 2008). We studied the effect of merlin-S10 phosphorylation on the morphology of isolated SCs and those co-cultured with sensory neurons. When merlin-S10A, a non-phosphorylated form was expressed in primary rat SCs, the majority of SCs were unipolar with long processes. In contrast, this phenotype was less frequently observed in SCs expressing WT and merlin-S10D. In SCs/Ns cultures, the number of axon-aligned bipolar SCs was lower when SCs expressed merlin-S10A than when SCs expressed WT and merlin-S10D. These results demonstrate that merlin-S10 phosphorylation regulates merlin-dependent changes in morphology and their ability to engage and align on axons.

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Small Molecule Inhibitors of Insulin-like Growth Factor-1 Receptor Kinase Autophosphoryiation Suppress NF1 MPNST Proliferation

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The insulin-like growth factor-1 receptor (IGF-1R) is a transmembrane, ligand-activated, tyrosine kinase. IGF-1R mediated signaling promotes cell proliferation, motility, and cell survival. Expression of the IGF-1R is commonly up regulated in many tumor types including plexiform neurofibromas and malignant peripheral nerve sheath tumors (MPNST). In the current study we investigated the potential therapeutic effects of two small molecule inhibitors of IGF-1R autophosphorylation (i.e., cyclolignan picropodophyllin [PPP] and NVP-AEW541) on the growth and viability of six Neurofibromatosis type 1 (NF1) MPNST cell lines (S462, S462TY, TsNF96.2, NF02.2, NF90-8, ST88-14) and one non-NF1 MPNST cell line (STS-26T). Exposure of all 7 MPNST cell lines to $\geq$ 50 nM PPP resulted in a concentration-dependent suppression of cell proliferation, with $\geq$ 250 nM PPP completely inhibiting cell proliferation. Cell cycle analyses indicated that PPP induced the accumulation of cells with tetraploid DNA contents. A large percentage of that tetraploid population arrested in metaphase, rounded-up, and subsequently underwent apoptosis. The remaining tetraploid cells eventually developed distinct nuclei, but did not execute cytokinesis, which resulted in binucleated cells. A majority of these binucleated cells had irreversibly withdrawn from the cell cycle and died with nonapoptotoic features. Concentrations of NVP-AEW541 $\geq$ 100 nM and $\leq$ 5 micromolar were partially cytostatic to S462TY cells. These effects were associated with an accumulation of cells in G1, and no accumulation of binucleated cells. Higher concentrations of NVP-AEW541 were very cytotoxic and rapidly induced apoptosis. Immunoblot analyses of IGF-1R autophosphorylation (at tyrosines 1131, 1135 and 1136) in cultures grown in medium supplemented with serum yielded very weak signals, which raises the issue of whether IGF-1R signaling is operative in the MPNST lines under standard culturing conditions, and thus whether the observed potential therapeutic activities of these small molecule inhibitors might actually represent off-target effects. Irrespective of the answer, PPP was very effective at killing the MPNST cell lines, and phase II trials in the context of other cancers show that the agent is well tolerated in humans.

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Antioxidant Rescue of Nf1/Ras-induced Myelin and Vasculature dysfunction

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Human brain imaging suggests altered myelin may contribute to the macrocephaly found in *NF1* patients. We modeled white matter enlargement by inducing Nf1 loss or HRas hyperactivation in oligodendrocytes using an inducible *PLP-CreERT; Nf1 floxed* or a new *CNP-HRasG12V* mouse models. Electron microscopy revealed myelin decompaction, concurrent axon enlargement, and alterations in connexin localization within myelin. Non-cell autonomous effects included the expansion of perivascular astrocytic endfeet, blood brain barrier leakiness, and loss of gap junctions between astrocytic endfeet. Extracellular nitric oxide (NO) has previously been shown to cause gap junction disruption in cultured astrocytes, and nitric oxide synthases (NOS1, 2, & 3) were upregulated in affected white matter tracts. Furthermore optic nerve size and all cellular phenotypes were reversed after treatment of CNP-HRasG12V mice with the antioxidant N-Acetyl Cysteine (NAC) *in vivo*. We therefore suggest that the ability of Nf1/Ras to regulate gap junction formation through NOS may be a fundamental biological process, and dysregulation of this system causes pathologic symptoms that may be reversible using antioxidant therapy.

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An *in vitro* model of schwannomatosis-related pain

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Many individuals with schwannomatosis have loss of function mutations in both alleles of *NF2* and *INI1/SMARCB1*, resulting in decreased production of merlin and INI1, respectively. Loss of these proteins may change the homeostatic influence of Schwann cells on sensory neurons that initiate pain signaling and underlie the severe pain that is a prominent symptom in schwannomatosis. An *in vitro* model of schwannomatosis was created to examine interactions between Schwann cells and sensory neurons. Schwann cells and sensory neurons were isolated from adult mouse sciatic nerve and dorsal root ganglia, respectively. While in culture, the expression of merlin or INI1 were reduced in Schwann cells using siRNA technology. After siRNA treatment, conditioned media from Schwann cells was collected and the effect of Schwann cell-conditioned media (SCCM) on the release of calcitonin-gene related peptide (CGRP) from sensory neurons was measured. Since CGRP is a peptide transmitter for a subclass of sensory neurons involved in pain signaling, changes in CGRP release allows monitoring of the sensitivity of sensory neurons and the chemical influence of Schwann cells on these neurons. Reduced expression of merlin or INI1 was confirmed using Western immunoblotting. SCCM from Schwann cells with reduced expression of either merlin or INI1 did not alter the resting or stimulus-evoked release of CGRP, suggesting that the reduction of both genes may be necessary to alter the influence of Schwann cells on sensory neurons. Ongoing studies will examine this possibility and identify morphological changes in Schwann cells with reduced INI1 and merlin expression. Examining the interaction between Schwann cells and sensory neurons will allow better understanding of the molecular mechanisms underlying schwannomatosis-related pain.

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Silvestrol, a novel inhibitor of protein translation with potent activity against NF2$^{-/-}$ tumors

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Inactivating mutations in the *Neurofibromatosis 2* (*NF2*) tumor suppressor gene commonly cause vestibular schwannomas (VS) and meningiomas. Treatment options for patients with these tumors are limited to surgical resection and radiation therapy, making the development of effective chemotherapeutics highly desirable. In this study, we screened a natural compound library for molecules with sub-micromolar potency at inhibiting the growth of cultured VS and meningioma cells. Out of the 23 compounds tested, four (silvestrol, episilvestrol, cucurbitacin D, and bruceantin) inhibited the growth of primary VS and meningioma cells and Ben-Men-1 cells at sub-micromolar concentrations. The 50% inhibitory concentration (IC_{50}) values for all four were less than 300 nM, with silvestrol being the most potent ($IC_{50} \sim 20$ nM for primary meningioma cells and 10 nM for VS and Ben-Men-1). In contrast, 4 commonly used natural compounds, CAPE, curcumin, resveratrol, sulforaphane, had low potency. Silvestrol is reported to inhibit cap-dependent translation initiation. Silvestrol treatment of Ben-Men-1 cells resulted in a marked dose-dependent accumulation of cells in the G2/M phase. Consistently, Ben-Men-1 cells treated with silvestrol showed dramatically decreased levels of PCNA and cyclin A, molecules essential for progression through S and G2 phases. In addition, silvestrol treatment noticeably reduced the phosphorylation of ERK1/2 and AKT, two key mitogenic proteins. In conclusion, silvestrol is a novel natural compound that interferes with several signaling pathways critical for the growth of meningioma cells. The potent antiproliferative effect of silvestrol indicates that it merits further investigation as a potential therapy for *NF2*-associated tumors.

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TC21/R-Ras2 acts through TGF-beta to mediate the *NF1* oncogenic switch

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Ras proteins are involved in transforming growth factor beta (TGF beta) mediated development, and paradoxically in both tumor suppression and tumor progression. Whether specific Ras GTP-binding proteins integrate with TGF beta signaling pathways in vivo is unknown. We activated all Ras proteins *in vivo* by absence of the Nf1 GTPase activating protein (GAP). In mice lacking both *Nf1* and the Ras-related protein *TC21*/R-Ras2 benign neurofibroma formation was delayed. Conversely, in *Nf1* deficient models *TC21* loss accelerated growth of brain tumors and sarcomas. This duality implicated TGF beta, and elevated TGF beta mRNA and protein expression in *Nf1* Schwann cell precursors were reversed by *TC21* loss. *TC21* loss also blocked an *Nf1*/TGFβRII/AKT dependent autocrine precursor survival loop and decreased precursor numbers, implying that delayed benign tumor formation resulted from effects on tumor initiation. Increased $Nf1^{-/-}$ sarcoma size induced by *TC21* loss was also TGF beta dependent, but mainly non-cell autonomous. Gene expression analysis, RNA, and protein analyses demonstrated increases in TGF beta ligands and absence of TGF beta RII in human malignant peripheral nerve sheath tumors (MPNST), with effects of TGF beta on endothelial cells and myofibroblasts. Thus, the Ras-related protein *TC21* is a critical *in vivo* regulator of TGF beta.

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LIM Kinase, a Potential Therapeutic Target for NF2

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Loss of function mutations in the Neurofibromatosis 2 (*NF2*) gene causes Neurofibromatosis Type 2 (*NF2*). The *NF2* gene encodes a tumor suppressor protein named merlin. Loss of merlin function is associated with increased levels of active Rac and p21-activated kinases (PAK). The serine/threonine LIM kinases (LIMK1 and 2) are substrates for PAK. LIMK regulates actin dynamics and thus cytoskeletal organization in cells by phosphorylating and inactivating cofilin at serine 3. Cofilin is a direct actin depolymerizing factor. LIMK also phosphorylates the cyclic-AMP responsive element-binding protein (CREB) transcription factor at Ser133. Here we report that that the protein levels of LIMK1 and 2 in human schwannomas and mouse Schwann cells with inactivated Nf2 by deletion of its exon2 ($Nf2^{-/-}$) are increased as compared to normal human and mouse Schwann cells. Additionally, mouse $Nf2^{-/-}$ Schwann cells have increased levels of active LIMK1/2 as revealed by higher levels of phosphorylation at threonine 508/505 compared to normal mouse Schwann cells. We tested the effect of a small molecule LIMK inhibitor, BMS-5 on mouse $Nf2^{-/-}$ Schwann cells. We found that BMS-5 decreased the levels of phosphorylated cofilin-Ser3 and phosphorylated CREB-Ser133 dimer in a dose dependent manner. Concurrently, BMS-5 decreased the viability of mouse $Nf2^{-/-}$ Schwann cells in a dose dependent manner as well. The loss of viability of mouse $Nf2^{-/-}$ Schwann cells was not due primarily to caspase3/7 dependent or independent apoptosis, but rather to inhibition of cell cycle progression. Our results indicate that LIMKs are a potential new target for *NF2* therapy.

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Sexual dimorphic and neurofibromin-dependent alterations in the adenylyl cyclase/cAMP cascade

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The *NF1* gene product, neurofibromin (NF), is known to be an important modulator of adenylyl cyclase (AC) activity and cAMP concentrations within several model systems. Decreasing NF levels reduces cAMP concentrations, suggesting NF may be a positive regulator of the AC/cAMP cascade. The effects of NF on the AC/cAMP signal cascade within sensory neurons could have significant effects on neuronal sensitization and function. Furthermore, there are recent observations that murine astrocytes from males have greater AC capacity and reduced phosphodiesterase activity compared to astrocytes from female mice, resulting in higher resting cAMP levels in the astrocytes from males. These findings suggest that regulation of the AC/cAMP cascade is different between males and females. To investigate the sexual dimorphic effects of NF on the AC/cAMP cascade, resting and stimulated cAMP concentrations were measured in dorsal root ganglia (DRG) from wild-type and $Nf1^{+/-}$ mice. Resting conditions were measured in the presence and absence of 3-isobutyl-1-methylxanthine (IBMX) (2 mM), a phosphodiesterase inhibitor, to establish if cAMP degradation is deregulated after NF depletion. Resting cAMP concentrations in the absence of IBMX were not different between genotypes, but cAMP concentrations were significantly lower in both wild-type and $Nf1^{+/-}$ DRGs from males compared to cAMP concentrations in DRGs from females. However, when resting cAMP concentrations were measured in the presence of IBMX, there were no significant differences between the genotypes or sexes. This suggests that phosphodiesterase expression and/or activity may be higher in DRGs from males than from females. A low concentration of forskolin (100 nM), a direct AC activator, significantly increased cAMP concentrations in DRGs from wild-type females but not in DRGs from $Nf1^{+/-}$ females or DRGs from males of either genotype, suggesting that DRGs from wild-type females are more sensitive to cAMP stimulation. This data also suggests that the loss of NF within DRGs from females results in deregulation of the AC/cAMP cascade, and NF's actions as a positive regulator of the AC/cAMP cascade may be sex-dependant. Elucidating sexually dimorphic effects of NF within the AC/cAMP cascade will enhance our understanding of the cellular functions of NF, which may aid in our understanding of some of the symptom variability of the disease.

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High resolution melting analysis of DNA methylation status of the genomic segment at the verge of a CpG island in the *SMARCB1* gene

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Schwannomatosis is an autosomal dominant disorder characterized by the development of multiple schwannomas, without the occurrence of bilateral vestibular schwannomas (VS), however unilateral VS has recently been reported in schwannomatosis patients (Smith et al. 2011. Am J Med Genet A. doi: 10.1002/ajmg.a.34376). Most cases of schwannomatosis are sporadic and SMARCB1 has been identified as a schwannomatosis-predisposing gene. Involvement of this tumor suppressor gene in ~50% of familial and ~10% of sporadic cases was found independently by several groups. Clearly, the genetic underlying cause for the majority of schwannomatosis patients remains unknown as of today. Therefore, regulatory mechanisms altering expression levels of the *SMARCB1* gene, such as epigenetic silencing through DNA methylation, have been considered as possible causal factors.

As the core of the *SMARCB1* promoter CpG island was inaccessible for reliable and unbiased amplification of methylated DNA, we designed a High Resolution Melting (HRM) assay to study the methylation status of a 252 bp genomic segment located at the verge of the CpG island (chr22:24129666-24129917, hg19). This GC rich segment contains 12 CpG sites that are potential subject to DNA methylation. The assay was optimized to allow detection of partial methylation, i.e. >25%. *In vitro* methylated human DNA standard was used as a control. The HRM analysis was carried out on bisulfite converted DNA samples which were derived from 29 schwannomatosis patients who had no germline DNA mutations in the *SMARCB1* and NF2 genes. The analysis included 11 tumor and 27 blood DNA samples.

We found no CpG methylation in the studied DNA segment in any of the samples. Therefore other genetic or epigenetic factors at different loci are likely responsible for schwannomatosis phenotype in patients without *SMARCB1* and *NF2* mutations.

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Trp53 loss modifies *EGFR*-driven peripheral nerve sheath tumorigenesis

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Analysis of human MPNSTs has identified some of the common genetic drivers of this tumor type. One common event is the biallelic inactivation of the *NF1* gene. However, *NF1* loss alone is not sufficient for MPNST formation based on results from genetically engineered mouse models. Besides *NF1* loss, deletions and/or point mutations of *TP53* occur in ~40-75% of human MPNSTs. These mutations rarely inactive both alleles of *TP53* suggesting that haploin sufficiency is sufficient for MPNST formation in the context of accumulating other genetic mutations. In addition to genetic losses in human MPNSTs, gene amplifications also commonly occur. Epidermal Growth Factor Receptor (*EGFR*) gene amplification occurs in ~25-85% of human MPNSTs. Mouse models overexpressing human *EGFR* in Schwann cells show increased incidence of benign neurofibroma formation, but no MPNST formation. This suggests cooperating mutations are needed for MPNST progression.

Here, we describe a new mouse model of sporadic neurofibroma and MPNST formation. Mice overexpressing *EGFR* in Schwann cells/precursors and were heterozygous for *Trp53* developed Schwann cell tumors at a ~60% penetrance with median time of tumor harvest of 316 days. Schwann cell tumors histologically resembled human neurofibromas and MPNSTs. Immunhistochemical analysis of tumors demonstrated activation of the Mek/Erk pathway and p21 expression suggesting *EGFR* was activated and that *Trp53* signaling was intact. Genetic analysis of tumor-derived cell lines demonstrated loss of the *Trp53* wildtype allele (~80%) and high incidence of aneuploidy. Moreover, tumor-derived cells injected into immune compromised mice developed tumors that histologically resembled MPNSTs.

In summary, loss of Trp53 function cooperates with overexpression of *EGFR* in Schwann cells and their precursor cells to form MPNSTs in mice. Additionally, this system provides a new model for sporadic MPNST development that resembles the human disease. Currently, we are modulating *EGFR* and *TP53* expression in immortalized human Schwann cells and MPNST cell lines to identify molecular interactions between *EGFR* and *TP53* pathways in human Schwann cell tumorigenesis.

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Identification of novel MPNST genes by an insertional mutagenesis screen

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To elucidate mechanisms of Schwann cell transformation to MPNSTs (Malignant Peripheral Nerve Sheath Tumors), we used the *Sleeping Beauty* (*SB*) transposon-based somatic mutagenesis system in mice. We targeted *SB* transposon mutagenesis to Schwann cells and their precursors using the Schwann cell specific Cre-recombinase transgene, *CNPase-Cre*, and a conditional *SB* mutagenesis system. Since loss of *TP53* function and/or overexpression of *EGFR* are associated with MPNSTs, *CNPase-hEGFR* and conditional dominant negative $Trp53^{R270H}$ alleles were also included. Mice harboring both the *CNPase-hEGFR* and $Trp53^{R270H}$ alleles developed MPNSTs at a ~23.8% penetrance with a median time of tumor harvest at 362 days. Importantly, mice that also undergo *SB* mutagenesis have increased frequency of MPNST development (63.2%) with reduced latency (median 299 days).

Transposon insertion site analysis of *SB*-derived MPNSTs using high-throughput Illumina GAIIx sequencing identified 35 common insertion site (CISs) associated genes. These included known Schwann cell tumor suppressor genes, *Nf1*, *Nf2*, and *Pten*, which validated the screen. Moreover, many of the CIS-associated genes were enriched in Wnt/Beta-Catenin, PI3K/Akt/mTor, and growth factor receptor signaling pathways, some of which are altered in human MPNSTs. In addition, we identified several novel proto-oncogenes including *FOXR2*.

Immunohistochemical analysis of a tissue micoarray comprised of 30 MPNSTs and 60 neurofibromas (dermal and plexiform) demonstrated a significant increase in *FOXR2* expression in MPNSTs compared to neurofibromas. Human MPNST cell lines demonstrated a significant increase in *FOXR2* expression compared to immortalized humans Schwann cells (iHSCs). *In vitro* experiments overexpressing *FOXR2* in iHSCs led to increased proliferation, migration, and colony formation indicating anchorage independent growth. This phenotype was confirmed using *shRNA* knockdown in human MPNST cell lines resulting in a reduction in proliferation, migration, and colony formation.

In summary, *SB* mutagenesis in concert with overexpression of *EGFR* and *Trp53* loss of function accelerated and increased MPNST formation. Sequencing analysis identified known genes and new gene candidates in MPNST formation that may provide new therapeutic targets for treatment of MPNSTs.

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Application of MEK inhibitor therapy to a mouse model of tibial pseudarthrosis featuring localized double inactivation of the *Nf1* gene

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Congenital tibial dysplasia (CTD) is a severe orthopaedic condition that occurs sporadically in children with Neurofibromatosis type 1 (*NF1*). Stevenson et al. reported in 2006 that children with CTD exhibited local double-inactivation of *NF1* locally within their osseous lesions (Am J Hum Genet. 79:143-8, 2006). We have developed a mouse model that recapitulates the localized double inactivation of Nf1 in open and closed tibial fractures.

We are able to generate reproducible models of fracture healing in the mice tibia with closed fractures or open osteotomies (with periosteal stripping) performed. We have created a model of localized *NF1* double inactivation by injecting $NF1^{flox/flox}$ mice with a Cre expressing adenovirus (AdCre) at the fracture site. This creates delayed or non-unions in both closed fractures (100% union in controls vs <40% in $NF1^{null}$ fractures, $P<0.05$ at 3 weeks), and open fractures (75% union in controls <30% in $NF1^{null}$ fractures, $P<0.05$ at 3 weeks). The $NF1^{null}$ state was associated with a significant increase of up to 15-fold more fibrotic tissue and this tissue was found to be transduced by AdCre using a Z/EG (conditional GFP) reporter transgene. In this model no statistically significant differences were seen with between $NF1^{flox/flox}$ and $NF1^{flox/-}$ mice suggesting that a heterozygous background is not critical for impaired healing in this model.

NF1 deficiency affects the Ras-MAPK signaling pathway, which has been demonstrated *in vitro* to impair osteoblast differentiation. We hypothesized that differentiation of the cells at the fracture site may be impaired, and this may be specifically reversed using a MEK inhibitor PD0325901 (10-25mg/kg/day). In wild type mice this was found to promote chondrogenesis and prevented cartilage removal during fracture repair, although ceasing dosing at 10d post-fracture enabled conventional cartilage removal. Preliminary data from the $NF1^{null}$ fracture model indicates that 10mg/kg/day PD0325901 during the early stages of bone repair led to increased chondrogenesis, but did not impair fibrosis and did not significantly improve union rates.

In summary, we have developed a localized $NF1^{null}$ fracture model that recapitulates many of the features of human tibial pseudarthrosis that can be used for therapeutic evaluation. Preliminary experimentation suggests that PD0325901 alone may not be sufficient to rescue the healing defect in this model, but combination therapies with rhBMPs and/or other signaling inhibitors and different delivery systems may yield improved outcomes.

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Cancer Stem Cell-Like Cells Derived from Malignant Peripheral Nerve Sheath Tumors

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This study aims to examine whether or not cancer stem cells exist in malignant peripheral nerve sheath tumors (MPNST). Cells of established lines, primary cultures and freshly dissected tumors were cultured in serum free conditions supplemented with epidermal and fibroblast growth factors. From one established human MPNST cell line, S462, cells meeting the criteria for cancer stem cells were isolated. Clonal spheres were obtained, which could be passaged multiple times. Enrichment of stem cell-like cells in these spheres was also supported by increased expression of stem cell markers such as CD133, Oct4, Nestin and Nerve Growth Factor Receptor, and decreased expression of mature cell markers such as CD90 and Neural Cell Adhesion Molecule. Furthermore, cells of these clonal S462 spheres differentiated into Schwann cells, smooth muscle/fibroblast and neuron-like cells under specific differentiation-inducing cultural conditions. Subcutaneous injection of the spheres into immunodeficient nude mice led to tumor formation at a higher rate compared to the parental adherent cells (66% versus 10% at $2x10^5$). Whole genome micro array analysis showed different expression of approximately 15.000 genes (30% of genes examined) between the stem cell-like cells and their adherent parental MPNST 462 cells. One of the top 20 increased candidates with highest signal log ratio was Ras-Related Nuclear Protein, a component of the Wnt-signaling pathway. Pathway-analysis further revealed alteration of additional components of this pathway, suggesting its involvement in stemness of the MPNST cells. These results provide evidence for the existence of cancer stem cell-like cells in MPNST. Studies are in progress to further characterize these cancer stem cell-like cells and to identify targets for therapy development.

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Reduced expression of the SWI/SNF-subunit BAF170 in neurofibromatosis associated and sporadic peripheral nerve sheath tumors

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SWI/SNF (SWItch/Sucrose NonFermentable) is an ATP-dependent chromatin-remodelling-complex which allows compacted DNA to become accessible for transcription factors and RNA polymerase. It regulates processes such as DNA methylation, reparation, recombination and replication. The evolutionary highly conserved complex consists of nine essential and variable subunits some of which possess tumor suppressor activity.

Several studies demonstrated mutations of the SWI/SNF protein SMARCB1 (SWI/SNF-relatedmatrix-associated actin-dependent regulator of chromatin subfamily B member 1) (INI1 (integrase interactor 1)) in malignant tumors such as rhabdoid tumours, AT/RTs (Atypical teratoid rhabdoid tumor), and sarcomas as well as in benign tumors like meningiomas and schwannomas, the latter being associated with schwannomatosis.

To elucidate the role of the SWI/SNF complex for development of peripheral nerve sheath tumors, we characterized other SWI/SNF subunits than INI1 and identified loss of BAF170 (BRG1-associated factor 170) in a high percentage of INI1 expressing schwannomas by immunohistochemistry. To identify the molecular mechanisms for reduced expression of BAF170 in those tumors, we investigated RNA and protein expression, and also carried out sequencing and methylation analysis. Furthermore, BAF 170 expression was analysed in various human tissues.

In conclusion, we identified a reduced expression of the SWI/SNF-subunit BAF170 in peripheral nerve sheath tumors assuming a potential tumor suppressor role of this protein.

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Identification of Novel Pathogenic Missense Mutations in the GTPase-Activating Protein (GAP)-Related Domain of the Neurofibromatosis Type-1 (*NF1*) gene using functional, bioinformatic and structural analyses

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Neurofibromatosis type-1 (*NF1*), characterised by the development of benign and malignant peripheral nerve sheath tumours, is caused by constitutional mutations of the *NF1* gene. Whilst ~85% of inherited *NF1* microlesions constitute truncating mutations, the remaining ~15% are missense mutations with unknown pathological relevance. The GTPase-activating protein-related domain (GRD) of the *NF1* protein, neurofibromin, serves to define its major function as a negative regulator of the Ras-MAPK signalling pathway. At least 150 of the known *NF1* gene lesions are located within the GRD of neurofibromin, including 25 missense mutations. Of the 25 known missense mutations in the GRD, only four have so far been functionally characterized *in vitro*. We have established a functional assay to assess the potential pathogenicity of 16 non-synonymous mutations (11 novel) identified in the *NF1*-GRD. Individual mutations were introduced into an expression vector containing the *NF1*-GRD, and activated Ras was assayed by two independent assays: enzyme-linked immunoabsorbent assay (ELISA) and phosphorylated ERK levels. Eleven *NF1*-GRD variants were deemed to be pathogenic by virtue of significantly elevated levels of activated GTP-bound Ras in comparison to wild-type *NF1* protein. In sporadic cases, DNA from normal parental samples (where available) were checked for the sequence change. Our findings received broad support from both bioinformatic analysis and molecular modeling and serve to improve our understanding of *NF1*-GRD structure and function. This information will be useful for counselling and molecular diagnosis of *NF1* patients. A combination of functional, bioinformatic and structural analyses in this study therefore extends previous work in identifying the sites of *NF1*-GRD/Ras interaction and improving their definition.

David N. Cooper, Mark Richards, Matthew Mort, Laura Thomas .

Improved detection of type-2 *NF1* microdeletions and identification of breakpoint clusters

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Neurofibromatosis type-1 (*NF1*) is an autosomal dominant inherited disease that occurs with a frequency of 1:3000. Whereas 95% of all *NF1* patients harbour mutations within the *NF1*-gene, 5% of *NF1* patients exhibit large deletions of the NF1-gene and its flanking regions (termed *NF1* microdeletions). Four types of *NF1* microdeletions have been identified (type-1, type-2, type-3 and atypical) that differ with respect to breakpoint localization and the underlying causative mechanism. Multiplex-ligation-dependent probe-amplification (MLPA) has frequently been employed to identify *NF1* microdeletions. However, the unambiguous typing of *NF1* deletions is impossible in many instances due to the spacing of the probes included in the currently available MLPA-kit (P122-C1, MRC-Holland). Indeed, distinguishing between type-2 and certain atypical *NF1* deletions is impossible using this MLPA-kit. In this study, we have developed an improved set of MLPA-probes that allows the unambiguous identification of type-2 *NF1* deletions and potentiates breakpointmapping by PCR. Using a combination of this improved MLPA-technique and breakpoint-spanning PCR, we analysed 30 *NF1* microdeletions initially considered to be type-2 deletions according to results obtained with the MLPA-kit P122-C1. We determined that 25 of the 30 deletions were indeed classical type-2 microdeletions, with breakpoints located in the *SUZ12* gene and its pseudogene *SUZ12P*. However, 5 deletions turned out to be atypical exhibiting only one of both breakpoints within the *SUZ12* sequences. Taken together with 17 previously identified type-2 deletions whose breakpoints have been localized, the analysis of a total of 40 type-2 *NF1* deletions revealed a significant clustering of breakpoints within the *SUZ12* sequences.

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Canonical Wnt/Beta-Catenin Signaling Plays a Role in Malignant Peripheral Nerve Sheath Tumor Development and Progression

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While many genetic changes are required for the formation and progression of Schwann cell tumors, the pathways that drive this process remain elusive. The current standard of treatment for patients with Malignant Peripheral Nerve Sheath Tumors (MPNSTs) is high dose, non-specific chemotherapy, and the 5 year survival rate remains low, exemplifying the need for the identification of drug targets that can be targeted for therapy in these patients. Here, we show that canonical Wnt/Beta-Catenin signaling is activated in a subset of MPNSTs, plexiform neurofibromas and dermal neurofibromas by gene expression microarray and human tissue microarray (TMA) immunohistochemical analyses. Our functional studies also show that activation of Wnt signaling is sufficient to oncogenically transform normal human Schwann cells, and down-regulation of this pathway can reduce the tumorigenic phenotype in human MPNST cell lines. Therapeutic agents that target Wnt signaling are effective at inhibiting proliferation *in vitro* in MPNST cell lines, with little effect on normal human Schwann cells. These Wnt-targeted therapies show synergistic effects with previously identified therapies targeting the mTOR pathway, in which either drug alone is highly cytostatic, but combination therapies synergistically induce apoptosis. These results demonstrate that Wnt signaling could be a novel therapeutic target for patients with MPNSTs.

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CPI-17 in proliferation control: pushing Ras activity from two sides

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The tumor suppressor protein merlin is an important regulator of cell proliferation and mutations in the respective *Nf2* gene are found in various types of tumors. Our lab has previously shown that upon sensing cell contact merlin becomes activated by dephosphorylation which is catalyzed by the MYPT1-PP1delta phosphatase. We could furthermore show that the activated merlin inhibits the activation of the small GTPases Ras and Rac leading to inhibition of cell proliferation and we propose that this is a part of merlins tumor suppressive activity. In this tumor suppressing cascade the phosphatase inhibitor CPI-17 was identified as a potential oncogene that specifically inhibits MYPT1-PP1delta thereby blocking merlin activation. We hypothesized that enhanced CPI-17 levels might be an alternative mechanism for tumor development compared to *Nf2* mutations. Indeed, in a cellular model overexpression of CPI-17 increased the level of phosphorylated, inactive merlin with subsequent increase in Ras activation and transformation. Interestingly, we found that ERM proteins (ezrin, radixin, moesin), which are closely structurally related to merlin, are also a target of MYPT1-PP1delta and their phosphorylation status is similarly increased upon CPI-17 overexpression. In contrast to merlin, ERM proteins are activated upon phosphorylation and are in fact counterplayers of merlin in Ras activation. Using *in vitro* cellular transformation assays, CPI-17 induced transformation via loss of functional merlin as well as gain of ERM function driving Ras activity. We propose that the oncogene CPI-17 acts by inhibiting MYPT1-PP1delta leading to loss of merlins tumor suppressor function and switching to a tumor promoting activity of ERM proteins. The relevance of this dual effect of CPI-17 on cellular transformation for human tumor development is demonstrated by our finding that CPI-17 is abnormally expressed in several human tumor cell lines and tumor samples, including astrocytomas and melanomas. To verify the role of CPI-17 in tumorigenesis we now aim to overexpress CPI-17 in an in vivo model and ask whether CPI-17 expression through modulating merlin and ERM activity contributes to tumor formation providing a basis for a cancer therapy targeting the oncogene CPI-17 and ERM proteins.

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EGFR-STAT3 signaling promotes NF1 peripheral nerve tumorigenesis and transformation

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Neurofibromatosis type 1 (*NF1*) patients form benign neurofibromas and malignant peripheral nerve sheath tumors (MPNSTs). Growth factor receptors, particularly *EGFR*, have been implicated in *NF1*-driven neurofibroma formation and malignant transformation, but their precise roles and relevant signaling pathways remain unknown. To test the relevance of *EGFR* expression to benign neurofibroma formation and malignant transformation and to identify possible pathways and genes that might contribute to neurofibroma formation, we bred *Nf1*$^{flox/flox}$*;DhhCre* mice, 100% of which form neurofibromas (Wu et al., 2008), to *CNP-hEGFR* mice and to *Wa2* mice, an *EGFR* hypomorphic allele. We used *sleeping beauty* (*SB*) insertional mutagenesis and ingenuity pathway analysis to predict pathways and genes that might contribute to neurofibroma formation. *EGFR* modified neurofibroma-initiating cell number and promotes transformation to aggressive GEM-PNST. *SB* identified a correlated STAT3 pathway. Immunohistochemistry demonstrated phosphorylated STAT3 (Tyr705) in human and mouse tumors. A specific JAK2/STAT3 inhibitor blocked neurofibroma-sphere formation *in vitro*, and reduced neurofibroma growth *in vivo*; STAT3 knockdown by shRNA fully prevented MPNST formation while FLLL32 delayed MPNST formation *in vivo*. Finally, reducing *EGFR* activity strongly reduced pSTAT3. Thus, an *EGFR*-STAT3 pathway regulates neurofibroma number and neurofibroma growth, and promotes transformation. These findings suggest that STAT3 may provide a novel therapeutic target for *NF1*-related tumors. Further, efficacy of the FLLL32 pharmacological inhibitor in reducing neurofibroma growth suggests a therapeutic treatment strategy. (Supported by R01 NS28840 to N.R., P50 NS057531 to N.R. and D.L. OSUCCC Pelotonia Grant and DAMD New Investigator Award NF100053 to J.W.)

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Analysis of *NF2* isoform 2 function in the organism using a mouse knockout model

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Most cases of neurofibromatosis type 2 (*NF2*) show loss of the *NF2* gene. The tumor suppressor Merlin is expressed from the gene NF2, which, via alternative splicing, generates two major isoforms differing at the very Cterminus. Isoform 1 is generally thought to be the main tumor suppressor while the role of isoform 2 is currently unknown. Since almost all mutations causing *NF2* are non-sense mutations leading to premature abort of translation and subsequent degradation of the protein product, both isoforms are frequently lost in *NF2* patients. In order to deepen our understanding of Merlin biology we have used a knockout approach of either *NF2* isoform 1 or isoform 2 in the mouse (generated by Marco Giovannini) in order to screen for functional difference between these two isoforms. Aged *NF2* isoform 2 KO mice develop prostate lesions up to *in situ* carcinoma stage IV and testicular lesions. Additionally we found a neuronal function of isoform 2 [presented by A. Schulz]. In order to complement the *in vivo* work we use MEFs generated from the animals and established cell lines to analyze the effect of *NF2* isoform 2 on various signaling pathways. We found tumor suppression like inhibition of Ras-MAPK and activation of Hippo are equally regulated by both isoforms. However, and in contrast to Ras and Rac1, which are inhibited by both isoforms, the small GTPase Rho is activated specifically by isoform 2. We show that isoform 2 binds to RhoGDI, an inhibitor of Rho. Currently we are working on the molecular details of this regulation: We hypothesize that Merlin isoform 2 acts as a GDI displacement factor, releasing Rho from inhibition by sequestering RhoGDI. Our overarching goal is to show that Rho regulation by Merlin isoform 2 is responsible for the cellular phenotypes seen in the KO animals and learn about the cellular signaling circuits that depend on isoform 2.

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Is there a relationship between head size and severity of autistic mannerisms in patients with neurofibromatosis type 1?

Maria T. Acosta, M.D.
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It is recognized that children with autism spectrum disorder (ASD) have a larger head circumference (HC) early in life than the general population. Likewise, macrocephaly is found in 30-50% of children with Neurofibromatosis Type 1 (NF1). Our group described a 8.9% frequency of clinically elevated ASD symptomatology in our patients with NF1, which is 9.5 times higher than in the general population. However, the relationship between ASD symptomatology and HC in patients with NF1 has not been explored. We aim to correlate ASD symptom severity with head size in this population.

Medical records were reviewed for 84 patients with a diagnosis of NF1. Participants with reported T scores > 60 on the Social Responsiveness Scale (SRS) were selected for analysis. Analyses of covariance (ANCOVA) were performed between HC and ASD symptom severity to evaluate the relationship between head circumference (range = 40-60.5 cm) and ASD symptomology, as quantified by SRS scores. Analyses were controlled for age (<6, 6-12, >12 y/o) and gender.

It was determined that individuals with NF1 and a T score > 60 in the autistic mannerisms domain of the SRS have, on average, a HC 1.47 cm smaller ($P<0.05$) than those who had T scores < 60. Therefore, increased autistic mannerisms were associated with a smaller HC.

We found that the presence of clinically elevated autistic mannerisms in patients with NF1 is associated with decreased HC as compared to children with pure NF1. Increased head circumference has also been described in children with ADHD. Interestingly, a study by Cutting et al. (2002) reported that males with NF1 and ADHD had a smaller HC, on average, than those with pure NF1. The corresponding association between HC and ASD symptoms in children with NF1 has not previously been described. Therefore, future studies should evaluate HC in patients with NF1 and ASD longitudinally and explore the neurophysiological factors that contribute to head size in this population.

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Patients with neurofibromatosis type 1 are at increased risk to develop vincristine-induced peripheral neuropathy

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Background: Chemotherapy-related toxicity in children with neurofibromatosis type 1 (NF1) is not well-described. Anecdotal observations suggest that children with NF1 may be at higher risk of developing chemotherapy-induced peripheral neuropathy (CIPN). Partial deficiency of neurofibromin, the NF1 gene product, is found in all cell types, including peripheral nerve. Previous studies suggest that neurofibromin may influence peripheral nerve integrity and response to inflammation and injury. In this study, we evaluated the incidence of CIPN in children with NF1 treated with vincristine for a low grade glioma.

Method: We conducted an IRB approved retrospective review of patients <21 years with NF1 treated with vincristine for low grade gliomas and a subset of age- and gender-matched non-NF1 patients. Data collection included indication for treatment, duration of treatment, cumulative vincristine dose, number of omitted/reduced vincristine doses, and signs/symptoms of neurotoxicity.

Results: 18 patients were identified with NF1 (56% male) who were treated with vincristine and carboplatin for a low grade glioma with median age at time of treatment of 5.9 years and median treatment duration of 1.1 years. The comparison population included 18 patients without NF1 (50% male); median age at treatment for this cohort was 4.3 years with median treatment duration of 1.2 years. Gender, age at treatment, and duration of treatment were not statistically different ($p = .74$, $p = .16$ and $p = .29$, respectively). 15/18 patients with NF1 required dose-reductions of vincristine due to CIPN compared with 6/18 patients without NF1 (83 versus 33%, $p=0.01$). Mean number of scheduled doses for patients with and without NF1 did not differ (28.7 and 33.5, $p=0.16$); however, the average percentage of cumulative vincristine treatment received for patients with NF1 was less than for patients without NF1 (88.5 versus 96.6%, $p=0.037$). Signs and symptoms of toxicity included leg pain, jaw pain, constipation, irritability, ptosis, other cranial nerve impairment, and distal weakness.

Conclusion: These results support the hypothesis that children with NF1 are at increased risk to develop CIPN. Further investigation of the pathophysiologic contribution of neurofibromin haploinsufficiency in vincristine chemotherapy-related toxicity is warranted.

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Speckled Lentiginous Nevi in Children and Young Adults with Neurofibromatosis Type 1

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Background: Speckled Lentiginous Nevi (SLN) have been reported in 1-2% of the general population, but the incidence of SLN in individuals with neurofibromatosis type 1 (NF1) is unknown. Little is known about the clinical behavior of SLN, if the lesion disappears with age or if it has a propensity to malignant transformation. Methods: Patients with NF1 enrolled on the NCI NF1 Natural History Study undergo longitudinal detailed skin examinations. Documented findings include (1) SLN: macular or papular speckles of dark-pigmented color within a macular café au lait-like lesion, (2) hypopigmented macule/patch (3) number of café au lait macules (CALM) ≥ 5 mm (4) and subtypes of CALM (reticulated: irregular borders; and variegata: two toned). The incidence of SLN is described and compared to age, number of CALM, hypopigmented macule/patch, and the subtypes of CALM in this patient population. Results: The NF1 Natural History study enrolled 118 patients, most patients had plexiform neurofibromas or other NF1 morbidity. Skin manifestations are summarized in the table. The median age of patients with and without SLN was 17.9 years (range 8.1-24.8) and 14.9 years (range 3.0-45.7), respectively (p=0.11). Patients with and without SLN had a median of 25 (range 6-48) and 19 (range 2-55) CALMs, respectively (p=0.040), a significant difference. There was no association between presence/absence of SLN and presence of reticulated, variegate, or hypopigmented macule/patch. Conclusion: The incidence of SLN in the NF1 patients studied is higher (19%) than previous studies have shown in the general population (1-2%), a finding that has not been previously reported to our knowledge. Confirmation of this finding in a non-select population of individuals with NF1 should be considered. Presence of SLN was significantly correlated with number of CALM, but not with age or the subtypes of CALM. Because the median age of patients enrolled on the natural history study was 15.4 years, longer follow-up will be necessary to establish if SLN decreases with increasing age, as previously suggested. The clinical significance of SLN in the NF1 population is unknown. The NF1 Natural History study provides a unique opportunity to follow these patients longitudinally and establish the clinical behavior of SLN.

Patients with detailed skin exam	67
Age (yrs.) Median (range)	15.4 (2.9-45.7)
No. of CALM ≥5 mm, Median (range)	20 (2-55)
Pts. with skin manifestations n (%)	
CALM	67 (100%)
SLN	13 (19%)
Hypopigmented macule/patch	12 (18%)
Subtype of CALM	
Reticulated	21 (31%)
Variegata	6 (9%)
Location of SLN n (%)	
Upper extremities	4 (30%)
Lower extremities	6 (46%)
Trunk	3 (19%)

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Infantile Myofibromatosis in a Patient with Neurofibromatosis Type 1

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A rare case of neurofibromatosis type 1 (NF1) and multicentric infantile myofibromatosis without visceral involvement is reported. Patient presented at 4 weeks-of-age with multiple subcutaneous nodules. Brain MRI revealed an enhancing lesion in the frontal lobe (11 x 15mm). Biopsy of a forearm lesion was initially interpreted as a spindle cell sarcoma, although it lacked the ETV6 rearrangement typically seen in infantile fibrosarcoma. Chemotherapy was initiated. The subsequent development of café-au-lait macules prompted a Genetics consult and a second biopsy which was initially interpreted as a plexiform fibrohistiocytic tumor. Chemotherapy was discontinued. Genetic testing for NF1 mutation revealed a two base-pair deletion (1541_1542 AG) consistent with a truncating mutation, confirming the diagnosis of NF1. *NF1* LOH of the tumor samples was negative excluding the possibility that the nodules represented an atypical presentation of NF1. Immunohistochemical screening of tumor tissue for proteins involved in DNA mismatch repair (PMS2, MLH1, MSH2, and MSH6) was normal. Chromosomal microarray was normal. Review of more recent biopsies and re-review of the previous specimens is most consistent with infantile myofibromatosis, a benign soft tissue tumor not uncommonly misdiagnosed as infantile fibrosarcoma. [1]. De Schepper *et al* reported myofibromas in a patient with NF1, however their patient had only two myofibromas and no CNS involvement [2]. CNS involvement in infantile myofibromatosis is rare, but has been described [3]. Future studies could potentially reveal genetic variability to link the two disorders.

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References: 1. Alaggio R, *et al*. Morphologic Overlap between Inantile Myofibromatosis and Infantile Fibrosarcoma: A Pitfall in Diagnosis. Pedaitric and Developmental Pathology 11, 355-362, 2008. 2. De Schepper S, *et al*. Multiple myofibromas and an Epidermal Verrucous Nevus in a Child with Neurofibromatosis Type 1. Dermatology 209, 223-227, 2004. 3. Tamburrini G, *et al*. Infantile myofibromatosis of the central nervous system. Childs Nerv Syst, 19, 650-654, 2003.

Using a Continuous Performance Task (CPT) to measure attention of children with neurofibromatosis type 1: The influence of intelligence

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Children with NF1 are frequently characterised as having attentional problems, with the reported frequency of ADHD around 40%. Continuous performance tasks (CPT) are commonly used by clinicians to provide an objective measure of sustained attention, assist in the diagnosis of ADHD and evaluate response to stimulant medication. Few studies have utilised CPT in children with NF1 and results have been inconclusive. The role of intelligence on CPT performance has generally not been considered in these studies. One study of children with NF1 found that those children with below average IQ had poorer CPT scores than those with average IQ. However, this study had a very small sample size. The aim of this study was to examine the sustained attention of children with NF1 and the influence of intelligence on CPT performance.

Study Design & Analysis. A total of 79 children with NF1 and 46 unaffected siblings (8 -16 years old) completed a CPT - the Test of Variables of Attention (TOVA) and a measure of intelligence. Parent and teacher ratings of attention were also obtained. The performance of children with NF1 was compared to normative mean values and unaffected siblings using one sample and dependent t-tests respectively. For children with NF1, those with low IQ (70-84) were compared to those with average IQ using independent t-tests.

Results. Children with NF1 made significantly more commission (impulsivity) and omission (inattention) errors when compared to normative values. When compared to unaffected siblings, children with NF1 made significantly more omission errors. There was no significant association between TOVA variables and parent or teacher ratings of attention. For children with NF1, those with low IQ made significantly more omission errors, were significantly slower and more variable in their responses when compared to children with average IQ. The ability to sustain attention improved with age.

Conclusion. Children with NF1 showed elevated levels of impulsivity and inattention. Clinicians need to consider the effect of IQ and age when interpreting TOVA scores, particular for children with NF1 who experience a general lowering of intelligence.

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Patient with a deletion of 15q14, including SPRED1 gene, and duplication of 16p13.11

Deborah Copenheaver
Children's National Medical Center

A 9-year old female was referred to Genetics for evaluation for possible neurofibromatosis with multiple cafe-au-lait spots, relative macrocephaly and with axillary and inguinal freckling. She also had some atypical manifestations including the presence of a cleft palate and mildly dysmorphic features. A whole genome Single Nucleotide Polymorphism (SNP) oligonucleotide array and molecular testing for neurofibromatosis was requested. Due to insurance constraints only the microarray was performed. Results demonstrated two submicroscopic alterations with a 5.3 megabase deletion of chromosome 15q14 encompassing the SPRED1 gene producing Legius syndrome, in addition to a 1.02 megabase duplication of chromosome 16p13.11 of uncertain significance. Neither parent has been tested for either the deletion or duplication at this time.

The patient presents with macrocephaly, 10-15 cafe-au-lait spots, axillary and inguinal freckling, upslanting palpebral fissures, a broad forehead and broad nasal bridge. She was born with a cleft palate. There is a positive family history for possible autosomal dominant polycystic kidney disease on the paternal side. The patient has been diagnosed with ADD and is on Focalin. She also receives OT and ST in school. An MRI of the brain was normal with no stigmata that is associated with NF1, such as T2 lesions.

In a recent paper that reviewed 4 cases of deletions of the SPRED1 gene, patients had phenotypes varying from those similar to that seen in SPRED1 mutations to the more typical manifestations associated with neurofibromatosis, type 1. The deletion in this patient is larger than those previously reported. It is also more likely that all of the patient's physical variations are due to this contiguous gene deletion of chromosome 15q14.

This proband had an unexpected finding of chromosome abnormalities including a deletion of 15q14, and duplication of chromosome 16p13.11. The size of the deletion, which includes the SPRED1 gene, likely explains this unusual phenotypic presentation. Further testing in the parents and other family members may be considered.

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Bone Mineral Density in Pediatric Patients with Neurofibromatosis Type I

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Studies have documented a high prevalence of short stature and low bone mineral density (BMD) in Neurofibromatosis type 1 (NF1) patients. However many of these studies fail to account for differences in height especially among pediatric patients which can lead to erroneous interpretation.

To study bone mineral apparent density (BMAD) and bone mineral content (BMC) in pediatric patients with NF1 while accounting for gender, age, ethnicity, vitamin D status, and stature; as well as to assess the relationship between plexiform neurofibromas and BMAD.

Hologic dual energy x-ray absorptiometry scans (DEXA) obtained from 69 patients with NF-1 with a mean age 13.7 ± 4.8. BMD was normalized to derive a reference volume by correcting for height through the use of BMAD, an estimation of volumetric bone mass g/cm3, as well as the whole body BMC/ht. BMAD of the lumbar spine (LS 2-4), femoral neck (FN), and total body BMC/ht were measured and Z scores were calculated using a pediatric reference database. Impaired BMD was defined as a BMD score ≤ -2. Data was analyzed using paired t-tests and Pearson correlation coefficients; data was reported as mean ±SD where appropriate.

Forty-seven percent of patients exhibited impaired bone mineral density at any bone site, with 36% at the lumbar spine, 18% at the femoral neck and 20% total BMC/height. BMAD Z scores of the LS (-1.6 ± 1.26) were more impaired compared to the FN (-0.54 ± 1.58; p = 0.0003) and the whole body BMC/ht Z scores (-1.16 ± 0.9; p = 0.0072). Fifty-two percent of patients had moderate vitamin D deficiency, however, vitamin D status was not correlated with BMAD Z scores for any of the 3 sites. Ninety-one percent of our patients had plexiform neurofibromas; tumor burden as a percent of body weight was inversely correlated with LS BMAD Z scores (r = -0.36, p= 0.01).

In pediatric patients with NF1, LS BMAD was more severely affected than the FN BMAD or whole body BMC/ht. There was no correlation between vitamin D and BMAD.

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Longitudinal Assessment of Spinal Bone Mineral Density in Children with NF1 using Quantitative computed tomography (QCT)

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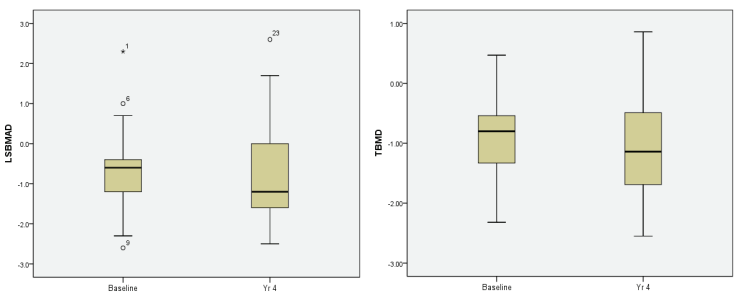
Scoliosis is a common skeletal problem affecting 10-30% of patients with NF1. NF1 patients have been shown to have reduced bone mineral density (BMD) which may play a role in the pathogenesis or progression of scoliosis. Our centre is one of four international centres currently evaluating the efficacy of various spinal imaging techniques and BMD as predictors for scoliosis in NF1.In our cohort we hypothesised that lumbar spine (LS) BMD and LS trabecular BMD (TBMD) would be reduced in children with NF1.

Clinical examination, spinal x-ray and bone densitometry was undertaken in 22 children with NF1 aged 6-9 years (12 female). This was repeated at year 4. BMD of L1-L4 was measured by dual energy absorptiometry; data was expressed as bone mineral apparent density (BMAD; g/cm3) and values transformed to Z scores using previously published normative data (ADC;2007;92(1):53-9). Volumetric TBMD (TBMD; mg/cm3) of L1-L3 was also measured using QCT; values transformed to Z scores using Mindways softwareTM (Austin,Texas). The mean difference between years 1 and 4 was calculated using a paired T test.

Year 1 mean Z score LSBMAD (-0.62±1.1; p=0.01) and TBMD (-0.86±0.7; p<0.001) were less than zero. Initial data for year 4 mean Z score LSBMAD (-0.75±1.3; p=0.01) and TBMD (-1.07±0.94; p<0.001) were also less than zero.

Mean difference in LSBMAD between year 1 and 4 is (-0.07±0.64; p=0.58) and for TBMD (-0.21±0.5; p=0.07).
Conclusion: Children with NF1 had reduced LS BMD which was more marked in the trabecular compartment .This appears to be a sustained reduction.



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Feasibility of Photodynamic Therapy Using Intratumoral Light in Children with Neurofibromatosis Type 1 and Plexiform Neurofibromas

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Plexiform neurofibromas (PN) cause serious morbidity in patients with neurofibromatosis type-1. Complete excision is the only known effective therapy. Photodynamic Therapy (PDT) is a locoregional therapy in which systemically administered photosensitizers (e.g. talaporfin sodium) are activated locally by illuminating tumor with a specific light wavelength. Activation of talaporfin sodium forms reactive oxygen species, leading to vascular thrombosis and tumor cell death. A novel PDT approach uses implantable LED light sources (LitxTM). Preclinical and clinical studies in adults with refractory solid tumors indicate that this strategy is tolerable and results in tumor volume reduction.

We performed a phase I study of PDT to determine the maximal tolerated dose (MTD) of light combined with talaporfin sodium for treatment of PN in children. Light dose was to be escalated from 50J/cm to 200J/cm using a 3+3 design. In one day, light source was placed by interventional radiology, talaporfin sodium delivered intravenously, light energy delivered, and light source removed. Post-treatment light exposure guidelines were mandated for two weeks.

Seven subjects were enrolled at two dose levels. One subject was inevaluable for dose escalation due to procedural-related toxicity (increase in neuropathic pain from baseline grade 1 to 3) with light source placement. Pain resolved to baseline after light source removal; subject received no study therapy. No dose-limiting toxicities were identified. Nine toxicities (grades 1/2) at least possibly related to photosensitizer/light treatment were seen. Three subjects had transient increases in pain from pre-treatment grade 1 to grade 2. Only one photosensitivity reaction (grade 1) occurred. No subjects had tumor response or evidence of tumor destruction in the region of light source placement.

Placement of light source and treatment with PDT was feasible and safe up to 100J/cm. The MTD was not reached. The study closed early due to expiration of study materials.

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The prevalence of autism in NF1: Evidence from a two-phase population based study

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Research over the last decade has highlighted the psychiatric morbidity in the NF1 population. Our recent epidemiological study found high rates of autism (29%) in a large representative population sample (n=109) of children aged 4-16 years using parent and teacher rated *Social Responsiveness Scale* (SRS). The aim of our current study is to undertake a phase 2 detailed characterization of questionnaire-positive children in order to estimate the prevalence of autism in the NF1 population.

A total of 109 children participated in the phase 1 study. A random stratified sampling strategy is being used to recruit a sample of 50 children from the phase 1 study sample. A proportion of children from each of the 3 groups- those who scored in high, mild-moderate and normal range of the SRS are being invited for the second phase study.

Measures: The Autism Diagnostic Interview (ADI) is being used to obtain a developmental history from the parent or the caregiver. The Autism Diagnostic Observation Schedule is being used to assess the child. In addition, we are using the Weschler Abbreviated Scale of Intelligence (WASI) to measure verbal IQ and the parent rated Vineland Adaptive Behaviour Scale (VABS) to measure adaptive functioning.

Preliminary data suggest that the prevalence of autism in NF1 is consistent with our earlier phase 1 study (around 30%). Data collection is ongoing, but conclusive results will be available for the Conference. The findings will have important implications for clinical management of patients with NF1. For autism studies, these findings will make NF1 an important single-gene model for autism symptoms.

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Fracture Risk in Neurofibromatosis 1

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Neurofibromatosis 1 (NF1, von Recklinghausen's disease) is an autosomal dominant neuro-cutaneous-skeletal syndrome. Low bone mineral density (BMD) and osteoporosis are common in NF1, but not necessarily influence the fracture risk. In the current study, the fracture risk in NF1 was evaluated by screening the hospital medical records of 460 Finnish patients with NF1. The control population included 3988 appendicectomy patients whose age and gender distribution was similar to that of the NF1 patients. Medical records of NF1 and control cohorts were screened for fractures according to ICD-10 between January 2000 and October 2011. BMD measures were available from 50 NF1 patients.

Patients with NF1 had increased fracture risk compared to controls. Specifically, patients with NF1 aged 41 years and older had risk ratio of x5.2 for fractures compared to controls. Children with NF1 had x3.4 risk ratio for fractures compared to children without NF1. In contrast, the fracture risk was not increased in NF1 patients aged 17-40 years. No gender related differences were observed. Furthermore, NF1-related osteoporosis was shown to be an additional risk factor for fractures. We recommend fracture risk assessment for all patients with NF1 aged ~40 or more, since fracture risk was increased five-fold in this age group. We also recommend to consider prophylactic measures, such as lifestyle advice, to prevent fractures from occurring if osteoporosis is found.

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The use of bevacizumab in the treatment of Schwannoma-related pain in a patient with Neurofibromatosis Type 2

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Bevacizumab, an antiangiogenic therapy that works as a vascular endothelial growth factor inhibitor, has been shown to be effective in the treatment of vestibular schwannomas in with Neurofibromatosis Type 2 (NF2). To date, there have been no reports of bevacizumab used to treat pain resulting from other schwannomas in this population. We present a report of a young adult with NF2 and complex spinal plexiform neurofibromas causing intractable pain successfully treated with bevacizumab.

Our patient is a 17 year old male with NF2 complicated by multiple schwannomas of the spine. He presented at 9 years of age with lumbar spine schwannomas that resulted in significant back pain. Debulking was performed with improvement in pain. He again presented at 14.5 years with increasing back pain and shooting pain in his legs. He was unsuccessfully treated with medications for neuropathic pain as well as narcotics and by 15 years imaging showed increasing size of spinal schwannomas and he was started on imatinib. He experienced stability of lesions while on imatinib however he had worsening of his pain and deterioration of his quality of life. He experienced intractable pain while on imatinib despite increased narcotics and pain blocks and imaging demonstrated growth of spinal schwannomas. Given reports of effectiveness in the treatment of NF2-related schwannomas, patient was started on bevacizumab. Since starting therapy 10 months ago, he has experienced imaging stability of his schwannomas. He has been able to wean off pain medications he had been on for over 2 years with significant improvement in his quality of life. He has had no significant side effects from treatment. This is the first report of successful use of bevacizumab for pain control and these findings warrant exploration of the use of Avastin in patients with NF2 and schwannoma-related pain.

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Evaluating Social-Emotional Cognition in Children with NF1: Utility of a Brief Computerized Task

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Children with Neurofibromatosis Type 1 (NF1) often experience social impairments, which are most often measured by parent- and teacher-report; however, concordance between adult ratings of social impairment and peer-based sociometrics is often poor. Therefore, there is a critical need for additional assessment tools to evaluate aspects of social functioning in at-risk children. Performance-based measures may provide additional information regarding children's cognitive processing of facial expressions.

Children with NF1 (n=13 to date; mean age=11.0±2.58; 46% male) enrolled in a computerized cognitive training program completed a baseline assessment, including parent-reported measures of behavior, performance-based measures of attention and memory, and the computerized CogState assessment battery. The CogState battery included a task called the Social-Emotional Cognition Task (SECT), which employs a series of human-like faces in an odd-man out paradigm. Specifically, children were asked to identify the face that was different from the others as quickly and accurately as possible.

The majority of parents (80%) reported that their children with NF1 had at least 2 close friends; however, many parents also reported that their children are often teased (60%) and not liked by other kids (50%). On the SECT, children selected the correct response for stimuli involving full faces (mean=70%) more often than for those involving eyes only (mean=44%). Correct responses on the SECT were associated with full-scale IQ ($r=0.60$; $p=0.039$), Working Memory Indices ($r=0.55$; $p=0.063$), and parent ratings of participation in extracurricular activities ($r=0.62$; $p=0.078$). Speed of response on the SECT was also correlated with Working Memory Indices ($r=0.44$; $p=0.148$). Of interest, parent-rated social and attention problems were not associated with either speed or correct responses on the SECT.

For this small sample of children with NF1, the SECT appears to be a useful measure in assessing the social information processing of children with NF1. Further investigation into the use of SECT in both children with NF1 and a comparison group is warranted. Computerized measures have the potential to provide important methodology for objectively assessing cognitive and emotional cognition.

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Health flow care of Neurofibromatosis type 1 patients with peripheral neurofibroma or malignant peripheral sheath tumor

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Neurofibromatosis type 1 (NF1) is autosomal dominant, neurocutaneous disorder caused by genetic alteration of the NF1 gene that encodes for neurofibromin, a tumor suppressor protein located on chromosome 17q11.2. This disease is characterized by growth abnormalities in a wide variety of tissues derived from embryonic neural crest, including peripheral neurofibroma (PN) and malignant peripheral nerve sheath tumors (MPNST). We investigated the flow of care for patients with either PN or MPNST to gain insight on the clinical presentation and outcome of patients who require surgery for either PN or MPNST at the University of Alabama (UAB) Health System. Medical record review was used to screen patients with NF1 who had tumors surgically removed at UAB during 2005-2010 to assess patient management and outcomes. In both sets of patients with PN or MPNST, care is usually referred from a primary care physician (PCP) to UAB Surgery or Neurosurgery Clinic for evaluation and/or surgery after long-standing observation of NF1 progression by the PCP. A limited number of patients were also referred and managed in NF Clinic at UAB. Patients with PN present to UAB for a variety of complaints that include pain, bleeding, numbness, etc. Patients with PN enter the UAB Health System via PCP referral for pain or other complaints, undergo surgery, and follow-up with surgery briefly before returning to PCP. In contrast, the majority of patients with MPNST generally present with chief complaint of visible mass or pain. Due to the aggressive nature of MPNST, patients undergoing surgery for MPNST are more likely to be compliant with medical advice and follow-up management than patients treated for PN. Notably, patients with MPNST are more prone to resection and metastasis, and thus undergo radiation oncology treatment than patients with PN. Our study reveals a difference in extent of care, presentation, and outcome for NF1 patients with PN or MPNST.

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Convergence and divergence of executive function profiles in neurofibromatosis type 1, ADHD, autism spectrum disorder, and healthy comparison groups

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Research has shown that children with autism spectrum disorders (ASD) have especially pronounced executive function deficits in flexibility and response inhibition, while children with NF1 and ADHD have more universal executive function (EF) deficits. We sought to determine EF convergence and divergence across these three clinical populations compared to a healthy control group to gain insight into impairments in socialization specifically associated with EF deficits.

The parent report BRIEF was used to assess EF in our NF1 (n=50) group and three comparison groups (ASD, ADHD, Controls, $n=50$ for each group). This is a measure that provides data on everyday executive functioning across 8 EF domains. A profile analysis was conducted as a mixed repeated measures ANCOVA, with scores on each of the eight BRIEF scales as the within-subjects measures and participant group (Control, ASD, ADHD, NF1) as the between-subjects variable, controlling for age at assessment.

A within-subjects main effect was found for participant group ($F = 4.93$, $p = .000$, $\eta 2 = .07$). Diagnostic groups were significantly different in overall EF deficit elevation regardless of individual BRIEF subscales, with the ADHD and ASD groups showing significantly higher overall elevations than the NF1 group. A moderate between-subjects interaction effect was also found between participant group and the individual BRIEF subscales ($F=52.48$, $p = .000$, $\eta 2 = .44$) suggesting unique profiles of EF impairments between the clinical groups. Specifically, the ADHD group showed the greatest deficits in working memory and planning and organization, the NF1 group in monitoring and working memory, and the ASD group in shifting and emotional control.

Our findings indicate that overall EF deficits in children with ADHD and children with ASD are similar in severity, but differ in which domains are predominantly impaired. Our NF1 group showed a pattern more closely resembling that of the ADHD group, but with less severity. The similarities between the NF1 and ADHD groups (versus the ASD group) suggest that NF1 patients may benefit from interventions for ADHD, with an emphasis on working memory and self-monitoring. Future research should investigate the relationships between executive function profiles and impairments in socialization in these clinical groups to help develop targeted social interventions.

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Funding Source: The Jennifer and Daniel Gilbert Neurofibromatosis Institute

Identification of Novel Biomarkers & Sub-Clinical Vasculopathy in Neurofibromatosis Type 1 Patients

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Vasculopathy is one of the most serious, non-neurocutaneous manifestations of NF1. NF1 vasculopathy is usually asymptomatic, and the first clinical manifestation may be a life-threatening or fatal event. The pathogenesis of NF1 vasculopathy is not clearly understood but is thought to result from haploinsufficiency of neurofibromin in vascular smooth muscle cells, endothelial cells and bone marrow derived inflammatory cells. Our prevous research in genetically engineered mice indicated that *Nf1*$^{+/-}$ bone marrow derived macrophages directly contribute to vasoocclusive disease and that *Nf1*$^{+/-}$ mice have evidence of vascular inflammation. Pro-inflammatory monocytes have been linked to inflammatory cytokine production and increased endothelial transmigration of macrophages in other studies of vascular disease. A pilot study from our group demonstrated a group of 8 patients with NF1 had evidence of vascular inflammation and evidence of vascular endothelial dysfunction.

Based on the these observations, we designed a larger study to examine asymptomatic vascular disease in 20 younger patients 18-40 years of age with NF1 and unselected for vascular disease status. All patients underwent flow-mediated vasodilation (FMD) and glyceryl-trinitrate-mediated dilation (NMD) to assess endothelial and smooth muscle cell function, carotid ultrasound to access vessel thickness and plaque formation, and a clinical exam with empahasis on the cardiovascular system and lab tests such as cholesterol to assess statndard risk factors. Upon clinical exam none of the patients had evidence of vascular disease, but two patients had been previously seen by a cardiologist. Interestingly, in this study we found 12/20 patients (60%) had reduced FMD suggestive of endothelial dysfunction while 3/20 (15%) had reduced NMD suggestive of smooth muscl dysfunction. The intima-media thickess of the carotid artery was increased (defined as above the 75th percentile for their age and gender) in 14/20 patients (70%) and 3/20 (15%) had plaque present. Standard lab tests demonstrated high total cholesterol in 11/19 patients (58%), high homocysteine in 3/20 (15%), high LDL cholesterol in 8/20 (40%), high red blood cells in 9/20 (45%), and high monocytes in 7/20 (35%), low vitamin D in 15/20 (75%), and low fasting insulin in 9/20 (45%). Glucose, calcium, trigylcerides, HDL, C reactive protein, and cystatin C were within the range range for all indiviuals. Some young adults with NF1 appear to have evidence of subclinical vascular disease. Several biomarkers for vascular disease are increased and may be associated with abnormal vascular function in this group.

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Development of an international registry and genomic DNA repository for understanding cancer etiology in individuals with Neurofibromatosis Type 1 (NF1)

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NF1 is one of the most common hereditary cancer syndromes in which affected individuals are at risk for the development of several cancers, especially pediatric brain cancers. Despite the high frequency of tumorigenesis in this population, factors that predict cancer development and outcomes are poorly defined. Over the past year, we launched the web-based NF1 Patient Registry Initiative (NPRI) (https://nf1registry.wustl.edu) and the NF1 Genome Project to advance understanding of genetic and environmental factors that influence cancer development and health outcomes in individuals with NF1. All individuals with NF1 are eligible to participate. For the registry, participants provide contact information and complete a 30-45 minute questionnaire that captures demographic data, medical history, social history, and inquires about interest in participation in future research studies. Over ~10 months, 220 individuals have participated in the registry. To date, participants include residents of 44 U.S. states, the District of Colombia, and 14 countries spanning six continents. The mean age of the participants is 27.9 years (range 1-77 years), where 61% are female and 84% are white (race). Approximately one third of all participants reported a family history of NF1. The most common cancers reported by participants to date are pediatric brain tumors (n=30) followed by malignant peripheral nerve sheath tumors (n=6). Over 98% of participants have indicated their willingness for future contact. Over a period of 15 months, a total of 191 participants have provided DNA samples and clinical data for the NF1 Genome Project. Based on encouraging preliminary GWAS data, future sequencing-based investigations are planned to identify genetic risk modifiers of pediatric brain tumor susceptibility. Together, the NPRI and NF1 Genome Project provide unprecedented and viable mechanisms for assembling patients with rare diseases from across the world to discover genomic and non-genomic factors that influence cancer development and progression in a cancer predisposition syndrome. This work is supported by an American Cancer Society Institutional Research Grant.

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Radiation Therapy in Management of Sporadic and Neurofibromatosis Type 1 (NF1) Associated Malignant Peripheral Nerve Malignant Peripheral Nerve Sheath Tumors (MPNST)

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MPNST are rare, highly aggressive sarcomas with a high rate of local recurrence and distant metastases. Complete surgical resection with negative margins is required for cure. Radiation therapy (RT) and chemotherapy are additional treatment options. However, the role of RT in MPNST remains inconclusive. This study examined the utility of both palliative and adjuvant RT in sporadic and NF1 associated MPNST.

Materials/Methods: A retrospective review of all patients with MPNST evaluated at the NCI between 1990 and 2011was performed. Pathologic features, treatment and outcomes were reviewed. Survival was calculated using the Kaplan-Meier method and survival comparisons were analyzed using the log-rank test for NF1 versus sporadic, extent of resection, histology grade, gender, and RT treatment.

Forty-seven patients were reviewed and due to incomplete records 37 pathologically confirmed MPNST patients were used for this study. NF1 (n=20) and sporadic MPNST (n=17) were diagnosed at an average age of 27 (range 1.25 -76 yrs) years presenting with low grade (n=7) and high grade (n=29) disease. Treatment was with surgery alone (n=1), chemotherapy and surgery (n=10), RT and surgery (n=3), and with chemotherapy, RT, and surgery (n=15). Five patients were treated with palliative RT, chemotherapy, and surgery. In total 28 lesions were treated with RT in 23 patients, 18 patients of which were adjuvant. Modalities were external beam (78%), brachytherapy (13%), both external and brachytherapy (4%), or protons (4%). Twelve patients (52%) treated with RT had NF1. Tumor location in the adjuvant group location was: 67% primary extremity, 22% trunk, and 11% head/neck. Seven patients with incomplete resection (R1 or R2) and 11 patients with complete resection received adjuvant RT with a mean dose of 56.85 Gy in 1.86 Gy per fraction. The mean dose for palliative RT was 40.8 Gy in 2.6 Gy fractions. After adjuvant RT 6 patients had no evidence of disease, 4 had local recurrence, and 8 had distant recurrence. Six experienced RTOG acute grade toxicities. Mean survival of all patients was 35.7 months and 29.7% overall 5 year survival. NF patients had a median survival of 29.7 months while sporadic had a median survival of 64.3 months ($p=0.16$). Poor prognostic factors that were statistically significant ($p<0.05$) include incomplete resection and truncal tumor location. RT was not found to be a prognostic factor for overall survival ($p=0.53$).

Conclusion: Our analysis shows that RT can be effective in achieving local and symptomatic control for sites with manageable toxicities. Our study is limited by its retrospective nature, small sample size, and heterogeneity in the patient population analyzed and their treatment.

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Parent Perspectives on Executive Functioning in Preschoolers with NF1: Comparison to Typically Developing Controls and Teacher Ratings

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Children with NF1 are at increased risk for attention problems and executive functioning challenges, but there is little research with young children. In this study, parent report of executive functioning in everyday contexts was examined for preschool-aged children using the Behavior Rating Inventory for Executive Functioning – Preschool Form (Gioia, Espy, Isquith, 2003). Parent report was compared to teacher report, and to rating of typically developing (TD) children. Participants were 68 children ages 3 through 5 (Mean age = 4.52, SD = .88), 26 with NF1 (17 boys, 9 girls) and 37 TD children (15 siblings, 22 community children; 23 boys, 14 girls). While on average ratings of emerging executive functioning were in the normal range, children with NF1 were rated significantly higher than the normative mean by their parents on the Working Memory scale and on the Emergent Metacognition Index, and these scales showed the greatest number of children with difficulties (46% and 50% respectively). Teachers rated children with NF1 significantly higher than the normative mean on Planning and Organization (PO), Working Memory (WM), Emergent Metacognition Index (EMI), and General Executive Composite (GEC), and these scales showed the greatest number of children with difficulties (38%, 50%, 42%, 27% respectively). There were no significant differences between parent and teacher mean ratings. A significant correlation between parent and teacher ratings of WM was found ($r = 4.79$, $p = .013$), with no other significant correlations between raters. The TD sample did not differ significantly from the normative mean on any scales based on parental report. Based on parental report, the children with NF1 showed significantly more difficulties than the typically developing controls on the WM scale ($t(61) = 2.60$, $p < .05$) but did not differ on any other scales. Rates of difficulties were similar across the groups for the majority of the scales, with the exception of WM (46% NF1, 24% TD), EMI 50% NF1, 22% TD), and GEC (31% NF1, 16% TD). Working memory in everyday contexts emerged as a consistent area of difficulty for young children with NF1 based on parent and teacher report and in comparison to typically developing peers, indicating that some emergent executive functioning difficulties can be observed even in the preschool years.

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A study of signaling pathways in human schwannomas

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Recent studies have established that Merlin, the product of the NF2 suppressor gene control cellular proliferation by regulating the expression and activity of growth factor receptors at the cell surface. Merlin was also shown to be a regulator of the Hippo signaling pathway. Although these mechanisms have been well studied in cellular models, the spectrum of growth factor receptors and signaling pathways that are activated in human schwannomas remains to be better characterized.

We have analysed a series of 40 human schwannomas. The activity of 42 receptors tyrosine kinase (RTK) was evaluated by RTK arrays. In parallel, 50 proteins comprising RTK, major signaling pathways and proliferation markers were analysed by two complementary approaches. Reverse Phase Protein Array (RPPA) was used to measure the expression and activity of signaling molecules and evaluate potential correlation between them and proliferation markers such as Ki67. Immunohistochemistry was performed to evaluate the expression, the proportion of positive cells and the subcellular localization of a various receptors and signaling molecules.

We observed that four RTKs were activated in more than 40% of the tumors. Her3 was the most frequently activated. EGFR is essentially absent from schwannomas whereas ErbB2 and 3 are always detected. Nevertheless, IHC showed that RTKs expression was extremely variable in term of intensity and percentage of positive cells.

RPPA analysis showed that signaling pathways such as MAPK, AKT and STAT3 are essentially co-regulated. Ki67 is correlated to the level of YAP expression and inversely to the ratio of phospho-Yap/Yap suggesting that the Hippo signaling is indeed a important determinant of schwannoma proliferation. Other observations will be discussed.

Using complementary proteomic approaches, we have generated an extensive study of signaling pathways in human schwannomas. Our results should help to precise which signaling molecule may or may not represent a potentially interesting therapeutic target.

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Cognition and Executive Function in Children with NF1: A Population-Based Study

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Studies investigating the cognitive phenotype of neurofibromatosis type 1 (NF1) in childhood have revealed problems with academic achievement, attention, executive functioning and language (e.g. Ferner et al., 1996, Hyman et al, 2005; Krab et al., 2008). However, no population-based studies have been conducted so far. Our study aimed to investigate the cognitive phenotype of NF1 in a population-based sample of children with NF1.

The study benefited from the large, population-based cohort of NF1-patients followed in Genetic Medicine, St. Mary's Hospital, Manchester, UK. There were 198 children aged 6-16 years on the departmental register; a hundred children were randomly selected and invited to do a cognitive assessment battery. The randomisation involved stratifying for age and socio-economic status. Forty-nine children took part in the cognitive assessment phase. There were two control groups: 19 siblings of the children with NF1 (without NF1 themselves) and 29 community controls, matched for age and socio-economic status.

Measures: The cognitive assessment included measures of intelligence, visual learning, academic achievement, executive function, visuospatial perception, attention and facial emotion recognition. Participants were seen either at home or in the hospital.

Children with NF1 performed significantly more poorly than sibling controls on measures of intelligence, academic achievement, executive function and visuospatial perception. Their scores were also lower on attention and visual learning, although differences were not significant. There were no differences between groups in recognition of facial emotions. Children who had inherited NF1 from one of their parents had lower socioeconomic status than children who were sporadic. Comparisons to community control group were not made, as this group differed from the other groups too much in terms of demographics.

The results of this population-based study mostly agree with previous findings, although the differences between children with NF1 and controls for the attention and visual learning tasks are weaker than expected. For NF1 physical problems, population based studies usually show a lower frequency than clinic based cohorts. This is not the case for learning and behaviour. The lower socioeconomic status in familial cases is likely to result at least in part from the impact of NF1-related learning and behaviour problems in the parent. It is essential to develop improved ways of assisting NF1 children to maximise their potential.

References: Ferner, R., Hughes, R., & Weinman, J. (1996). Intellectual impairment in neurofibromatosis; [1] Journal of the Neurological Sciences, 138, 125- 133. Hyman, S., Shores, A., & North, K. (2005). The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. Neurology, 65, 1037-1044. Krab, L., Aarsen, F., de Goede-Bolder, A., et al. (2008). Impact of Neurofibromatosis 1 on school performance. Journal of Child Neurology, 23, 1002-10.

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Attitudes About Internet Support Groups Among Adolescents and Young Adults with Neurofibromatosis Type 1 and their Parents

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Youth with NF1 are at risk for negative psychological outcomes, including depression, anxiety, and social problems. Social support from people experiencing similar problems, such as through an internet support group (ISG), could benefit individuals with NF1 and their parents. The current survey assessed attitudes about ISGs among adolescents and young adults with NF1 and their parents.

Eligible patients included individuals ages 12 to 25 years with NF1 who were enrolled on a natural history or treatment protocol at the National Cancer Institute (NCI). Their parents or legal guardians were eligible for the parent survey. The anonymous surveys each contained 24 multiple choice, Likert, and open-ended items assessing attitudes about ISGs. Items were developed by the NCI Neurobehavioral Group and the NF medical research team. Thirty patients and 30 caregivers completed the survey during an outpatient clinic visit; two parents and one patient declined participation.

Among patients, the most common reason for not currently using an ISG was not knowing of any (69%). Patients indicated they would be "likely" or "very likely" to use an ISG in the future to connect with others with NF (48%), to get answers to questions about NF (45%), to find out about research studies (34%), and to talk about problems or worries (31%). When asked about ISG format, most patients indicated that they would prefer a chat room that would allow them to communicate with others with NF in real time (73%). Topics of interest included treatment studies (81%), physical effects of NF (70%), social-emotional topics (41%), and cognitive/academic problems (33%). Fifty-four percent reported interest in a discussion board where they could post messages for others with NF; there also was interest in a chat room (50%) or discussion board (50%) where they could communicate with a health professional.

Among parents, the most frequent reason for currently not using an ISG was not knowing of any (58%). Parents reported being "likely" or "very likely" to use an ISG to find out about research studies (87%), to talk to other parents of children with NF (67%), and to get answers to their questions about NF (50%). Topics of interest among parents included treatment studies (90%), physical effects of NF (73%), social-emotional topics (60%), and cognitive/academic problems (57%). Parents were interested in a discussion board to post messages for other parents of children with NF (67%) and for a health professional (67%). There also was interest in a chat room where they could talk to a health professional (67%) and other parents (53%).

Adolescents and young adults with NF1 and their parents are interested in using an ISG for both educational and supportive purposes. Studies assessing the potential effects of an ISG in this population are needed and underway at the NCI.

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Impact of ADHD in adults with Neurofibromatosis Type 1 (NF1): associated psychological and social problems

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To analyze the psychological phenotype of attention deficit hyperactivity disorder diagnosis (ADHD), and the effect of ADHD upon life satisfaction, personality, and emotional functioning in adults with Neurofibromatosis Type 1 (NF1).

Adult NF1 patients without (n=26) and with (n=22) ADHD, and adults with ADHD only (n=27) completed questionnaires on personality traits, emotional functioning, and life satisfaction using standardized measures. ADHD was diagnosed according to DSM-IV criteria. Between groups differences were analyzed.

Participants with NF1 and ADHD present a similar emotional instable psychological phenotype as adults with ADHD only, which differed significantly from that in adults with NF1 only. Both groups with ADHD presented higher values in the personality traits excitability ($p<.001$), aggressiveness ($p<.002$), tension/stress ($p<.001$), and somatic distress ($p<.002$), and were more emotional instable ($p<.001$) than participants with NF1 only. Participants with NF1 and ADHD had significantly lower overall life satisfaction ($p<.001$) than NF1 participants without such symptoms, especially affecting general health ($p<.003$), self character ($p<.006$), sexuality ($p<.03$), and family/acquaintances ($p<.001$).

Our findings show that ADHD symptoms in NF1 patients can persist in adulthood. These NF1 patients display similar problematic personality traits, and corresponding emotional and social problems as adults with ADHD only. This finding is highly relevant to understand the psychological phenotype and associated problems in adult NF1 patients with attention problems. We need to offer psychological and/or medical treatment for these patients.

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Psychological burden in adult neurofibromatosis type 1 patients: impact of disease visibility on body image

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Objectives: To evaluate the impact of subjectively perceived disease visibility on psychological stress factors in adult neurofibromatosis type 1 (NF1) and explore how adult NF1 patient experience and appraise their own body (body image).

Method: A total of 228 adult NF1 patients in Germany completed the study questionnaire in this cross-sectional survey. The questionnaire assessed subjectively perceived disease visibility, other NF1 specific clinical data and patients' body image. Outcome parameters were depressiveness, distress and quality of life measured by standardized instruments. Mediation models were performed to test if body experience mediated the effect of disease visibility on psychosocial burden.

Results: Adult NF1 patients' had a negative body image, expressed by bodily insecurity/uneasiness and fewer feelings of attractiveness and bodily self-confidence. Compared to body image in patients with other disfiguring diseases (e.g. psoriasis), patients with NF1 patients felt less attractive and self-confident (women: $p<0.001$; $d=0.76$; men: $p\leq0.001$, $d=0.77$) and more insecure/uneasy and sexually dissatisfied with their body (men: $p<0.001$; $d=0.83$; women: $p\leq0.12$, $d=0.28$). As expected, perceived disease visibility had an impact on psychological stress (depressiveness $r=0.21$**) and on psychosocial burden (distress $r=0.33$** and quality of life $r=0.37$**). The effect of perceived disease on psychological stress was completely mediated by patients' body experience. The effect of disease visibility on psychosocial stress was partly mediated by NF1 patients' body experience.

Conclusions: Our study shed light on the importance of how NF1 patients' experience and appraise their own body. We found a negative body image in adult NF1 patients which was higher in patients who reported high perceived disease visibility. We also revealed that NF1 patients' body image was an important link between perceived disease visibility and psychological and psychosocial burdens. This means that body image is an important issue in adult NF1 patients affecting psychological well being. How patients experience and appraise their body can be improved with psychotherapeutic interventions. Knowledge in these areas may help develop additional guidelines for psychosocial support in NF1 patients.

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Mutational Spectrum and Search for Genotype-Phenotype Correlations in a Cohort of NF1 Patients with Plexiform Neurofibromas

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Background: Few NF1 genotype-phenotype correlations have been found to date. Methods: NF1 patients, enrolled on the NF1 Natural History study at the NCI, undergo comprehensive clinical evaluations for NF1 tumor and non-tumor manifestations including detailed skin examination and whole body MRI. Comprehensive NF1 mutation analysis in these patients is performed at the Medical Genomics Laboratory (MGL) UAB. The mutational spectrum of NCI patients with [3]1 plexiform neurofibroma is compared with the spectrum found in a cohort with comparable phenotype, submitted for clinical testing to MGL as well as with the overall mutation spectrum as previously described in 1770 unrelated NF1 patients 1. In addition, the phenotype of the probands from the NCI cohort carrying a missense, a 1-2 amino-acid deletion or an in-frame splice mutation is compared with the phenotype of patients with the same mutation, as identified through clinical testing at UAB.

Results: 73 unrelated probands from the NCI study, all fulfilling NF1 NIH diagnostic criteria, were analyzed so far. An NF1 mutation was identified in 70/73 (detection rate 96%): more specifically in 30/30 familial cases, in 36/37 of sporadic cases and in 4/6 with unknown family history. In 67/70 probands with $\geq$1 plexiform neurofibroma (median total tumor burden of 2.9% of body weight) a mutation was identified in the blood. A mutation resulting in a premature stopcodon, expected to result in nonsense-mediated RNA-decay, was found in 51/67 (76%) cases, 3 of them being deep intronic splice mutations that would be missed by gDNA based exon-by-exon sequencing. A Type 1 total gene deletion was found in 2/67 probands. A mutation that may lead to a mutant neurofibromin was found in 14/67 probands: 7 different missense (including 2 not previously found in the UAB cohort), 5 in-frame splice and two 2-amino acid deletions were identified. Four of 5 NCI patients with specific missense alterations had substantial plexiform neurofibroma burden and other NF1 related morbidity.

Conclusions: A high NF1 mutation detection rate was confirmed in this carefully phenotyped cohort. A comparison of the NCI phenotypic data and mutational spectrum with findings from patients undergoing NF1 mutation analysis for clinical indications at UAB is ongoing. Preliminary results indicate that some missense alterations are associated with a severe phenotype including heavy tumor burden.

Reference: [1] Messiaen L. and Wimmer K. – NF1 mutational spectrum, in Monographs in Human genetics, the Neurofibromatoses, editor D. Kaufmann, Karger, 2008, vol 16: 63-77.

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Frequency and Severity of Specific Cognitive Deficits in NF1 Brazilian Individuals

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Introduction: Neurofibromatosis Type I (NF1) is a single-gene disorder characterized by a high incidence of complex cognitive symptoms (Shilyansky, Lee, & Silva, 2010) that have a significant impact on quality of life in individuals affected by NF1 (North et al.,1997).

Objective: To assess the frequency and severity of specific cognitive deficits in NF1 Brazilian sample.

Methods: Fifteen11-to-56 years old (28.73 ± 14.9) informed consent individuals diagnosed with NF1 (NIH criteria, 8 male, 7 female) were assessed using an extensive neuropsychological battery including measures of intelligence, executive functions, perceptual skills, language and motor coordination. Their performance was compared with normative data considering education (9.07 years ± 3.63) and sex when necessary, and then converted in 'z' standard scores.

Results: IQ scores were in normal range (95 ± 17), exceptely for one case with mental retardation (IQ=66), and in general had no discrepancies between verbal and performance IQ. Ninety-three percent of the participants had moderate to severe impairment in one or more areas of cognitive functioning. The most frequently deficit was visuoconstruction (60%), but the major severity impaired cognitive domain was executive functions (attentional processes and planning) with 53% frequency on this sample. Others frequently specific deficits were in fine motor coordination (47%), andvisual and verbal immediate recall (53% and 20% respectively). No deficits were finding in language, working memory or short term memory. The variability within participants' performance was high (5.66 ± 3.13 effect sizes) mostly in perceptual skills, and automatic process.

Conclusion: There is an extremely high frequency of cognitive deficits in Brazilian individuals with NF1. Further multimethodologic studies are needed to better understand the etiology of this complex picture of variable cognitive deficits.

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References: [1] North KN, Riccardi V, Samango-Sprouse C, Ferner R, Moore B, et al. 1997.Cognitive function and academic performance in neurofibromatosis 1: consensus statement from the NF1 Cognitive Disorders Task Force. Neurology 48:1121–27 [2] Shilyansky C, Lee YS, Silva AJ. (2010). Molecular and cellular mechanisms of learning disabilities: a focus on NF1. Annuals Reviews of Neuroscience, 33, 221-243.

Neurofibromatosis Type I and Scoliosis: A Multicenter Study to Determine Radiographic Predictors of Dystrophic Scoliosis

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Scoliosis in Neurofibromatosis type I (NF1) can manifest as non-dystrophic or dystrophic, dystrophic scoliosis can cause rapidly progressive deformity.

Scoliosis radiographs of 122 NF1 patients from multiple institutions were graded by NF-experienced spine surgeons as dystrophic or non-dystrophic based on eight radiographic characteristics: vertebral wedging, vertebral rotation, sharp angular curve, rib penciling, vertebral scalloping, widened interpedicular distance, atypical location, and spindling of transverse processes. Of the 122 cases, 83 (68%) were classified by the contributing institution as dystrophic and 39 (32%) were classified as non-dystrophic. Logistic regression was used to model the odds of an x-ray being dystrophic as a function of the 8 radiographic characteristics. Backward elimination, forward elimination, and stepwise selection were used to determine which characteristics were most predictive of dystrophic status.

Modeling indicates that rib penciling; vertebral rotation, vertebral wedging and atypical location are strongly associated with dystrophic status (p-values < 0.001). The other four characteristics were not significantly associated with dystrophic status, given the presence of the first four characteristics in the model (p-values > 0.4). The odds of an x-ray being dystrophic were 2.43 times higher when rib penciling was present. Similarly, the odds ratio for dystrophic curves were: vertebral rotation – 2.98, vertebral wedging – 2.37, atypical location 3.00. If all 4 characteristics patterns were present there would be a 51 times higher risk of dystrophic curve pattern.

Dystrophic scoliosis in NF1 patients can be best predicted by the following radiographic findings – vertebral wedging, rotation, rib penciling, and atypical curve location. Further research to predict dystrophic curve patterns should focus on these radiographic markers. If all four factors are present, there is a 51 times increased risk of a dystrophic curve.

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Pineoblastoma (Pineal Primitive Neuroectodermal Tumor (PNET)) Associated with Neurofibromatosis; Type 1 (NF1)

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Introduction: NF1 predisposes to the development of central nervous system neoplasms, primarily low-grade gliomas, most commonly optic pathway pilocytic astrocytomas, plexiform neurofibromas and malignant peripheral nerve sheath tumors. Patients are also at a heightened risk for the development of extra-neural malignancies, such as myelomonocytic leukemia. While there have been rare reports of children with NF1 developing medulloblastoma, we are not aware of supratentorial PNET occurring in NF1.

Report: A 4 year-old girl with no clinical stigmata of NF1 presented with headaches and vomiting. Neuroimaging revealed an enhancing, hemorrhagic, 2.7 x 2 x 1.6 cm mass in the region of the pineal gland, with associated tectal plate compression and obstructive hydrocephalus. Spine imaging revealed nodular metastatic disease. Preoperative CSF cytology was negative for malignant cells and germ cell tumor markers were not detectable. A biopsy from the pineal mass was obtained during performance of endoscopic third ventriculostomy to manage hydrocephalus. Histopathologically, the neoplasm was densely cellular and made up of a monomorphic population of small-round cells, some containing melanin, exhibited a tendency to rosette formation, and had foci of hemorrhage and necrosis, all consistent with a diagnosis of pineoblastoma (a.k.a., pineal PNET). The spinal metastatic lesions were not biopsied. The patient was subsequently treated with a contemporary era high-risk (Chang M+ disease) CNS PNET Children's Oncology Group trial, using craniospinal irradiation and combination chemotherapy. No primary mass resection was performed. Two years off therapy, she remains in radiographic remission. Physical examination at the end of treatment for her pineoblastoma revealed numerous café-au-lait macules and she met clinical diagnostic criteria for a diagnosis of NF1. There was no evidence of an optic pathway glioma.

Discussion: The most common neoplastic CNS manifestation in children with NF1 is the optic pathway glioma, almost always a slow-growing, low-grade pilocytic astrocytoma with a usually indolent, self-limited clinicoradiographic course. Rarely, higher-grade glial neoplasms, such as glioblastoma, are encountered in NF1. Medulloblastomas (infratentorial PNET) have been infrequently reported, even from large childhood NF1 cohorts. The occurrence of pineoblastoma, a type of supratentorial PNET, suggests that embryonal childhood CNS tumors may be associated with NF1. Awareness and further reporting of this observation is suggested.

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Auditory processing disorders correlate with learning disabilities in Neurofibromatosis Type 1

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Introduction: It has already been described a high prevalence of learning disabilities in individuals with NF1, resulting in poor academic performance. It is known that language is learned through hearing which is considered a functional system responsible for receiving sound information and to convert them into specific transduction signals along the nerve fibers to the cerebral cortex. Auditory processing disorder associated with learning disabilities has been described in different diseases and it was confirmed for the first time in a patient with NF1 evaluated in our service.

Objectives: To verify the neurological processing of the auditory information and its possible association with language and learning disabilities in patients with NF1.

Methods: Descriptive-comparative study with 25 individuals with NF1 (14 female and 11 male) and 22 controls (15 female and 7 male) aged 10 to 34 years (sex- and age- matched). We analyzed the performance in audiometric evaluation and auditory behavioral tests: sound localization (SL), sequential verbal memory (SVM), sequential non-verbal memory (SNVM), frequency patterns (FP), duration patterns (DP), Gap-in-Noise (GIN), speech in noise (SN), dichotic digits (DD), staggered spondaic word (SSW) and dichotic nonverbal (DNV). In addition, the results of School Performance Test, phonologic and syntactic awareness were also analyzed. Abnormal acoustic hearing in audiometric evaluation was an exclusion criterion. Statistical analysis was performed using: t-Test, Mann-Whitney test, Fisher's Exact test and the Pearson correlation. Probability values <0.05 were considered statistically significant.

Results: All participants presented normal peripheral acoustic hearing, but significant differences between NF1 and control groups were observed in the tests: SVM ($p = 0.009$), SNVM ($p = 0.028$), FP ($p = 0.001$), DP ($p = 0.000$), GIN ($p = 0.000$), SN Right Ear (RE) ($p = 0.017$) and Left Ear (LE) ($p = 0.003$), DD RE ($p = 0.004$) and LE ($p = 0.000$), SSW RE ($p = 0.039$) and LE ($p = 0.000$), DNV attention to RE ($p = 0.027$) and in the phonologic ($p = 0.000$) and syntactic ($p = 0.000$) awareness. Positive correlations were observed between the dichotic tests and the school performance subtests ($p < 0.05$). PF and PD showed positive correlation with the phonologic awareness test ($p < 0.05$).

Conclusion: Patients with NF1 displayed auditory processing disorders which can be associated to language and learning disabilities.

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Granting agencies: CNPq, CAPES, FAPEMIG

Ras-Driven Transcriptome Analysis Identifies Aurora Kinase A as a Potential Malignant Peripheral Nerve Sheath Tumor Therapeutic Target

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Patients with Neurofibromatosis Type 1 (NF1) develop malignant peripheral nerve sheath tumors (MPNST) which are often inoperable due to location and do not respond well to current chemotherapies or radiation. The goal of this study was to utilize comprehensive gene expression analysis to identify novel therapeutic targets.

The *NF1* gene encodes neurofibromin, a RasGAP protein, and nerve Schwann cells and/or their precursors are the tumorigenic cell types in MPNST. We created a transgenic mouse model, CNP-HRas12V, expressing constitutively-active HRas in Schwann cells and defined a Ras-induced gene expression signature that was used to drive a Bayesian factor regression model analysis of differentially expressed genes in mouse and human neurofibromas and MPNSTs. We tested functional significance of Aurora kinase A (AURKA) over-expression in MPNST *in vitro* and *in vivo* using Aurora kinase A shRNAs as well as compounds that inhibit Aurora kinases.

We identified 2000 genes with probability of linkage to nerve Ras signaling of which 339 were significantly differentially expressed in mouse and human NF1-related tumor samples relative to normal nerves, including AURKA. AURKA was dramatically over-expressed and genomically amplified in MPNSTs but not neurofibromas. Aurora kinase shRNAs and Aurora kinase inhibitors blocked MPNST cell growth *in vitro*. Furthermore, an AURKA selective inhibitor, MLN8237, stabilized tumor volume and significantly increased survival of mice with MPNST xenografts.

Integrative cross-species transcriptome analyses combined with preclinical testing has provided an effective method for identifying candidates for molecular-targeted therapeutics. Blocking Aurora kinases represents a novel and potentially effective treatment strategy against MPNST.

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Neurofibroma in Greater Occipital Nerve As A Second Clinical Criterion For Neurofibromatosis type I in Infants and Children

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To emphasize the use of a clinical method for detecting neurofibroma in young neurofibromatosis Type 1(NF1) patients.

NF1 is a dominantly inherited neurocutaneous disorder that is commonly associated with cutaneous lesions, learning disabilities, orthopedic and vascular manifestations, benign and malignant tumors. The incidence of NF1 is about ~1 in 3000 live births. The clinical diagnosis of NF1 requires the presence of at least two of the seven clinical criteria. Since 50 percent of NF1 cases can arise by spontaneous mutation in infants café-au-lait spots may be the first and only manifestation. Optic glioma in infants and young children can cause significant visual loss so early diagnosis of NF1 is important.

Patients <6yrs old who presented only with café-au-lait spots were identified from electronic medical records. For those who subsequently met the criteria for NF1 we identified the second criterion that allowed the diagnosis of NF1.

A total 34 patients were identified using the criteria of more than five café au lait spots >/= 5 mm in size and age < 6years old. There were 6/34 patients diagnosed with NF1 based on 6 or more café-au-lait spots over 5 mm in greatest diameter and bilateral occipital subcutaneous neurofibroma. There were 2/6 who had T2 weighted MRI brain changes and 1/6 had an optic glioma consistent with NF1.

In this study, we found that the diagnosis of NF1 could be reached in a significant percentage of children who only had café-au-lait spots by finding subcutaneous neurofibromas in the greater occipital nerves. The subcutaneous neurofibroma can be found before an infant or young child manifested other clinical criteria for NF1. In 3/6 case T2 weighted MRI brain changes were found including one patient with an optic glioma. Palpating early subcutaneous neurofibroma in the occipital region is possible because the thin overlying tissue and the skull allow better definition of a change in the nerve diameter. The infants or young children who presents only with café-au-lait spots can be diagnosed with NF 1 by utilizing this inexpensive clinical method. This method for diagnosis also allows ruling out an alternative diagnosis of Legius syndrome. Early diagnosis provides a timely window for detection of complications of NF1 and better genetic counseling.

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Local-global processing in children with neurofibromatosis type 1: trees before forest?

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It is generally accepted that children with NF1 have a heightened vulnerability to distraction from the environment and are affected by information that is irrelevant to their task at hand. Qualitative evidence also suggests that children with NF1 may process visual information in a manner different to their healthy peers, giving emphasis to stimulus fragments at the expense of the whole. Navon's paradigm of local-global processing has been used to examine the allocation of visual selective attention according to hierarchical levels of visual processing. Navon stimuli consist of large "global" characters made up of smaller "local" characters and participants are directed to identify the stimuli at either the global or local level, ignoring those at the other. The relationship between the two spatial levels is manipulated such that the same character can appear at both the local and global level (congruent) or different characters can appear at each level (incongruent). The aim of the current study was to determine whether children with NF1 demonstrate evidence of an abnormal local and/or global processing bias compared to age-matched controls. Using Navon stimuli, we compared reaction times (RT) and error rates of NF1 ($n = 25$) and control ($n = 25$) children on a task requiring them to name congruent and incongruent stimuli at local and global levels. Preliminary results reveal that NF1 participants experienced difficulty inhibiting irrelevant stimuli, such that they were slower and made more errors when naming incongruent stimuli compared to controls. Results are discussed within the context of an abnormal local-global processing bias, which may account for, at least in part, poor performance on measures of visuospatial ability.

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Transcranial Doppler in subclinical vasculopathy in children with neurofibromatosis-1 (NF1)

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Cerebral vasculopathy occurs in pediatric Neurofibromatosis-1 patients, (prevalence 2.8% - 24%, depending upon ascertainment methodology). We screened with Transcranial Doppler (TCD) to establish that whether patients with abnormalities in flow velocity by sonography have correlative confirmed cerebral vasculopathy, to confirm that vasculopathy can be reliably identified using the combination of screening TCD and confirmatory MRA in the pre-/asymptomatic NF-1 patient, or not. Our cross-sectional single-center study included 80 NF-1 children age 6 to 21 years. Participants underwent 6 vessel cerebrovascular study. Participants with time-averaged mean maximum velocity (TAMMV) readings > 100 cm/s in one or more vessels or vessels that could not be insonated underwent MRI/MRA. Of 80 subjects, 72.5% had normal TAMMV in all vessels, 11.3% had high flow velocity in one or more vessels, 13.8% could not be insonated in one or more vessels; Failure to insonate and high flow velocity occurred in 2.5%. Of 22 patients with abnormal TCD's, 6 failed to complete MRA. Only 2/ 16 remaining abnormal TCD subjects had abnormal MRA findings (12.5%): Stenosis, 1 (6.25%); Occlusion, 1 (6.25%). There was not an association between cerebral vasculopathies and optic pathway or CNS gliomas. Positive predictive value of TCD overall was 22.5%.TCD is not a practical screening tool in pediatric NF-1 for subclinical intracranial cerebral vasculopathy, because of failures to adequately/ fully insonate cerebral vessels. We believe this to be primarily due to signal difficulties of differential bone density and signal interference

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MAPK Levels as a Biomarker of Cognitive Deficits in Neurofibromatosis Type 1

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Cognitive dysfunction is seen in approximately 70% of individuals with NF1 and is a major cause of morbidity. In the NF1 mouse model, the loss of neurofibromin regulation leads to increased Ras/MAPK signaling which causes deficits in working and long-term spatial memory. Similar mechanisms may be responsible for the deficits seen in the NF1 human population but have not been thoroughly investigated.

NF1 mutations are expressed in blood cells and produce transcriptional changes in critical downstream pathways. We hypothesized that accessing MAPK blood levels would inform us of its expression in the brain and help make predictions about the severity of NF1 cognitive phenotypes. It was anticipated that higher blood MAPK levels would correlate with poorer cognitive function.

Phosphorylated MAPK (p-MAPK) Western blots were performed on blood samples from 14 patients with NF1. Protein concentration was measured by performing BCA protein assay and equal amounts of proteins were loaded to SDS-PAGE gels. After electrophoresis, protein was transferred to nitrocellulose membrane followed by blocking with 5% BSA. The membrane was incubated overnight at 40C. After washing with TBS-T, the membrane was incubated with an HRP-conjugated secondary antibody. The signal was detected by using chemiluminescence. Blood MAPK levels were then correlated with frontally-mediated cognitive tasks performed in these patients.

Strong correlations were observed between MAPK levels and multiple cognitive measures. Pearson correlations (r) of blood MAPK values for the cognitive tasks were as follows: Vocabulary = -0.73; Response inhibition/attention (Cancellation) = -0.37; Working memory (Letter number sequencing) = -0.33; Set switching (Verbal fluency-switching condition) = -0.64. All of the relationships were negative as would be predicted given that mutations in the NF1 gene lead to pathologically elevated Ras activity which is associated with cognitive deficits in the NF1 mouse model. Poorer performance on several neurocognitive assessments correlated with elevated peripheral blood MAPK levels in patients with NF1. While the results of this study are preliminary, if these methodologies are validated, the use of peripheral MAPK levels as a biomarker for cognitive dysfunction in NF1 would serve as a powerful, non-invasive and economical tool for determining prognosis. In addition, pharmacologic manipulation of MAPK levels could potentially ameliorate cognitive deficits in this patient population.

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Volume of vestibular schwannoma does not correlate with hearing loss in pediatric patients with Neurofibromatosis II

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Introduction: Vestibular schwannomas are common tumors in individuals affected by Neurofibromatosis type II (NF2). NF2 is often diagnosed in the third and fourth decades of life and as such there is little known about pediatric patients with NF2. Hearing loss is a common result of vestibular schwannomas in adults, but much remains unknown about the correlation between the size of vestibular schwannomas and their effects on hearing in the pediatric population.

Methods: MRI data and hearing data were obtained for eight children with NF2. All patients were between the ages of 10 and 21 at evaluation. Vestibular schwannoma tumor volumes were measured, and hearing scores were calculated based on the CTCAE version 4.0.

Results: The mean age of the population assessed was 15 years. Six of eight children were found to have hearing loss in at least one ear. Four of eight were noted to have hearing loss of 20db at 3Hz and above. The analysis of the data found no correlation between the size of the vestibular schwannoma and the degree of hearing loss. It was noted that tumors as small as 92mm3 were associated with grade II hearing loss while tumors as large as 904mm3 were associated with no loss of hearing.

Conclusions: We found no correlation between size of vestibular schwannomas and the degree of hearing loss associated with these tumors in children. Often children with small vestibular schwannomas are not screened for hearing loss. Our findings suggest that using tumor size on MRI as a criterion for hearing screening could result in missed diagnoses of potentially salvageable hearing loss. At our institution, we recommend that all children with NF2 should have regular hearing screens regardless of tumor size. These findings represent a single institution's experience and need to be replicated on a larger scale.

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Aftermath: Managing NF Related Stigma Following Surgical Intervention

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Irving Goffman posited that stigma is an attribute that can damage one's self worth and efficacy.[1] He referred to stigma as a spoiled identity. Stigmatization is a social process. The identity of individuals with an undesirable attribute is devalued. This devaluation often results in prejudice, discrimination, and social exclusion.[2]

Physical distinction[3] or a disfiguring / deforming and negatively valued attributes are prevalent among those affected with one of the neurofibromatoses. Visible physical distinctions, particularly those that involve the face, are stigmatizing because the social identity of the individual is disrupted. The affected individual is judged by "perceived normal appearing others" to be spoiled or flawed and subsequently, unable to fulfill the obligations of routine social interaction. The affected individual does not meet society's expectations of appearance and may be subject to social isolation, as physical perfection is the gold standard for social inclusion. Surgical interventions to ameliorate or manage tumor growth have been reported in the literature. For example, surgical excision of plexiform neurofibromas of the face is complex and may require several medical interventions to debulk tumor growth; however, the "cosmetic" result is often disappointing.[3]

Few, if any, studies focus on the psychosocial sequelae for those who must manage an impermanent and hostile social milieu. Of course there are case studies or personal experiences about living with NF1 but these are often presented within the context of overcoming adversity. A blueprint or model for understanding NF-related stigma will be presented. Components of this model include the ways in which NF-related stigma contributes to threats to social identity, social isolation, prejudice, and discrimination within a cultural context. Psychosocial sequelae and coping strategies that individuals with neurofibromatosis employ in managing NF related stigma will be presented. The model is based on 10 years of research on stigma research with other disenfranchised groups.

[1] Goffman E. Stigma: *Notes on the management of spoiled identity*. Englewood Cliffs, NJ: Prentice-Hall; 1963. [2] Richman, L.S., Leary, M.R., Reactions to Discrimination, Stigmatization, Ostracism, and Other Forms of Interpersonal Rejection: A Multi-motive Model. Psych. Rev., 2009, 116, 2, 365-383. Bull. [3] Solomon, S E. On an island by myself: Women of Color with Facial Distinctions. Jour Burn Care Rehab, 1998:19: 268-278. [4] Cobhan-Karatas M, Altan-Yaycioglu R, Bal N, & Akova YA. Management of facial disfigurement in orbito-temporal neurofibromatosis. Opthal Plas Surg, 2009. 26:2:124-126.

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Pulmonary function in individuals with NF1: A Preliminary Report

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Background - Reduced maximal voluntary handgrip muscular force (P=0.001) (Souza et al, 2009) and reduced exercise capacity (maximal oxygen uptake/VO2max) (P=0.02) (Souza et al, 2011) has been observed among individuals with NF1 attended at the Neurofibromatosis Outpatient Reference Center of Minas Gerais Federal University (CRNF). It is known that exercise capacity is determined by general muscle tonus and strength and also by pulmonary gas exchange capacity (Albouaini et al, 2007). However, to our knowledge, pulmonary function and respiratory muscle strength has not been quantified among individuals with NF1.

Aim - To compare pulmonary function (spirometry testing) and respiratory muscle strength (Maximal Inspiratory Pressure - MIP and Maximal Expiratory Pressure - MEP) of individuals with NF1 with sex- and age-matched, healthy individuals.

Methods - Eight volunteered patients with NF1 (NIH criteria, 3 male, 5 female), aged between 20 and 39 years, without heart and/or lung involvement, were matched by sex and age to 8 healthy individuals. All volunteers signed the informed consent protocol. Spirometry testing (Vital Capacity - VC, Forced Vital Capacity - FVC, Forced Expiratory Volume of 1.0 seconds – FEV1, Forced Expiratory Flow 25-75% - FEF 25-75%, Maximal Voluntary Ventilation – MVV), MIP (a measure of the strength of respiratory muscles, obtained by having the volunteer inhale as strongly as possible with the mouth against a mouthpiece) and MEP (a measure of the strength of respiratory muscles, obtained by having the volunteer exhale as strongly as possible against a mouthpiece) . All variables were measured in accordance with Brazilian Society of Pneumology and Tisiology guideline. Data was presented as mean and standard deviation of the percentage of predicted values and variables were compared using Wilcoxon's nonparametric test. Probability values < 0.05 were considered statistically significant

Results- The difference between spirometry testing results (percentage of predicted values) of healthy individuals (VC 94.5 ± 9.7, FVC 93.4 ± 8.6, FEV1 93.8 ± 10.4, FEF 25-75% 89.4 ± 20.2, MVV 95 ± 16) and individuals with NF1 (VC 98.5 ± 12.5, FVC 97.6 ± 13.1, FEV1 100.4 ± 10.6, FEF 25-75% 103.2 ± 30, MVV 89.5 ± 18.8) (P=0.3, 0.3, 0.1, 0.3, 0.5) was not statistically significant. However, MEP (percentage of predicted values) was greater in healthy controls (57.3 ± 14.3) then in NF1 individuals (37 ± 3.5) (p= 0.025). There was a tendency of a lower MIP in NF1 individuals (MIP healthy individuals 85.7 ± 23.8; MIP NF1 69.1 ± 21; P= 0.09).

Conclusion – Individuals with NF1 presented normal pulmonary function but respiratory muscle weakness.

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Granting agencies: FAPEMIG, CAPES and CNPQ.

References: [1] Albouaini K, Egred M, Alahmar A, Wright DJ. Cardiopulmonary exercise testing and its applications. Heart 2007; 83 (985): 675-82. [2] Souza JF, Passos, RLF, Guedes ACM, Rezende NA, Rodrigues LOC. Muscular force is reduced in neurofibromatosis type

[1] J Musculoskelet Neuronal Interact 2009; 9(1):15-7. [3] Souza JF, Araujo CG, Rezende NA, Rodrigues LOC. Aerobic capacity is reduced in neurofibromatosis type 1: a preliminary report. In: Annals of 2011 NF Conference, The Children's Tumor Foundation. Jackson Hole: USA, p 52.

Moyamoya Syndrome Associated with Neurofibromatosis Type 1 in Children: Perioperative and Long-Term Outcome After Pial Synangiosis

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Children with neurofibromatosis type 1 (NF1) are at greater risk to develop clinical and radiographic findings of a progressive arteriopathy consistent with moyamoya syndrome, a condition characterized by progressive stenosis of the bilateral supraclinoid internal carotid arteries. Although symptoms of moyamoya may be managed medically, the severity of symptoms, extent of disease, and risk of further progression may necessitate surgical revascularization to prevent or limit ischemic injury and development of fixed neurologic deficits. This study aims to evaluate the clinical, radiologic, and angiographic characteristics, the surgical course, and outcome of surgical intervention among children with NF1 and moyamoya.

Available clinical and radiographic records were retrospectively reviewed in patients with NF1 who were diagnosed with moyamoya syndrome between 1990 and 2010.

34 patients had NF1 and moyamoya syndrome with 28 undergoing surgical revascularization with pial synangiosis. 18/28 patients (55%) evidenced ischemic symptoms, including transient ischemic attacks, strokes, and seizures. 17/28 (52%) had radiographic evidence of prior stroke at time of diagnosis of moyamoya. 20/28 patients (69%) were symptomatic prior to surgery. No patients presented with hemorrhage. All 9 patients treated with cranial irradiation presented with bilateral disease at time of initial diagnosis. The average age at first surgery was 7.9 years (range 1.3-15.5 years). At time of first surgery, perioperative complications included stroke, transient ischemic attack and infection (N=1 each). 20/21 patients (95%) demonstrated stable or improved neurologic status with an average clinical and radiographic postoperative follow-up period of 69.9 months (range 9.4 – 253.3 months). Stroke rate was reduced 19.7-fold (66.9% per patient-year preoperatively versus 3.5% per patient-year postoperatively).

Children with moyamoya syndrome associated with NF1 are often diagnosed prior to development of fixed neurologic signs or symptoms, frequently as an incidental finding on imaging studies obtained for other intracranial manifestations of NF1. Prior cranial irradiation therapy is associated with a greater extent and severity of disease at presentation. The clinical, radiographic, and angiographic features are otherwise comparable to primary moyamoya disease. Surgical revascularization for these patients appears safe and is protective against further ischemic and neurologic damage with a significant reduction in stroke rate.

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Case Report: Congenital Glaucoma Associated with Eyelid Neurofibromas in NF1

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Congenital glaucoma is a rare manifestation of NF1. This case is a 3-year-old boy referred for an evaluation of congenital glaucoma and NF1. He is the 6th child of healthy parents, and was noted to have large, cloudy corneas at birth. He was treated with oral Diamox, and at 4 days of age, under anesthesia, pressures measured by Tono-pen were 21 in each eye and corneal diameters were 12.5mm on the right and 12mm on the left. A trabeculotomy was performed on the right and goniotomy on the left. At the time of initial evaluation and treatment, there was no mention of café-au-lait spots or asymmetry of the eyelids or face. Glaucoma has been difficult to treat, requiring a total of 5 operative procedures including placement of Ahmed valves. At 11 months of age, a head CT was read as normal, without bone abnormalities. Over the next 2 years he developed bilateral upper eyelid plexiform neurofibromas and right-sided facial hypertrophy compatible with an extensive plexiform neurofibroma based on MRI. He was also noted to have bilateral sphenoid wing dysplasia. Brain MRI showed focal areas of signal intensities (FASI), but no optic nerve pathway tumors. His parents had noted café-au-lait spots in early infancy, and on exam he had 10 café-au-lait spots with a hint of freckling in the left axilla and left groin. He did not have evidence of other plexiform neurofibromas or orthopedic manifestations. It was difficult to appreciate sphenoid wing dysplasia. Height was 50th centile and OFC was 56.5cm (>>98th centile).

A number of patients with congenital glaucoma and NF1 have been reported, and 21 are well-reviewed in Payne et al. (*J Child Neurology* **18**;504-508, 2003). Of 14 patients who had neurofibroma involving the head, 11 had plexiform neurfibroma of the upper eyelid(s), An association of unilateral congenital glaucoma with ipsilateral upper eyelid neurofibroma and ipsilateral facial hypertrophy was reported by Francois and Katz in 1961 (*Ophthalmologica* **142**;549-571).

The finding of congenital glaucoma in the context of NF1 should alert health care providers to the high likelihood of developing eyelid neurofibromas and possibly facial neurofibromas as part of the Francois Triad (congenital glaucoma/NF1/facial plexiform neurofibroma – especially of the upper eyelid).

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Natural History Study of Scoliosis in NF1

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Dystrophic scoliosis affects about 2% of the NF1 population, generally progressing in mid-childhood. Less severe scoliosis is thought to affect up to 30% of individuals with NF1. In an effort to identify anatomic markers that could predict who might develop scoliosis in mid-childhood, we embarked on a multi-center, 4-year natural history study of pre-pubertal children with NF1. Inclusion criteria were age 6-9 years, Tanner Stage 1, NF1 by NIH diagnostic criteria, and radiographic spine study showing a curve of less than 20 degrees. Our primary outcome was to determine how many pre-pubertal children with NF1 develop scoliosis over a 4-year observation period. Secondary endpoints included identification of abnormal findings on spine radiography and thoracic MRI, changes in bone mineral density and peripheral quantitative computerized tomography, calcium intake, leisure activity levels, and urinary pyridinolium crosslinks. Year 4 follow-up evaluations are being completed in Spring 2012.

A total of 110 participants were enrolled from 4 sites, and 7 withdrew from the study before year-4 evaluations. To date, 1 participant progressed to scoliosis of more than 20 degrees. Almost half of all participants had an abnormality as detected by imaging studies. The abnormalities included the following; scoliosis greater than 9 degrees, paraspinal neurofibroma, verterbral body wedging or scalloping, rib-penciling, abnormal pedicles, and dural ectasia. Approximately 20% of the cohort had scoliosis between 9 and 18 degrees, and 25-30% had paraspinal neurofibromas. Mild scoliosis and paraspinal tumors occured separately more often than together. About 5% had dural ectasia. Seventeen participants progressed to Tanner Stage 2 during the course of this natural history study.

Preliminary analysis from this study indicates that medically significant scoliosis did not develop over a 3-year observation period in an NF1 study population of nearly 100 pre-pubertal children older than 6 years of age. We did not recruit children with scoliosis, therefore the dystrophic form of scoliosis may develop in children younger than 6 years. In addition, progression of scoliosis could advance more aggressively after onset of puberty. We anticipate ongoing follow-up of this cohort, and additional analyses of secondary endpoints are underway.

David Stevenson, M.D., University of Utah; Zulf Mughal, M.D., University of Manchester; Susan Huson, M.D., University of Manchester; Elizabeth Schorry, M.D., University of Cincinnati; Alvin Crawford, M.D., University of Cincinnati; Jan Friedman, M.D., Ph.D., University of British Columbia; Kathleen Murray, M.D., University of Utah.

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Characterizing social functioning in children with NF1: Associations with attention, executive functioning, and social cognition

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Problems with social functioning are prevalent in children with NF1; yet, little is known about factors related to these impairments. Of particular importance is how social difficulties in this population converge or diverge with factors known to affect socialization in other unique or comorbid developmental disorders such as autism spectrum disorders (ASD) and attention deficit disorders. We aimed to examine these processes in children with NF1 in three studies assessing 1) prevalence and profiles of symptoms associated with ASD 2) patterns of executive dysfunction in children with NF1, ADHD, ASD, and healthy controls, and 3) performance on a computerized task of social cognition.

Data from three ongoing studies of social cognition and factors associated with social impairments will be presented. First, symptom profiles associated with ASD were analyzed, including the association with ADHD in a sample of children with NF1 aged 4-20 ($n = 66$). Next, we compared profiles of executive functions in 50 children with NF1 with age and gender matched ASD, ADHD, and healthy control comparison groups. Finally, we evaluated facial affect recognition, social cognition, and other neurocognitive variables in a small sample of children with NF1 ($n = 15$) using a novel, computer-based paradigm.

In our first sample, the prevalence of elevated ASD symptomatology in children with NF1 exceeded that of the general population and that previously reported in the NF1 literature. Significant associations between ADHD symptomatology were found, but did not fully explain the range of symptoms documented in this group. Secondly, analysis of EF profiles between NF1, ADHD, ASD, and healthy controls indicated similarities in peak deficits between NF1 and both clinical groups, but with less severity in symptoms experienced by children with NF1. Finally, our sample of children with NF1 evidenced impairments in affect recognition. These problems were associated with aspects of executive dysfunction and parent reports of daily functioning. Explanation of the multidimensional aspects of impaired social cognition and functioning in children with NF1 will be presented with recommendations for future research and possible intervention strategies.

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Funding Source: The Jennifer and Daniel Gilbert Neurofibromatosis Institute

Neuroimaging findings in children with neurofibromatosis type 1 and gastrointestinal problems

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OBJECTIVE: This study estimates the frequency of central nervous system abnormalities in the pediatric neurofibromatosis population with gastrointestinal (GI) complications.

BACKGROUND: Although the neurological complications associated with neurofibromatosis type 1 (NF1) are widely known, GI complications have been largely undocumented though they can greatly affect morbidity in this population. The GI problems are not usually associated with an obvious cause, i.e. obstructive neurofibromas.

DESIGN: Chart review on all patients less than 18 years of age with NF1 known to the Comprehensive Neurofibromatosis Center at Johns Hopkins Hospital. Charts were reviewed for type of GI complications, magnetic resonance imaging (MRI) of the neuraxis obtained, and pertinent MRI findings including presence of optic pathway gliomas, presumed low grade gliomas, unidentified bright objects, spinal neurofibromas or plexiform neurofibromas.

RESULTS: 196 children met diagnostic criteria for NF1. Chart review was possible in 180 subjects. 52 subjects had at least 1 GI complaint (28.9%). Constipation was the most common complaint.(15%), followed by abdominal pain (12.8%) , vomiting (11.7%), GERD (7.2%), diarrhea (3.3%), hematochezia (1.7%), low gut motility (1.1%) and nausea (1.1%). MRI of the brain was obtained in 78.8% of patients with GI complaints. Optic pathway gliomas were noted in 13.5% and presumed low grade gliomas of the brain were noted in 9.6%. Unidentified bright objects were seen in 70.7% of the brain MRIs. Spine imaging was obtained in 16 of 52 children (30.8%). 5 children had spinal neurofibromas. A total of 9 children had plexiform neurofibromas, and only 1 had an abdominal plexiform neurofibroma. Imaging of the neuraxis was specifically obtained as a part of the evaluation of GI complaints in only 1 child.

CONCLUSIONS: GI complications are common in children with NF1. There are no particular findings on brain or spine MR imaging that appear to correlate with GI symptoms in this limited series. The frequency of brain and spine abnormalities seen is similar to that of the general NF1 population.

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Visual Outcomes in Young Children with Neurofibromatosis type 1 and Orbitotemporal Plexiform Neurofibromas

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Orbitotemporal plexiform neurofibromas (OTPN) in children with Neurofibromatosis type 1 (NF1) can cause significant ocular problems such as strabismus, refractive error and ptosis, putting young children at risk for amblyopia. Most OTPN studies have focused on surgical rather than visual outcomes, primarily in adults. We describe the visual morbidity associated with OTPN in children with NF1. Two NF1 centers queried their established clinical databases for children with OTPN. Visual acuity, alignment, refractive error, ambylopia and treatment history were abstracted. Extent of involvement was assessed clinically and with MRI, including volumetric MRI analysis, if feasible.

Twenty-eight children with NF1 and OTPN were identified (median age = 8 years at first evaluation, range 0.33 to 23 years). Six subjects were excluded for coexistent glaucoma or glioma, resulting in 22 evaluable subjects. OTPN location was classified as isolated eyelid (n=6), eyelid and orbit (n=8), orbit and temporal region (n=7) or diffuse orbit (n=1). Amblyopia secondary to OTPN was present in 13 (59%) subjects and caused by strabismus (n= 2, 9%), ptosis (n= 10, 45%) or anisometropia (difference in refractive error between eyes, n= 10, 45%) or a combination of factors (n=7, 32%). Ten subjects had their OPTN treated by ptosis surgery (n=2), OTPN removal from eyelid (n = 3), enucleation (n = 2) and medication (n = 5) due to amblyopia (n = 8), cosmetic appearance (n = 4), exposure keratopathy (n=1) or a combination of factors (n = 3). MRI derived OTPN volumes were measured in 12 subjects (median 81 mL, range 2.7-754 mL). 10 of 10 (100%) subjects with OTPN volumes greater than 10 mL experienced amblyopia. In our series, amblyopia occurs in more than half of NF1 children with OTPN, most commonly due to ptosis and anisometropia. OTPN clinical evaluations, treatments and clinic trial outcome measures should focus on preservation of visual acuity in young children. Full List Authors: Robert Avery DO MSCE1,2, Eva Dombi MD4, Kelly Hutcheson MD3, Andrea Gillespie RN4, Maria Acosta MD1,2, Andrea Baldwin, CRNP4, William Madigan MD3, Edmond FitzGibbon, MD5, Roger Packer MD1,2, Brigitte Widemann MD4.

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Please note that any registrations received after May 25th are not listed here.

CLOSING RECEPTION

The Bombay Club

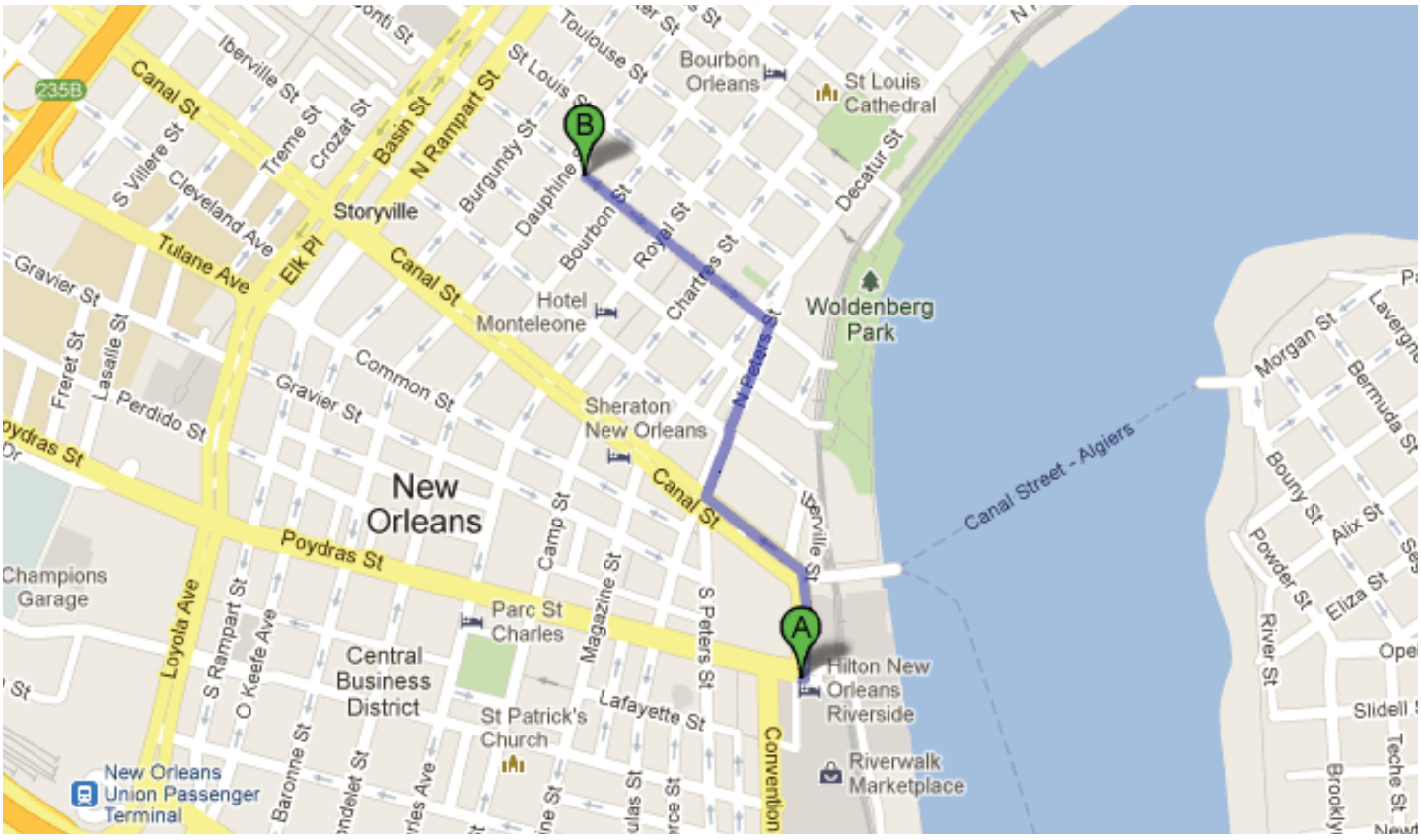
Closing Reception (June 12th, 8:00-10:30PM) at The Bombay Club

Walking Directions from the Hilton to The Bombay Club
A: The Hilton Hotel – Two Poydras Street, New Orleans, LA, 70130
B: The Bombay Club - 830 Conti Street, New Orleans, LA 70112

Hilton New Orleans Riverside

1. Head **north** on **Lafayette St.**
2. Turn right to stay on **Lafayette St.**
3. Continue onto **Convention Center Blvd.**
4. Slight left onto **Canal St.**
5. Turn right onto **N. Peters St.**
6. Turn left onto **Conti St.**
   Destination will be on the left

Bombay Club
830 Conti Street
New Orleans, LA 70112



SPECIAL THANKS TO THE 2012 NF CONFERENCE CHAIRS

Helen Morrison, Ph.D.,
Leibniz Institute for Age Research, Germany

Brigitte Widemann, M.D.,
National Cancer Institute

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Children's Tumor Foundation
CONFERENCE 2013
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See you in Monterey, CA in 2013!!