Fourth International

RASopathies Symposium

July 17-19, 2015
Doubletree by Hilton at SeaTac

Co-Chairs: David A. Stevenson, MD and Brigitte Widemann, MD
Investigators: Lisa Schoyer, PI and Lisa Schill, Co-PI

RASopathiesNet

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4th International
RASopathies Symposium

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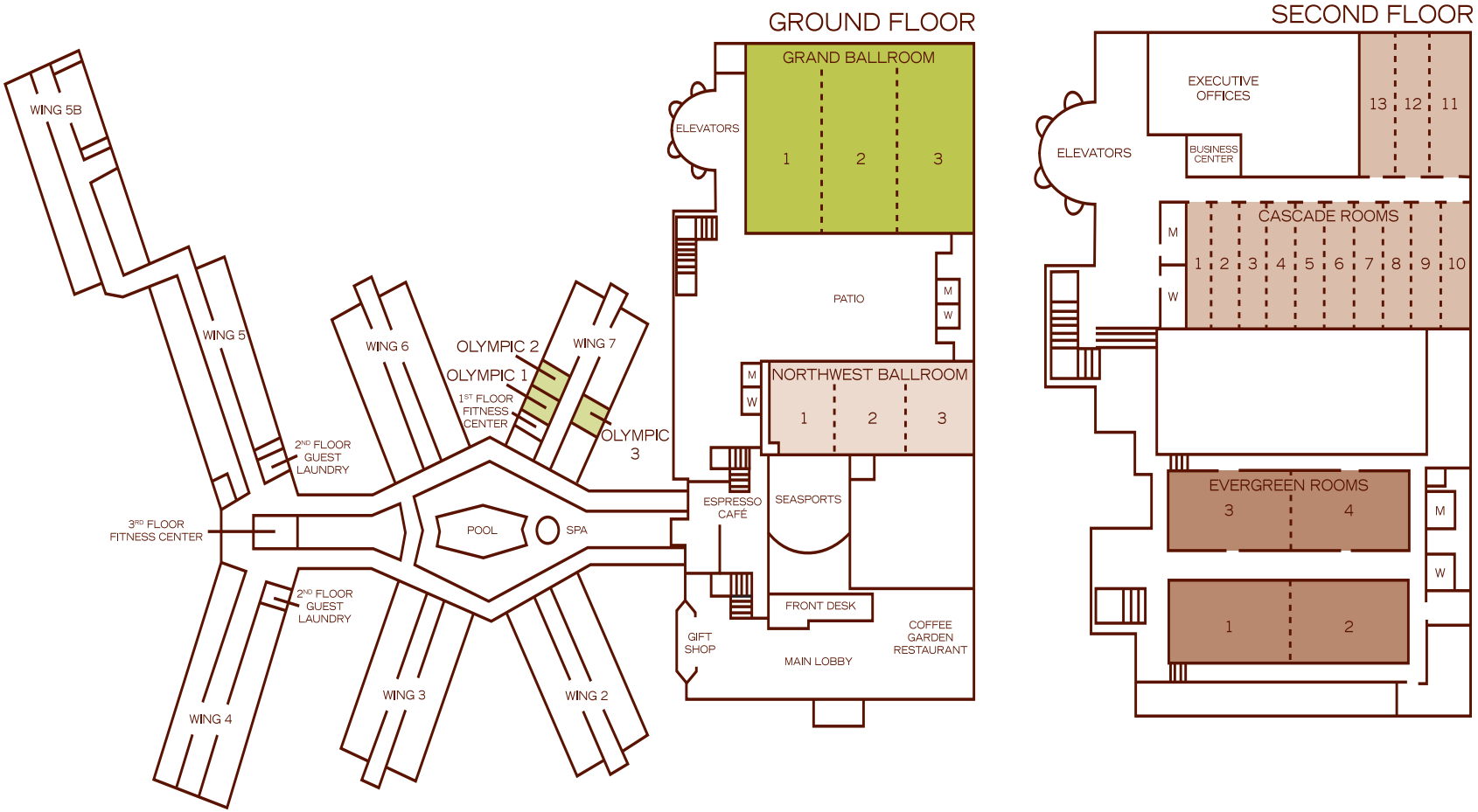
Doubletree by Hilton at Seattle Airport



4th International RASopathies Symposium

Agenda

July 17-19, 2015
Doubletree by Hilton at SeaTac

Summary of free sessions for families can be found at the end of this document.

DAY 1 – Friday, July 17, 2015 *-- Northwest 1 and 2*

8:00-10:00 pm	**Dessert and Scientific Poster Session** (Scientists and advocacy/family groups) Goal: Encourage collaboration between researchers and families in a non-clinical setting
8:00-8:15 pm	Welcoming Comments: David Stevenson, Brigitte Widemann, Lisa Schoyer

DAY 2 – Saturday, July 18, 2015 *-- Northwest 2 and 3*

7:00-8:00 am	Meet the Expert **Breakfast** ***(Grand 2)***	
8:00-8:15 am	**Introduction**: David Stevenson, Brigitte Widemann	
8:15-9:00 am	**Keynote Presentation**: "Ras-signaling and stem-ness" Frank McCormick (UCSF Helen Diller Family Comprehensive Cancer Center)	
9:00am-5:00pm	**Photo Shoot** in and around CFC International and CSFN meeting areas Rick Guidotti	
9:00-10:00 am	**RASopathy Phenotypes (Shared and Discordant)** Moderators: Bronwyn Kerr, Chiara Leoni Goal: Describe phenotypic overlap, with focus on translation of therapies and potential measurable endpoints for clinical trials	
9:00-9:15 am	Cancer and the RASopathies	Bruce Korf (University of Alabama, Birmingham)
9:15-9:30 am	Mechanisms underlying cognitive deficits in the RASopathies: from mice to trials	Ype Elgersma (Erasmus Medical Center, The Netherlands)
9:30-9:45 am	Cardiac Manifestations in RASopathy Syndromes	Kathryn Chatfield (University of Colorado)
9:45-10:00 am	Questions and discussion	
10:00-10:30 am	**Advocates' Panel** Moderator: Lisa Schoyer Representative from each syndrome group will address the pain they experience, to help identify endpoints for clinical trials: CFC: Judy Doyle; CS: Angel Thomas; NF1: Rosemary Anderson; NS: Michelle Ellis; NSML/LS: Tammy Bowers	
10:30-10:45 am	**Break**	

10:45-12:30 pm	**Preclinical Studies** Moderators: Florent Elefteriou, Anne Goriely Goal: Discuss models of RASopathies and approaches to preclinical drug discovery and testing	
10:45-11:05 am	Treating RASopathies	Katherine A. Rauen (University of California at Davis)
11:05-11:25 am	A Chemical Screen for H its for Noonan Syndrome Using a Fruit Fly Model	Bruce Gelb (Mt. Sinai School of Medicine)
11:25-11:45 am	Developing therapies for rare tumors: using mouse models of malignant peripheral nerve sheath tumors to complement rare human samples in drug screens. Screening for MPNST therapy	Karlyne Reilly (NIH National Cancer Institute)
11:45-12:05 pm	Splicing of HRAS exon 2 is vulnerable – Importance for Costello Syndrome phenotype and potential treatment by splice shifting oligonucleotides	Brage Andresen (University of S. Denmark)
12:05-12:20 pm	Treatment of stature using CNP	Florent Elefteriou (Vanderbilt University)
12:20-12:30 pm	Questions and Discussion	
12:30-1:30 pm	**Lunch Break -- *Grand 2***	
1:30-3:10 pm	**Clinical Trials in RASopathies: Design, Endpoints, and Results** Moderators: Scott Plotkin, Emma Burkitt-Wright Goal: Discuss ongoing and planned trials: Success, pitfalls, challenges	
1:30-1:50 pm	Therapeutics for NF1: "The promise of MEK"	Brigitte Widemann (NIH National Cancer Institute)
1:50-2:10 pm	Pilot Trial of Effect of RAS/MAPK inhibitors on Neurocognitive Function in NF1	Karin Walsh (Children's National Medical Center)
2:10-2:30 pm	Therapeutics for Rare and Neglected Diseases (TRND) and Noonan Syndrome with Multiple Lentigines (NSML)	Amy Roberts (Children's Hospital Boston)
2:30-2:50 pm	Individualized endpoint analysis for use in clinical trial of neurofibromatosis	Scott Plotkin (Massachusetts General Hospital)
2:50-3:10 pm	Questions and Discussion	
3:10-3:25 pm	**Break**	
3:25-5:30 pm	**Collaborations, Industry, and Funding** Moderators: Annette Bakker, Lisa Schill Goal: Discuss mechanisms for collaborations translating into clinical trials	
3:25-3:40 pm	Funding Patient-centered Outcomes Research for Rare Disorders	Suzanne Schrandt (PCORI)

3:40-4:00 pm	Agents targeting RAS/MAPK in clinical development: Current status	Michael Fisher (Children's Hospital Philadelphia)
4:00-4:15 pm	NCI Rare Tumors Initiative	Abby Sandler (NIH National Cancer Institute)
4:15-4:35 pm	Children's Tumor Foundation Business model	Annette Bakker (Children's Tumor Foundation)
4:35-5:30 pm	Panel discussion with industry representatives	**Alexion** - Andre Marozsan (Interim Director, Preclinical Pharmacology); **Cellerant** - Ram Mandalam (President & CEO); **Plexxikon** - Marguerite Hutchinson (Director, Business Development); **Synlogic** - Alison Silva (EVP, COO); **UC Davis** - Katherine A. Rauen (MD, PhD)
5:30pm Plus	**Free Time - Networking**	

Day 3 – Sunday, July 19, 2015

7:00-8:00 am	**Breakfast** -- ***Grand 2***	
8:00-9:00 am	**Abstracts by Young Investigator Finalists** -- ***Northwest 2 and 3*** Moderators: Karen Gripp, Amy Roberts Goal: Provide a forum for trainees to present their research and encourage participation	
8:15-8:30 am	*BRAF* knock-in mice provide a pathogenetic mechanism of developmental defects and a therapeutic approach in RASopathies	Shin-ichi Inoue
8:30-8:45 am	Social functioning in children with neurofibromatosis type 1 (NF1) and Noonan syndrome (NS)	Rene Pierpont
8:45-9:00 am	Taking a closer look into Costello Syndrome	Katherine Robbins
9:00-10:00 am	**Positive Exposure – Seeing Beyond Diagnosis** -- ***Northwest 1*** Rick Guidotti	
9:00-11:00 am	**RASopathy Cousins** -- ***Northwest 2 and 3*** Moderator: Dawn Siegel Goals: To discuss lessons learned from other conditions that intersect the Ras pathway	

9:00-9:30 am	PIK3CA -Related Overgrowth Spectrum (PROS): Clinical & Molecular Characterization, Evaluation and Potential Therapeutic Interventions	Kim Keppler-Noreuil (NIH National Human Genome Research Institute)
9:30-10:00 am	Clinical and Molecular Findings of RASA1-Related Disorders	Pinar Bayrak-Toydemir (University of Utah)
10:00-10:30 am	TSC	David Franz (University of Cincinnati)
10:30-11:00 am	PTEN Project Summary	Antonio Hardan (Stanford University)
11:00-11:30 am	**Final Comments and Discussion** David Stevenson, Brigitte Widemann, Lisa Schoyer	
11:30-12:30 pm	**Breakout Sessions with RASopathies Groups:** CFC: Katherine A. Rauen, MD, PhD ***-- Evergreen 2*** CS: Karen Gripp, MD, and David Stevenson, MD ***-- Evergreen 1*** NF1: Bruce Korf, MD, PhD ***-- Evergreen 3*** NS: Bruce Gelb, MD, and Amy Roberts, MD ***-- Evergreen 4***	
12:30-2:30 pm	**Research Networking Lunch** for academic and industry professionals	
12:30-3:00 pm	**NF Network's Post-Symposium Meeting-- *Evergreen 3*** – Lunch for NF1 families included Bruce Korf, MD, PhD and David Stevenson, MD	
2:00-4:00 pm	**Post-Symposium Noonan Syndrome Meeting** Rene Pierpont, PhD and Patroula Smpokou, MD ***-- Northwest 1***	

Summary of Free Sessions for Families

DAY 1 – Friday, July 17, 2015

8:00-10:00 pm	**Dessert and Scientific Poster Session – *Northwest 1 and 2*** For scientists and advocacy/family groups Goal: Encourage collaboration between researchers and families in a non-clinical setting

Day 2 – Saturday, July 18, 2015

9:00am-5:00pm	**Photo Shoot -- *around Evergreen 1, 2, 3 and 4*** Rick Guidotti

Day 3 – Sunday, July 19, 2015

9:00-10:00 am	**2015 Symposium RASopathies Photography** Rick Guidotti

1:30-12:30 pm	**Breakout Sessions with RASopathies Groups:** CFC: Katherine A. Rauen, MD, PhD – ***Evergreen 2*** CS: Karen Gripp, MD, and David Stevenson, MD ***Evergreen 1*** NF1: Bruce Korf, MD, PhD ***– Evergreen 3*** NS: Bruce Gelb, MD, and Amy Roberts, MD ***– Evergreen 4***

12:30-3:00 pm	**NF Network's Post-Symposium Meeting – *Evergreen 3*** – Lunch for NF1 families included Bruce Korf, MD, PhD and David Stevenson, MD

2:00-4:00 pm	**Noonan Syndrome Post-Symposium Meeting – *Northwest 1*** Rene Pierpont, PhD and Patroula Smpoku, MD

4th International RASopathies Symposium

Speakers' Abstracts

Ras signaling and stem-ness

Frank McCormick
University of California, San Francisco - Helen Diller Family Comprehensive Cancer Center
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Neurofibromin is a major negative regulator of RAS, and is frequently mutated in human cancer and in NF1. Neurofibromin binds directly to SPRED proteins, resulting in translocation to the plasma membrane. This interaction is essential for neurofibromin to act on RAS. In the presence of active growth factor receptors, binding of SPRED proteins to neurofibromin is disrupted, allowing RAS.GTP levels to accumulate. KRAS is significantly more potent than HRAS in tumorigenesis models, despite the fact these proteins share identical effector binding regions and activate canonical downstream pathways. We have investigated differences between cells transformed by KRAS and by HRAS to try and understand their distinct biological properties. Cells transformed by KRAS, but not be HRAS, cause a stem-like phenotype This is due to KRAS 4B's' unique ability to bind calmodulin, and so to inhibit calmodulin-dependent kinase. Low CaM kinase promotes wnt/TCF signaling and initiates a set of programs that confer stem-ness. Binding of K-Ras to calmodulin is prevented by phosphorylation of K-Ras on serine-181, by protein kinase C, directly or indirectly. Treatment of mice with a natural product, prostratin, that activates PKC and K-Ras phosphorylation disrupts KRAS binding to calmodulin and prevents initiation and growth of pancreatic tumors in vivo. Part of the "stemness" program initiated by K-Ras involves secretion of the cytokine LIF, an IL-6 family member with a unique role in maintaining stemness. Neutralization of LIF with a monoclonal antibody reduces stemness and sensitizes established pancreas tumors to gemcitabine. The existence of a new effector pathway specific for KRAS therefore offers new opportunities for therapeutic intervention.

Cancer and the RASopathies

Bruce R. Korf
Department of Genetics, University of Alabama at Birmingham

The RAS pathway is known to have an integral role in many forms of cancer, so an association of cancer in disorders due to mutation in genes along the pathway may not be surprising. The likelihood of malignancy and the types of cancers, however, are distinct for different disorders. The risk of cancer may be highest for those with neurofibromatosis type 1 (NF1), where malignancy affects 8-13% of affected individuals. The spectrum of NF1-related cancers includes malignant peripheral nerve sheath tumors, gliomas, rhabdomyosarcoma, and leukemia (especially juvenile myelomonocytic leukemia). An increased risk of breast cancer has also been reported in NF1 patients. Legius syndrome, in which skin pigmentary changes occur that resemble those of NF1, is due to mutation in the SPRED1 gene and does not seem to be associated with a significantly increased risk of malignancy. Myeloproliferative disorders or leukemia have also been reported in association with cardiofaciocutaneous syndrome (CFC) and Noonan syndrome (NS). Other cancer predispositions that have been reported include neuroblastoma (CFC, Costello syndrome, NS), meningioma (CFC), rhabdomyosarcoma (Costello syndrome, NS) and transition cell carcinoma (Costello syndrome). Recognition of the risk of malignancy is important in devising screening protocols for affected individuals and may be important in providing clues to the underlying pathogenesis of these disorders.

Mechanisms underlying cognitive deficits in the RASopathies: from mice to trials

Ype Elgersma
Scientific director ENCORE expertise center for neurodevelopmental disorders, Erasmus University Medical Center, Rotterdam, The Netherlands.

RASopathies are caused by mutations in the Ras/ERK signaling pathway, resulting in over-activation of this pathway. Disorders associated with this pathway (such as neurofibromatosis (NF1), Noonan and Costello syndrome (CS)) typically present with varying degrees of cognitive disability. Currently, there is no treatment for these disorders, although laboratory experiments have shown that statins can rescue the cognitive deficits in both (adult) mouse models of NF1 and Noonan syndrome. Unfortunately, our randomized, placebo controlled clinical trials did not show significant benefits of statin treatment in NF1 patients. NF1 is a very common Rasospathy presenting with relatively mild cognitive deficits. To investigate the molecular basis of NF1 we made use of a novel mouse mutant lacking a neuron-specific isoform of neurofibromin (Nf19a–/9a–mice). We identified the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel 1 as a neurofibromin interacting protein, and demonstrate that a selective attenuation of HCN current in parvalbumin-expressing interneurons is a cause of increased inhibition in Nf1 mutants. This phenotype is specific for NF1 and not dependent on the Ras-signaling pathway, as we saw no deficits in a mouse model of Costello syndrome. We further show that the HCN channel agonist lamotrigine rescues the synaptic plasticity and learning deficits in both Nf19a–/9a–mice and Nf1+/– mice. Together, our results highlight a critical role for HCN channels in the pathophysiology of NF1-associated cognitive deficit, and identify a novel target for clinical drug development. We are currently investigating the efficacy of this drug in a clinical trial. Costello syndrome (CS) is a rare RASopathy characterized by moderate to severe mental retardation, morphological brain abnormalities, craniofacial anomalies, increased birth weight, failure to thrive, short stature, cardiovascular dysfunction and a predisposition to develop tumors. CS is caused by germline point mutations in the H-Ras gene that render the protein constitutively active. To get insight in the molecular mechanisms underlying Costello syndrome, we made use of a mouse model, which is generated by a strongly activating mutation (H-RasG12V) in the H-RAS gene. We confirm that the H-RasG12V mouse is a suitable model to study CS by showing that these mice have a strong activation of the Ras/ERK pathway and a profound learning deficit. Remarkably, we found that most forms of plasticity were normal, with the notable exception of mGLUR-mediated LTD (long-term depression). MEK inhibitors rescued these LTD deficits but had no effect on cognitive performance when given to adult CS mice. We speculate that the structural brain changes seen in these mice may have prevented a phenotypic rescue. In summary, our data suggest that although Ras and NF1 mutations affect the same pathway and result both in a cognitive phenotype, the neuronal mechanisms underlying the cognitive deficits are only partially overlapping.

Cardiac manifestations in RASopathy syndromes

Kathryn C. Chatfield
Department of Pediatrics, Division of Cardiology, University of Colorado School of Medicine, Children's Hospital Colorado Heart Institute, Aurora, Colorado

Congenital and acquired cardiac defects are known to be a common finding in RAS-pathway disorders, and include specific types of congenital heart disease, cardiomyopathies, and arrhythmias. An ever-expanding number of RAS-pathway genes found to cause Noonan-spectrum disorders has led to recognition of genotype-phenotype correlations with respect to cardiovascular findings. This review will summarize the existing body of knowledge regarding the breadth of cardiovascular findings common to Noonan, Multiple-Lentigines Noonan (LEOPARD), Cardiofaciocutaneous, and Costello syndromes.

Genotype-phenotype associations related to the cardiovascular system will be covered; new treatments and targeted therapies will be discussed.

Treating RASopathies

Katherine A. Rauen
University of California Davis, Department of Pediatrics, UC Davis MIND Institute, Sacramento, CA

The RASopathies are a class of clinically related developmental disorders that are caused by germline mutations in genes that encode components, or regulators of the Ras/mitogen-activated protein kinase (MAPK) pathway. These syndromes include Neurofibromatosis type 1, Noonan, Noonan with multiple lentigines, capillary malformation-AV malformation, Costello, cardio-facio-cutaneous and Legius. As a group, the RASopathies are one of the largest recognizable patterns of malformation syndromes known, affecting greater than 1 in 1,000 individuals worldwide. The RASopathies each exhibit unique phenotypes; however, due to the common underlying mechanism of Ras/MAPK pathway dysregulation, they share numerous overlapping characteristics, including craniofacial dysmorphology, cardiac malformations, cutaneous, musculoskeletal, and ocular abnormalities, neurocognitive impairment, hypotonia and an increased cancer risk. Muscle weakness and hypotonia are significant quality of life issues and contribute to motor delays experienced to some degree by all RASopathies. Our study of systematically reviewing skeletal muscle biopsies from individuals with CS and CFC demonstrated abnormal muscle fiber size and variability with a presence of type 2 fiber predominance. In additional, we also demonstrated that RASopathy individuals have decreased handgrip strength compared to sibling controls.

The Ras/MAPK pathway has been attractive target in the treatment of cancer utilizing small molecule therapeutics that specifically inhibit or alter function of the pathway. Many are in development and several are currently undergoing clinical trials, with some already FDA approved. Because of such intense focus on pathway inhibition, small molecular inhibitors such as farnesyl transferase inhibitors that prevent posttranslational modification of Ras, MEK inhibitors and ERK inhibitors are being evaluated for cancer treatment and may be of therapeutic use for syndromes in this pathway. The RASopathies provide a powerful and unique model for studying how Ras dysregulation in development disrupts normal cellular proliferation, differentiation and growth. Skeletal muscle is an ideal system for studying intracellular signaling processes regulating cell proliferation and differentiation. The use of inhibitors as a possible treatment for RASopathies will be discussed.

Grant Funding: NIH/NIAMS R01AR062165

A Hits Screen for Hits for Noonan Syndrome Using a Fruit Fly Model

Céline Guichard,[1] Dhandapany Perundurai,[1] Sylvain Girardot,[1] Alexander Teague,[2] Susumu Hirabayashi,[2] Ross L. Cagan,[1,2] and Bruce D. Gelb[1,3]
[1]The Mindich Child Health and Development Institute, [2]Department of Developmental and Regenerative Biology, and [3]Departments of Pediatrics and Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

The RASopathies are pleomorphic disorders for which, in general, increased signaling through the RAS/MAP kinase cascade is crucial for pathogenesis. Certain characteristics of these disorders, including hypertrophic cardiomyopathy and neurocognitive dysfunction, appear amenable to small molecule therapy. Efforts at treating animal models to date have focused primarily on inhibitors of the canonical RAS pathway, a priority

area of drug development for cancer. For patients with RASopathies, it is unclear if such drugs will be both efficacious and possess sufficiently low side effect profiles to allow for their use for non-lethal indications and, possibly, long-term therapy. To identify potential novel therapies, we are taking a hypothesis-independent approach using a Drosophila melanogaster model of Noonan syndrome with a transgenic Raf-1 gain-of-function mutation. After considerable screen development, we identified a GAL4 driver and culturing conditions that result reliably in 100% pupal lethality. We are now screening a 14,400 chemical compound library (Maybridge Hitfinder) that broadly covers druggable space. To date, we have completed the screening of more than ½ of this library and expect to have early completed it by the time of the meeting. Our results, thus far, reveal one strong candidate compound and several other weaker hits, for which confirmation is in progress. In addition, we have discovered that our Noonan syndrome model fruit flies tolerate DMSO differently than wild-type flies, with higher concentrations engendering rescue from pupal lethality. This is consistent with prior literature suggesting that DMSO, which has been used therapeutically, inhibits signaling through the RAS pathway.

Developing therapies for rare tumors: using mouse models of malignant peripheral nerve sheath tumors to complement rare human samples in drug screens. Screening for MPNST therapy

Karlyne M. Reilly, Robert G. Tuskan, and Brigitte C. Widemann
NIH National Cancer Institute

Neurofibromatosis type 1 (NF1) is one of the most common genetic diseases of the nervous system, affecting 1 in 3500 people. NF1 patients are susceptible to malignant peripheral nerve sheath tumors (MPNSTs) in the peripheral nervous system, among other tumor types. The *NF1* gene, mutated in the disease, encodes a RasGAP protein that normally functions to downregulate active Ras. When *NF1* is mutated the Ras signaling pathway is hyperactivated, leading to increased tumor susceptibility.

MPNSTs occur in younger adults with an incidence of 1 in 100,000, with a higher incidence in NF1 patients. Due to a limited number of high quality human MPNST tumor lines, drug testing has been challenging both in vitro and in vivo. A genetically engineered mouse model carrying mutations in *Nf1* and *Trp53* develops MPNST spontaneously starting around 3 months of age. We have generated tumor lines from over 30 independent mouse MPNSTs, from different sexes and genetic backgrounds that we are using to test drugs in combination with available human MPNST lines. We find good concordance between the mouse and human MPNST lines in response to drugs. Furthermore, although the MPNST lines show good dose response and high maximum response to many targeted compounds, the concentrations of drug required to achieve inhibition is often high, suggesting that one of the difficulties in developing MPNST therapy is the inherent resistance of MPNST cells to drug inhibition.

We will present results of ongoing drug screens in panels of mouse and human MPNST cell lines.

Splicing of HRAS exon 2 is vulnerable – Importance for Costello Syndrome phenotype and potential treatment by splice shifting oligonucleotides.

Brage Storstein Andresen, PhD
University of Southern Denmark

Costello syndrome (CS) is most frequently caused by a c.34G>A (p.G12S) activating mutation in *HRAS* with modest transforming activity. p.G12V mutations have the highest transforming activity, the greatest

frequency in cancers, but are very rare in CS. So far, all CS patients with p.G12V mutations (c.35G>T; c.35_36delinsTT; c.35_36delinsTA), have had a severe, early lethal, phenotype.

Sequence analysis of a 12-year-old boy with an attenuated CS phenotype revealed, to our surprise, a new germline p.G12V mutation, c.35_36delinsTG, without evidence of mosaicism.

In silico analysis shows that exon 2 has a weak 3' splice site and that c.35_36delinsTG simultaneously abolishes exonic splicing enhancer (ESE) motifs and creates exonic splicing silencer (ESS) motifs indicating that it may disrupt splicing.

Analysis of patient *HRAS* cDNA showed that c.35_36delinsTG results in exon 2 skipping and consequently little mutant protein, explaining the attenuated phenotype and suggesting that exon 2 splicing is vulnerable to mutations.

We have employed *HRAS* minigenes to show that mutations in codon 12 and 13 result in different exon 2 splicing efficiency because they affect the function of a crucial ESE. We show that exon 2 inclusion is dependent on ESEs due to the suboptimal 3' splice site. By combining deletion analysis and antisense oligonucleotide walking we have identified other crucial ESEs in *HRAS* exon 2. By transfection of splice switching 2-O-Methyl-Phosphorothioate antisense oligonucleotides (SSOs) targeting crucial ESEs, we can mediate complete skipping of exon 2 from wild type and mutant minigenes, as well as from the endogenous *HRAS* gene in T24 and HepG2 cancer cells. SSO mediated skipping of exon 2 causes reduced proliferation of cancer cells and significant cell death.

Our study illustrates that the phenotype both in somatic cancers and Costello syndrome is not only determined by the transforming potential of the mutant *HRAS* protein, but also by the efficiency of exon 2 inclusion.

This has important implications for our understanding of the correlation between genotype and phenotype in diseases caused by *HRAS* mutations and for development of new therapeutic approaches based on SSO mediated exon 2 skipping.

Treatment of stature using CNP

Matthew R. Karolak[1] and Florent Elefteriou[1,2]
Vanderbilt University Medical Center
Departments of 1) Pharmacology and 2) Medicine

Short stature and craniofacial abnormalities are common findings in patients with RASopathies. However, the etiology of these conditions remains unknown, and available treatments to promote growth are not devoid of risk. Preclinical research focused on the skeletal maladies associated with FGFR3 activating mutations and neurofibromatosis type 1 (NF1) highlighted the importance of properly regulated RAS/ERK signaling during the process of endochondral formation, which give the skeleton its size and shape. These studies revealed that chronic activation of RAS/MEK/ERK signaling in growth plate chondrocytes, triggered by activating mutations in FGFR3 or NF1 loss of function, impairs their differentiation and function, leading to short stature and impaired bone healing. In both conditions, RAF-1 inhibition by C-type Natriuretic Peptide (CNP) improved bone growth in preclinical animal models; a pharmacokinetically stable, recombinant version of CNP is currently in clinical trials for the treatment of dwarfism caused by FGFR3 mutations. CNP thus represents a

potential targeted therapeutic drug to improve the stature of patients affected with disruption of the RAS/MEK/ERK pathway.

Therapeutics for NF1: "The promise of MEK"

Brigitte C. Widemann, MD
National Cancer Institute, Pediatric Oncology Branch

There is a great need for the development of effective medical therapies for NF1 related inoperable plexiform neurofibromas (PNs). PNs exhibit the most rapid growth in young children and therefore early intervention in children with growing PN may result in the greatest clinical benefit. A number of clinical trials directed at inoperable PNs have been conducted in the past. Of these, a phase II study of peginterferon alpha-2b for children and young adults with progressive PNs resulted in a doubling of the median time to progression compared to a historic placebo control group. In addition, imatinib resulted in shrinkage of small PN (less than 20 mL volume) in a phase II trial directed by Kent Robertson. Consistent volume decrease of large PNs has not been reported in the past.

The Ras/RAF/MAPK pathway is activated in NF1 related PNs. In a limited institution CTEP sponsored, NCI POB coordinated phase I trial of the specific oral MEK inhibitor selumetinib (AZD6244 hydrogen sulfate) for children 3-18 years old with inoperable PNs, the maximum tolerated dose was defined with 25 mg/m^2/dose on a BID continuous dosing schedule (approximately 60% of the adult recommended dose). Twenty-four patients with a median PN volume of 1634 mL (range 47-10,269 mL) enrolled on the study. Partial responses defined as PN volume decrease ≥20% compared to baseline have been observed in 16/24 patients. Disease progression defined as a ≥20% increase in PN volume compared to baseline, has not been observed to date. Anecdotal improvement in function, and reduction in PN related pain and disfigurement have been observed.

We hypothesize that selumetinib has the potential to alter the natural history of PN by 1) preventing the development of PN related morbidity in children with progressive PN, and 2) decreasing existing PN related morbidity in children with large, established PN. A selumetinib phase II registration trial is in development. MEK inhibition may impact the development of other NF1 related manifestations such as NF1 related gliomas, cognition, and bony manifestations.

The results of the phase I trial of selumetinib for NF1 related PNs in children will be presented and the design of future trials for NF1 PNs with selumetinib.

Pilot Trial of Effect of RAS/MAPK inhibitors on Neurocognitive Function in NF1

Karin S. Walsh, Psy.D.

This is an ancillary cognitive study to current and upcoming clinical trials in Neurofibromatosis Type 1 (NF1) using therapeutics targeting the Ras/MAPK pathway – specifically MEK and BRAF inhibitors. Prior research has established that the Ras/MAPK signaling cascade is involved in neuronal plasticity and long-term potentiation (LTP). In individuals with NF1, deregulation of this pathway is known to contribute to the development of tumors and is theoretically associated with neurocognitive deficits, specifically in the areas of memory, working memory, attention, and cognitive processing speed. Therapeutic trials targeting this pathway for the treatment of tumors in NF1 are underway and provide a unique opportunity to assess how the regulation of the Ras/MAPK pathway may impact neurocognitive functioning in children and adolescents with NF1. To accomplish this aim, we are implementing a multi-center pilot study using a novel,

computerized assessment approach (Cogstate), to examine change in cognitive function over the first 12 weeks of treatment.

Therapeutics for Rare and Neglected Diseases (TRND) and Noonan Syndrome with Multiple Lentigines (NSML)

Amy E. Roberts*, *MD*[1], Maria Kontaridis*, PhD[2], Bruce Gelb, MD[3], Benjamin Neel, MD, PhD[4]
[1]*Boston Children's Hospital,* [2]*Beth Israel Deaconess Medical Center,* [3] *Icahn School of Medicine at Mount Sinai,* [4]NYU School of Medicine

The TRND program supports pre-clinical development of the therapeutic candidates intended to treat rare or neglected disorders, with the goal of enabling an Investigational New Drug (IND) application.

NSML is a rare genetic disease affecting only about 200 patients worldwide. Nearly all cases of NSML result from mutations in a single gene, PTPN11. In the heart, the most common manifestation is hypertrophic cardiomyopathy (HCM). There is no existing treatment for NSML patients who have HCM, and end-stage heart failure can lead to early death. Dr. Kontaridis has shown that rapamycin can prevent and reverse HCM in animal models of NSML. The purpose of this project is to develop rapamycin or similar compounds as effective HCM therapies for NSML patients. TRND researchers are conducting additional animal efficacy studies. In addition, there are no published natural history studies with regard to NSML associated HCM with respect to expected course, severity, morbidity, mortality, or genotype phenotype correlations. We have undertaken a multi-center retrospective analysis of individuals diagnosed with NSML to analyze genotype, overall phenotype, medical history, and cardiac features as detailed by ECG, echocardiogram, cardiac MRI, and/or exercise testing. We will present available data to date and plans for the future.

*Dr. Roberts and Dr. Kontaridis will present jointly.

Individualized endpoint analysis for use in clinical trial of neurofibromatosis

Rebecca A Betensky, PhD[1] and Scott R. Plotkin, MD PhD[2]
[1] *Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA 02115*
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Objectives: Clinical trials for rare genetic syndromes present multiple challenges for investigators. Because these syndromes are rare, meeting targets for clinical trial accrual is difficult. In addition, the varied clinical presentation of these conditions forces investigators to design multiple independent trials for patients subgroups with different clinical presentations.

Methods: We developed a novel statistical clinical trial design to optimize the identification of patient subgroups in whom a study treatment is effective. We consider the scenario of a heterogeneous disease with patient-specific manifestation of symptoms and with associated endpoints for clinical trials. The design initially recruits all patient subtypes, each with its own, preselected endpoint. At an interim futility analysis, subgroups in whom the treatment appears ineffective are discontinued, and recruitment continues with the remaining subgroups. Using several simulations, we evaluate the operating characteristics of this design, including the probability of detecting an effective treatment and the probability of discarding a patient subgroup in which the treatment is ineffective. We compare this approach to standard trial designs in which (1) each patient subgroup is evaluated separately in clinical trials or (2) unselected patients are evaluated using a composite endpoint.

Results: We find that when a relatively rare subgroup responds to treatment, use of our proposed design can increase the overall power of the trial, though at the unavoidable expense of lengthening the time of the trial. Our design demonstrates superior power relative to the alternative of conducting separate trials for each patient subtype or using a composite endpoint for unselected patients.

Conclusions: In rare diseases with clinical heterogeneity among patients, innovative designs that improve efficiency by including as many patient subgroups as possible are of great interest. Our proposed individualized endpoint design, in conjunction with an interim analysis for futility, holds promise in some scenarios.

Agents targeting RAS/MAPK in clinical development: Current status

Michael J. Fisher, MD
Center for Childhood Cancer Research and Division of Oncology, Children's Hospital of Philadelphia

Next generation treatments for tumors are focusing on agents that target abnormalities in the molecular pathways of the tumors. Because the RAS pathway is commonly implicated in tumor development, agents targeting RAS and its downstream effectors are being evaluated widely. The current status of such agents will be discussed.

NCI Rare Tumors Initiative

Abby Sandler, Ph.D
Office of the Director, Center for Cancer Research, National Cancer Institute

The NCI Rare Tumors Initiative was launched in 2013 with the overall goal of better leveraging the expertise of NCI intramural investigators to more effectively translate potential new therapies for rare tumors. Two pilot projects involving a small network of NCI investigators were launched in an effort to better integrate early clinical studies with laboratory studies. The main focus of the Initiative is two-fold: to have laboratory-based investigators working alongside clinical investigators to help identify potential targets to inform clinical trial design; and to have clinical investigators provide biopsy materials or other tissue to laboratory-based investigators for genetic, genomic, and other analyses that can better in form future clinical trials. The two pilots consisted of one Ras-dependent tumor-type (malignant peripheral nerve sheath tumors and plexiform neurofibromas) and one Ras-independent tumor type (desmoid tumors). The status of the Initiative and these pilot projects, as well as the challenges inherent in studying rare tumors for which limited numbers of patients are available, will be discussed.

Children's Tumor Foundation Business model

Annette Bakker, Salvatore La Rosa, Marco Nievo, Hyerim Lee, Pamela Knight and Patrice Pancza
Children's Tumor Foundation

At Children's Tumor Foundation, we work to find cures for the approximately one in 3,000 people living with neurofibromatosis (NF), a title that encompasses three distinct genetic disorders (NF1, NF2 and schwannomatosis). NF can cause tumors to grow along various types of nerves and, in addition, can affect the development of non-nervous tissues such as bones and skin. It can cause tumors to grow on or in the body, and it manifests itself differently in every single patient—from blindness in one, to deafness in another, to

intense pain or even possibly cancer. We have realized that the traditional funding model—one that rather passively hands out grants to promising scientists—simply was not resulting in expedient effective treatments. We turned our funding model inside-out in order to build new mechanisms, create strategic partnerships, and foster a spirit of collaboration between the many different stakeholders inside and even outside the NF landscape: patients, researchers, clinicians, pharmaceutical companies and the biotechnology sector. And while we continue to provide grants to promising scientists for interesting research, we have rebuilt our foundation to fill the gaps that may hamper a fast and efficient translation of basic discovery into clinical benefit. All of the 'investment' choices were made with one goal in mind: to set in motion a process that promises to get treatments into the hands of our patients quickly.

Positive Exposure: Seeing Beyond Diagnosis

Rick Guidotti, Founder and Director
Positive Exposure

Rick will provide a brief history of his transition from Fashion photographer to Founder and Director of Positive Exposure and will introduce some of his amazing friends living with a variety of genetic conditions from around the globe.

CHANGE HOW YOU SEE, SEE HOW YOU CHANGE

PIK3CA -Related Overgrowth Spectrum (PROS): Clinical & Molecular Characterization, Evaluation and Potential Therapeutic Interventions

Kim M. Keppler-Noreuil, MD
National Human Genome Research Institute/ National Institutes of Health

Somatic activating mutations in the phosphatidylinositol-3-kinase/AKT/mTOR pathway cause the heterogeneous segmental overgrowth disorders. Historically, the clinical diagnoses in patients with PIK3CA activating mutations have included Fibroadipose hyperplasia or Overgrowth (FAO), Hemihyperplasia Multiple Lipomatosis (HHML), Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Nevi, Scoliosis/Skeletal and Spinal (CLOVES) syndrome, macrodactyly, Fibroadipose Infiltrating Lipomatosis, and the related megalencephaly syndromes, Megalencephaly-Capillary Malformation (MCAP or M-CM) and Dysplastic Megalencephaly (DMEG). Because of the extreme differences among patients, we sought to characterize the phenotypic spectrum associated with different genotypes and mutation burdens, including a better understanding of associated complications and natural history. [Keppler-Noreuil et al., 2014, AJMG 164A:1713-1733]. We studied 35 patients with segmental overgrowth and somatic PIK3CA mutations. The phenotypic data show that these previously described disease entities have considerable overlap, and represent a spectrum. While this spectrum overlaps with Proteus syndrome (sporadic, mosaic and progressive) it can be distinguished by the absence of cerebriform connective tissue nevi and a distinct natural history. Vascular malformations were found in 15/35 (43%) and epidermal nevi in 4/35 (11%) patients, lower than in Proteus syndrome. Unlike Proteus syndrome, 31/35 (89%) patients with PIK3CA mutations had congenital overgrowth, and in 35/35 patients this was asymmetric and disproportionate. Overgrowth was mild with little postnatal progression in most, while in others it was severe and progressive requiring multiple surgeries. Novel findings include: unilateral overgrowth that is predominantly left-sided, overgrowth that affects the lower extremities more than the upper extremities and progresses in a distal to proximal pattern, and in the most severely affected patients is associated with marked paucity of adipose tissue in unaffected areas. Based on these data, we conclude that somatic PIK3CA mutations are associated

with a spectrum of overgrowth phenotypes. While the current data are consistent with some genotype-phenotype correlation, this cannot yet be confirmed.

A workshop was convened at the National Institutes of Health (NIH) to discuss and develop a consensus document regarding diagnosis and treatment of patients with PIK3CA-associated somatic overgrowth disorders [Keppler-Noreuil et al., 2014, AJMG 167A:287-295]. Participants in the workshop included a group of researchers from several institutions who have been studying these disorders and have published their findings, as well as representatives from patient-advocacy and support groups. The umbrella term of "PIK3CA-Related Overgrowth Spectrum (PROS)" was agreed upon to encompass both the known and emerging clinical entities associated with somatic PIK3CA mutations including, macrodactyly, FAO, HHML, CLOVES, and related megalencephaly conditions. Key clinical diagnostic features and criteria for testing were proposed, and testing approaches summarized. Preliminary recommendations for a uniform approach to assessment of overgrowth and molecular diagnostic testing were determined. Future areas to address include the surgical management of overgrowth tissue and vascular anomalies, the optimal approach to thrombosis risk, and the testing of potential pharmacologic therapies. Based upon the progressive nature and degree of morbidity of PROS, this disorder is a potentially good target for a targeted drug treatment. We are currently conducting a nonrandomized, phase II pilot drug treatment trial with mTOR inhibitor, Sirolimus, of patients with a confirmed somatic PIK3CA mutation and evidence of progressive overgrowth.

CLINICAL AND MOLECULAR FINDINGS OF RASA1-RELATED DISORDERS

Pinar Bayrak-Toydemir
Department of Pathology, University of Utah

RASA1-related disorder is a vascular malformation syndrome characterized by hereditary capillary malformations (CM) with or without arteriovenous malformations (AVM), arteriovenous fistulas (AVF), or Parkes Weber syndrome. The CMs gene rally are atypical pink-to-reddish brown, multiple, small (1-2 cm in diameter), round-to-oval lesions sometimes with a white halo, and mostly localized on the face and limbs. AVMs and AVFs, which may be associated with overgrowth, have been observed in soft tissue, bone, and brain. Based on localization of these lesions, life-threatening complications from these malformations include hemorrhage, neurological consequences, congestive heart failure, and cutaneous ischemia requiring transarterial embolization or limb amputation can result. Symptoms from intracranial AVMs/AVFs seem to occur early in life.

Extracranial AVMs and AVFs are also prevalent and typically reported in skin, muscle, and spine; however, AVMs/AVFs have not been commonly reported in viscera. Identification of a RASA1 mutation in individuals with multifocal CMs would potentially be beneficial in order to screen for internal AVMs.

To date, the number of cases reported is relatively small, and the spectrum of phenotypes caused by mutations in the RASA1 gene is not well defined. I will discuss the clinical and molecular findings of 50 unrelated cases that tested positive for a RASA1 mutation. The RASA1 gene (located on chromosome 5q) encodes Ras p21 protein activator (GTPase activating protein) 1. Ras p21 protein activator 1 is involved in pathways regulating the growth, differentiation and proliferation of cells, likely during angiogenesis. Approximately 74% of individuals with a RASA1 mutation had CMs, and 50% of cases had an AVM or AVF. Detailed clinical findings of several cases expand the RASA1 phenotype. Our data suggest multifocal CMs are the key clinical finding to suggest a RASA1 mutation. Our data also suggest that screening for large RASA1 deletions and duplications in this disorder is important.

TSC

David Neal Franz, MD
Cincinnati Children's Hospital Medical Center

The protein kinase mTOR was first identified in 1993 in the laboratory of Dr. Michael Hall. In 2003, it became apparent that tuberous sclerosis complex (TSC) was associated with hyperactivation of mTOR. Based on this observation and existing clinical experience with these agents, we began our clinical trials of mTOR inhibitors for the manifestations of tuberous sclerosis, first with rapamycin and with everolimus. As a result of these and subsequent studies mTOR inhibitors are now FDA-approved and indicated for the treatment of subependymal giant cell astrocytoma, renal angiomyolipoma, and lymphangioleiomyomatosis (LAM). Cessation of therapy is frequently, but not always, associated with a recurrence of symptoms or regrowth of hamartomatous or neoplastic lesions. After an initial response it is often possible to reduce the dose of mTOR inhibitor for maintenance therapy without loss of efficacy. These agents appear to be well tolerated in children and adults even with long term treatment. There is a tendency for the incidence of adverse effects to decrease with long term use. Rapamycin and everolimus have also shown promise as topical treatments for facial angiofibromas and other cutaneous manifestations of tuberous sclerosis, and may improve epileptic seizures and cognition. Current research focuses on treating affected individuals at progressively earlier ages in the hope that many of the serious and life-threatening morbidities of tuberous sclerosis can be moderated or even prevented entirely. I will review the clinical development of mTOR inhibition for the treatment of tuberous sclerosis complex and prevsent current information regarding long term safety and efficacy for the systemic manifestations of this disorder.

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PTEN Project Summary

Antonio Hardan, MD
Stanford University

Germline heterozygous *Phosphatase and tensin homolog* (*PTEN*) gene mutations are associated with a spectrum of clinical disorders characterized by neurocognitive deficits, autism symptomatology, skin lesions, macrocephaly, hamartomatous overgrowth of tissues, and an increased risk of cancers. Historically, research

efforts have focused on examining the physical manifestations resulting from these mutations, but there has been little interest in the behavioral and cognitive features, and more specifically in the treatment of these abnormalities. In this investigation, we propose a 6-month randomized, double-blind, multi-site study to evaluate the safety and efficacy of a rapamycin analogue, everolimus, in 40 children and adolescents (age range 6-21 years) with a PTEN mutation. The mTOR pathway is affected in individuals with a PTEN mutation including lack of inhibition due to the fact that PTEN protein is defective. Therefore, the use of everolimus, a mTOR inhibitor, is warranted for the treatment of the core neurocognitive and social deficits observed in affected individuals. Everolimus has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation and has obtained marketing authorization in the US for several indications including renal cancer and subependymal giant cell astrocytoma associated with tuberous sclerosis complex. This pilot investigation will involve 3 sites: Stanford University, Cleveland Clinic, and Boston Children's Hospital. The study design will permit the evaluation of potential benefits of everolimus on working memory and processing speed deficits as well as any improvement on overall global clinical functioning, autism symptoms, and adaptive abilities. Furthermore, biologic measures assessing PTEN-associated pathway molecules (PI3K/AKT, mTOR, MAPK protein levels) will be obtained pre- and post-intervention. This will allow the exploration of whether baseline levels of any of these biological markers moderate treatment response and the assessment of the relationship between changes in their levels over the course of everolimus treatment and clinical improvements. If safety and efficacy of everolimus are established, this will represent a major advance in the treatment of individuals with PTEN mutations, as no medications currently exist that are believed to be effective in treating the core cognitive and social deficits. Additionally, if benefits are observed in patients with an associated autism spectrum disorder, it will allow the development of new disease-specific compounds that will help in the treatment of not only individuals with PTEN mutations but of those affected by autism as well. Finally, the use of everolimus in this investigation holds great promise for informing future research on the role of the mTOR pathway in the pathophysiology of PTEN mutations.

Post-Symposium Noonan Syndrome Meeting

Patroula Smpokou, MD
Division of Genetics & Metabolism Children's National Health System

This session will focus on the clinical presentation and medical issues encountered in infants, children, and adults with Noonan syndrome with a focus on answering specific parent/family inquiries, questions, and concerns. The first part of the session will include a short presentation on the clinical findings and management of those in Noonan syndrome, which will be followed by addressing specific family inquiries. The session will end with an open discussion of families' experiences and general questions.

Dr. Rene Pierpont
University of Minnesota

A wide variety of possible cognitive and behavioral complications are associated with Noonan syndrome. Dr. Pierpont will provide a brief overview of what is known about neuropsychological features of NS, including areas such as cognition, language, social, adaptive skills and mental health. Recommendations for assessment and monitoring, educational support, and behavioral treatments will be provided. A Q&A portion of this breakout session will address participant's questions on these topics.

4th International RASopathies Symposium

Young Investigator Finalists' Abstracts

BRAF knock-in mice provide a pathogenetic mechanism of developmental defects and a therapeutic approach in RASopathies

Shin-ichi Inoue[1], Mitsuji Moriya[1], Tetsuya Niihori[1], Daiju Oba[1], Yoichi Matsubara[6], Yoko Aoki[1]

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Activation of the RAS-MAPK pathway has been implicated in oncogenesis and developmental disorders, RASopathies. Germline mutations in the proto-oncogene B-Raf gene (*BRAF*) cause cardio-facio-cutaneous (CFC) syndrome, which is characterized by heart defects, distinctive facial features and ectodermal abnormalities. To define the pathogenesis and to develop a potential therapeutic approach in CFC syndrome, we here generated knock-in mice (here $Braf^{Q241R/+}$) expressing the *Braf* Q241R mutation, which corresponds to the most frequent mutation in CFC syndrome, Q257R. $Braf^{Q241R/+}$ mice manifested embryonic/neonatal lethality, showing liver necrosis, edema and craniofacial abnormalities. Histological analysis revealed multiple heart defects, including cardiomegaly, enlarged cardiac valves, ventricular noncompaction, hypertrabeculation and ventricular septal defects. $Braf^{Q241R/+}$ embryos also showed massively distended jugular lymphatic sacs and subcutaneous lymphatic vessels. Prenatal treatment with a MEK inhibitor, PD0325901 or MEK162, rescued the embryonic lethality with amelioration of craniofacial abnormalities and edema in $Braf^{Q241R/+}$ embryos. Unexpectedly, one surviving pup was obtained after treatment with a histone 3 demethylase inhibitor, GSK-J4, or NCDM-32b. Combination treatment with PD0325901 and GSK-J4 further increased the rescue from embryonic lethality, ameliorating enlarged cardiac valves. These results suggest that our *BRAF* knock-in mice recapitulate major features of CFC syndrome and that epigenetic modulation as well as the inhibition of the ERK pathway will be a potential therapeutic strategy for the treatment of RASopathies.

Finally, $Braf^{Q241R/+}$ mice survived to adulthood when these mice (C57B/6J background) were crossed with ICR/CD-1 mice. We have also initiated studies to examine the phenotype of $Braf^{Q241R/+}$ mice on an ICR background.

Social functioning in children with neurofibromatosis type 1 (NF1) and Noonan syndrome (NS)

Pierpont, Elizabeth I; Hudock, Rebekah L; Foy, Allison MH; Semrud-Clikeman, Margaret; Pierpont, Mary Ella; Sommer, Katherine M; & Moertel, Christopher L.

Department of Pediatrics, University of Minnesota Medical School

Background: Gene mutations within the RAS-MAPK signaling pathway, which cause neurofibromatosis type 1 (NF1), Noonan syndrome (NS) and related disorders ("RASopathies"), can affect neural development in multiple brain regions, leading to variable cognitive and behavioral difficulties. While functions such as intellectual ability, memory and attention have been previously investigated, development of social competence has been a relatively neglected area in RASopathies research. Recent studies indicate that children with these syndromes are at heightened risk for traits of autism spectrum disorder. Nevertheless, it is unclear whether social challenges are similar across the RASopathy syndromes. Furthermore, little is known about how different neurocognitive and emotional variables may contribute to social problems in these populations.

Aims and Hypothesis: The purpose of the current study was to compare social functioning of children with the two most common RASopathies, NF1 and NS. We hypothesized that children with these syndromes would demonstrate impairments relative to their unaffected siblings in overall social competence, and that social challenges facing children with NF1 and NS would be similar, due to similar underlying disease characteristics. A second aim of the study was to identify cognitive and emotional variables that are associated with reduced daily life social functioning in NF1 and NS. We examined the relative influence of four potential contributing factors on everyday social functioning in NF1 and NS: social language ability, attention regulation (i.e., ADHD symptoms), externalizing behaviors, and social motivation.

Methods: Data are presented from a cohort of 62 children and adolescents ages 8 to 16 years (M = 12.25 years; SD = 2.5 years). The sample included 21 individuals with NF1 (9 boys, 12 girls), 21 individuals with NS (8 boys, 13 girls), and 20 unaffected siblings (9 boys, 11 girls). The three groups did not differ significantly on gender distribution or chronological age. Parents and children completed a set of well-validated standardized neuropsychological measures to examine social and emotional development.

Results: Children with NF1 and NS demonstrated lower overall social competence relative to unaffected siblings on our primary outcome measure, the Social Skills Improvement System (SSIS). Social competence did not differ significantly between the NF1 and NS groups. In general, caregiver ratings of children with NF1 and NS indicated significantly greater social-emotional difficulties than children's self-ratings, suggesting a lack of self-awareness of their own challenges among children in these groups. Regression analyses indicated that social competence ratings were related to different predictors in the different RASopathy syndromes. In children diagnosed with NF1, problems with social-pragmatic language ability and externalizing behaviors were the strongest predictors of social skills. In children diagnosed with NS, social motivation and ADHD symptoms were most associated with social competence.

Conclusions: Social competence (i.e., the ability to interact successfully with peers and significant adults) is known to contribute significantly to the adjustment and well-being of children. This is the first controlled study to assess underlying factors that may contribute to social functioning in children with NS, and to compare these skills with those of individuals with NF1. These comparisons shed light on the relative specificity of the pathophysiology that underlies psychosocial challenges in the RASopathies, and may lead to development of more effective interventions for social impairments in these populations.

Taking a closer look into Costello Syndrome

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1) Center for Pediatric Research, AI duPont Hospital for Children Wilmington , DE.; 2) Medical Genetics, AI duPont Hospital for Children, Wilmington, DE; 3) University of Delaware Biological Sciences, Newark, DE

Our goal at Nemours/ A.I. duPont Hospital for Children is to be able to efficiently and effectively diagnose and treat children with rare disorders. Costello Syndrome (CS) is a rare autosomal dominant disorder, which results in failure-to-thrive, coarse facial features, short stature, skeletal abnormalities, and intellectual disabilities. CS is the result of heterozygous germline mutations in the proto-oncogene *HRAS*. In our laboratory, we established one of the largest of Costello Syndrome sample collections. Our CS cohort consists of 118 patients with a range of heterozygous *HRAS* mutations. The most common mutation found in 79 CS patients is a p.G12S. Molecular screening via PCR and sanger sequencing enabled us to discover less common mutations including: 5 p.G12A, 5 p.G12C, 1 p.G12D, 1 p.G12E, 13 p.G13C, 1 p.G13D, 3 p.T58I, 4 p.G60D, 2

p.S89C, 1 p.A146V. We have been able to demonstrate p.G12S mosaicism in three additional individuals that were previously undiagnosed because of milder phenotype presentation. We used allelic specific amplification to determine parent of origin in informative CS families: the *HRAS* germline mutation was paternally inherited in 75 patients while maternally inherited in 4 cases. Costello Syndrome patients have a predisposition to malignancies, specifically embryonal rhabdomyosarcoma (ERMS). Tumor samples available from seven individuals were screened at the molecular, transcriptional and cytogenetic levels and compared to tumors obtained from sporadic cases. All CS patients carried a paternally inherited HRAS mutation (5 p.G12S and 2 p.G12A), and their ERMS was characterized by a complete loss of the wild type maternally inherited G12 allele. This loss of heterozygosity (LOH) was not confined to the *HRAS* mutation or locus. Using chromosome 11 microsatellite markers, we observed for the first time complete uniparental disomy with loss of the maternal chromosome 11 allele in all but one CS ERMS. Strikingly, complete LOH was also observed in many sporadic tumors with or without RAS associated mutations, indicating that the LOH in itself is an important driver in this cancer. We were able to establish the first CS ERMS cell line to conduct functional studies, screen for new therapeutic agents that control cancer cell proliferation. Transcriptome analysis of drug resistant or sensitive subclones isolated from our patient ERMS cell line are ongoing and will identify actionable therapeutic targets.

Using CS as a syndromic model for ERMS uncovered novel findings furthering our understanding of ERMS tumorigenesis. This knowledge will ultimately guide drug development and precision medicine based drug selection.

4th International RASopathies Symposium

Poster Abstracts

Splice shifting oligonucleotide based targeting of important splicing regulatory elements in HRAS

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The RAS genes (*HRAS*, *NRAS* and *KRAS*) encode small GTPases, which are crucial for proliferation, growth and survival of cells. Aberrant regulation of RAS in somatic cells usually leads to cancer, whereas germline mutations lead to severe congenital syndromes. Approximately 30% of all cancers harbor activating Ras mutations which affect the codons for glycine 12 or 13 of exon 2 and result in a constitutively active Ras protein.

Our previous studies using Costello syndrome (CS) patient cells and *HRAS* minigenes revealed that the different activating mutations in *HRAS* codon 12 and 13 splice differently thereby affecting the amounts of oncogenic protein formed. Employing a *HRAS* minigene, we could show that exon 2 has an intrinsically weak 3' splice site. Therefore inclusion of *HRAS* exon 2 into the mRNA depends on a finely tuned balance between exonic splicing enhancer elements (ESE) and exonic splicing silencer (ESS) elements. Blocking the accessibility of splicing regulatory proteins to essential splicing regulatory sequences (SREs) in *HRAS* exon 2, would result in exon 2 skipping and consequently translation of little or none oncogenic mutant protein.

In the present study we wanted to identify and characterize important SREs in *HRAS* exon 2, which could be targeted by Splice Shifting Oligonucleotides (SSO) to promote exon skipping. We performed a SSO walk covering the entire exon 2 and employed *HRAS* minigenes with serial deletions. Using this approach we identified a new SRE region and confirmed the importance of the previously identified ESE located in codon 12 and 13. Using RNA-affinity purification we have investigated binding of splicing regulatory proteins to the new SRE.

Based on the localization of the essential SREs, we have designed new SSOs, which successfully mediate exon 2 skipping in T24 bladder cancer cells harboring the p.G12V mutation. The SSO induced exclusion of *HRAS* exon 2 disrupts HRAS protein function and causes a decrease in proliferation and/or cell death.

To obtain more efficient exon skipping, we further improved the SSOs by attaching a nucleotide tail containing exon splicing silencer (ESS) motifs, shown to inhibit *HRAS* exon 2 inclusion. This ESS motif recruits the splicing inhibitory factors hnRNP F/H and hnRNP A1 thereby further increasing the level of exon 2 skipping. Moreover, using combinations of SSOs targeting different SREs, we observe an additive or synergistic effect and consequently more pronounced exon 2 skipping.

We hope that our SSO based approach can be further developed into a future potential therapy for CS and cancer.

Genotype-phenotype correlation in NF1: Missense substitutions in NF1 associated with a lack of cutaneous neurofibromas

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Background: Café-au-lait macules (CAL) are the commonest presenting feature of neurofibromatosis type I (NF1), and hence a primary indicator of risk for future NF1-related pathology. Few genotype-phenotype correlations in NF1 are known. Predictors of mild, 'CAL only' phenotypes allow for better prognostication and patient care; p.(992del Met) is the only longstanding known example of a variant in NF1 securely associated with this phenotype.

Method: We examined genotypes of a cohort of 463 patients with molecularly confirmed NF1 for evidence of variants predicting a mild phenotype.

Results: Cutaneous neurofibromas (NFs) appear significantly less common in patients with missense variants than in those with truncating, frameshift or splice variants (p=0.0027, two-tailed Fisher's exact test). p.(Arg1809Cys), as previously identified by other groups, including those of Ludwine Messiaen and Alessandro de Luca, is a recurrent substitution (7 individuals from 4 families) in patients without NFs in the Manchester cohort. Further recurrent substitutions seen in three generation pedigrees without NFs include p.(Arg1276Gln), in 10 individuals from two unrelated families, and p.(Met1440Leu), identified in two families. Other variants clustered around the Ras-binding domain were also observed in smaller pedigrees where no affected person in the family had NFs, but an absence of NFs in a smaller number of individuals, where the majority may be young, is harder to interpret.

Discussion: Missense substitutions in *NF1* predict a lower cutaneous NF burden. It is likely that further specific genotype-phenotype correlations will emerge with testing of larger numbers of patients and international collaborations to share molecular and clinical data. Exclusion of rare complications will require such approaches and the long term follow up of patients.

Conclusion: Certain substitutions in *NF1* appear to segregate with very mild disease. Molecular diagnosis can inform prognosis and management of these patients.

Selfish de novo mutations in the paternal germline: implications for human disease

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As mutations are at the origin of all genetic variations, understanding the factors that influence the apparent rate at which de novo mutations occur is crucial to the study of genome heterogeneity, evolution and disease. Although it is well established that point mutations initially arise as random miscopying events, preferentially from the paternal germline, we have described a new mechanism which predicts that some pathogenic mutations may hijack the way sperm production is controlled to their own advantage. In doing

so, these 'selfish' mutations become progressively enriched in the testis as men age and are therefore associated with an increased risk of transmission to the next generation.

The concept of selfish spermatogonial selection was originally proposed to account for the unusual presentation of a group of rare Mendelian diseases, which we collectively called 'paternal age-effect (PAE) disorders'. It relies on principles similar to oncogenesis to explain why some paternally-derived mutations, such as those causing Apert (FGFR2) Costello (HRAS) and Noonan (PTPN11) syndromes or achondroplasia (FGFR3), occur spontaneously at levels up to 1000-fold higher than the genomic background rate. The evidence – gathered originally through direct quantification of these ultra-rare pathogenic mutations in human sperm – suggests that selfish mutations, although occurring rarely, confer a selective advantage to mutant spermatogonial stem cells, leading to their clonal expansion and progressive enrichment in sperm over time.

To explore further the link between selfish selection and human disease, we have quantified the levels in sperm of spontaneous mutations around p.G12 of HRAS, the codon most frequently affected both by germline Costello syndrome mutations and by somatically acquired oncogenic mutations. Our results show that although selfish selection shares many of its characteristics with oncogenesis, we also observe differences between these 2 processes, including mutational profiles that are specific to the germline. We have also used a statistical modeling approach to show how both the intrinsic mutation frequency and the selfish selective advantage conferred by different mutations contribute to the de novo mutational load borne by the paternal germline.

Our understanding of this process so far suggests that molecularly selfish selection relies on the activation of the growth factor receptor-RAS signaling pathway, which is a key regulator of stem cell homeostasis in the testis. As RAS is required in many different cellular contexts, we will discuss to which extend dysregulation of this pathway is likely to be relevant to the pathology of common disorders, including cancer predisposition and neurodevelopmental disorders, such as schizophrenia and autism - for which paternal age-effects have been described epidemiologically.

Transgenic *Drosophila* models of RASopathy-causing MEK mutations

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Transient Ras/ERK signaling is activated in specific regions of the early *Drosophila* embryo by two different Receptor Tyrosine Kinases (RTKs), Torso and Epidermal Growth Factor Receptor (EGFR). Torso-mediated signaling specifies the non-segmented regions of the future larva and EGFR-mediated signaling is needed for cell differentiation in the central nervous system. Previous studies from our lab have established quantitative assays to study the dynamics of ERK signaling and its functional consequences in the early embryo. MEK, a kinase directly upstream of ERK, can be mutated in various domains resulting in unique effects on signaling, such as either faster signal activation or slower signal decay, or uniformly increased signaling levels over time. To study the precise effects of mutations that are known to be involved in RASopathies in an *in vivo* setting, we generated six transgenic fly lines with Gal4-driven expression of wild-type or mutant Dsor1, a fly ortholog of MEK. Fly embryos with overexpressed wild-type Dsor1 develop normally and hatch without any visible defects, suggesting no side effects of overexpression of the wild-type protein. However, overexpression of

both Cardio-facio-cutaneous (CFC) syndrome and cancer causing mutations leads to high embryonic lethality. Furthermore, the cuticles of dead embryos exhibit fused and reduced number of abdominal segments and have slight defects in the head and tail structures. To understand the biochemical basis of these observed defects, we are characterizing the signaling dynamics and gene expression patterns in these mutant embryos.

Exploring the role of the FGF Signaling Pathway in Asymmetric Heart Development

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Three of the most common forms of human congenital heart defects (CHD) are the result of aberrant asymmetric cardiac morphogenesis. CHDs, which affect 60-85% of patients with RASopathies, are the leading cause of all infant mortalities arising from birth defects. Given the conservation between heart formation in zebrafish and humans, the zebrafish heart is a particularly suitable model in which to evaluate the relationship between cellular organization and asymmetric organ formation. Understanding the mechanisms involved in asymmetric morphogenesis of the zebrafish heart will help us understand how defects occur in this process.

In zebrafish, heart morphogenesis is governed by two asymmetric events: cardiac jogging and cardiac looping. Jogging positions the atrial cells to the left and anterior of ventricular cells, resulting in a leftward shift of the cardiac cone. This displacement occurs simultaneously with the conversion of the cone into a linear heart tube. During looping, the tube bends and the ventricle is positioned to the right of the atrium. Our lab has demonstrated that Nodal signaling asymmetrically increases migration rates specifically on the left of the cardiac cone; this L/R asymmetry in migration rates leads to a leftward jog. Microarray analysis revealed upregulation of Fibroblast Growth Factor (FGF) receptors and transcriptional targets in response to Nodal signaling.

We find that treatment of embryos with the FGFR inhibitor SU5402 during rotation of the cardiac cone delays cardiac jogging. Analysis of the expression of Nodal target genes *lefty2* and *spaw* reveals that the delay is not reflective of defects in L/R patterning. *ntl* mutants express *nodal* bilaterally in the embryo, and thus exhibit an increase in cell velocities on both sides of the cardiac cone leading to a midline heart tube. Inhibition of FGF signaling in these mutants also results in a delay in cardiac jogging without altering the bilateral expression of *lefty2* or *spaw*; however, tubes eventually form and are positioned at the midline. This suggests that although Nodal is the laterality cue, FGF signaling might be required permissively for cell movements in the heart. Given that the FGF signaling pathway activates the RAS/MAPK pathway, a comprehensive understanding of the mechanisms through which the FGF pathway affects heart development during embryogenesis might yield answers regarding the etiology of CHD in RASopathy patients.

Response to neurotransmitter replacement therapy in a patient with BRAF mutation and low CSF levels of biogenic amines

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We are following an 8 year old boy in clinic who presented in infancy with failure to thrive, severe GI dysmotility, autism, anxiety, severe self-injury, and sleep disorder. He always had a distended abdomen and

severe constipation. He is mostly G-tube fed. At age 4 years he developed a seizure disorder, and endocrine abnormalities (precocious puberty). He had a lumbar puncture for CSF neurotransmitters fairly early in the diagnostic work-up and was found to have low levels of dopamine and serotonin metabolites; HVA:172 nmol/L (ref. 280-852) and 5HIAA: 42 nmol/L (ref. 66-388). Empiric treatment with neurotransmitter replacement therapy was started with L-dopa/carbidopa (Sinemet: 5 mg L-dopa/1.25 mg carbidopa/1 ml) and 5-hydroxytryptophan (5.6 mg/1 ml).

Following the initiation of 5-HTP and Sinemet, the patient's behaviour was reported to have markedly improved, the frequency of head hitting, outbursts of screaming and aggression decreased. His anxiety, sleep and attention have also significantly improved on treatment. The patient became more mobile, have improved his gait and communication. Additional benefits include improvement of chronic constipation, and temperature regulation. These improvements were almost immediately noticeable after administration of 5-HTP and Sinemet; however, deterioration of behaviour occurs approximately 3.5-4 hours after dose.

There was a period of 6 months when the neurotransmitter replacement therapy was on hold, because of interactions with his motility medication (causing significant elevations of prolactin levels) and he clearly deteriorated, loosing all skills that he gained. The therapy was restarted when a different promotility agent was prescribed and the improvements observed before were noticeable again, right at the start of the treatment.

To achieve sustained improvement in behaviour and quality of life each of the medications are administered five times a day according to the following schedule 0700: 3 ml, 1130: 3 ml, 15:30: 3 ml, 19:30: 4 ml, 24:00: 5 ml.

On clinical examination his facial features are suggestive but not diagnostic of Cardio-facio-cutaneous syndrome (CFC). He has abundant curly scalp hair, minimal cutaneous findings and no cardiac involvement. The diagnosis was made with Whole Exome Sequencing, which found him to carry the C244A missense variant in the *BRAF* gene. This mutation has not been previously reported, however bioinformatics predict that it is pathogenic.

There is no report in the literature of low CSF neurotransmitters in CFC, and the pathomechanism is not well understood. RAF family kinases are key components of intracellular signaling and interact with many signaling molecules and proteins. The *BRAF* gene encodes for a serine/threonine protein kinase that participates in the MAPK/ERK signaling pathway and plays vital role in cancers and developmental syndromes. Proteins are phosphorylated by kinases and hence activated. Enzymes in the biogenic amine synthetic pathway are heavily phosphorylated when activated. Lack of phosphorylation might lead to reduced levels of enzyme activity and ultimately biogenic amine synthesis and CSF levels.

Although the pathomechanism is not yet clear, this case illustrates the importance of identifying secondary CSF neurotransmitter deficiencies in patients with CFC and their treatment with biogenic amine precursors.

The Effects of MEK1 RASopathy Mutations on Zebrafish Morphological Phenotypes

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As more individuals with RASopathies are being sequenced, the number of known causative mutations in MEK1 is growing. However, it is not entirely clear what levels of pathway activation are associated with each of the RASopathy MEK1 variants, nor is it well understood how these levels compare to the levels of pathway activation in cancer MEK1 variants. As a first step towards addressing these questions, we asked whether different disease-related MEK1 mutations can be ranked according to their effects on multiple aspects of zebrafish embryogenesis, a sensitive system for studies of developmental signaling. We use Ras/ERK-dependent developmental processes in the early zebrafish as quantitative assays to assess the effects of the currently known MEK1 mutations. We overexpress RASopathy MEK1 variants by injecting mutant MEK1 mRNA into embryos at the 1-cell stage and report the effects on two phenotypes affected by overactive Ras/ERK signaling in zebrafish: oval shape of the embryos at 12 hours post fertilization (hpf) and lethality of the embryos at the end of embryogenesis at 48 hpf. We find that MEK1 RASopathy variants result in oval embryos of varying degree and lie between the negative control of wild-type MEK1, which is essentially circular in shape, and the positive control of the melanoma-causing MEK1 variant, which is the most oval in shape. In the lethality assay, the same relative ranking is roughly preserved. We seek to correlate these phenotypes with changes in ERK activation patterns. Since aberrant cardiogenesis is a common symptom in individuals with RASopathies, this same overexpression technique will be used to assess the effect on heart size and asymmetric heart morphogenesis in zebrafish, which is readily visualized because of the transparency of the embryo.

Splice shifting oligonucleotide (SSO) mediated skipping of *KRAS* exon 2

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The RAS genes (*HRAS* ,*KRAS* and *NRAS*) are highly homologous and encode small 21kDa GTPase proteins that play a central role in coupling extracellular signals with complex intracellular pathways. The Ras proteins function as molecular switches for a variety of critical cellular processes, such as proliferation growth and survival. Activating mutations in the RAS genes, which create constitutively active Ras proteins, result in oncogenic transformation and progression of tumor cells. *KRAS* is mutated in approx. 30% of all tumors and is the most frequently mutated RAS isoform in cancer.

Similar to *HRAS*, where we have shown that mutations in codon 12 and 13 affect inclusion of the vulnerable exon 2, the vast majority of oncogenic mutations in *KRAS* are also located in codon 12 and 13 of exon 2. Based on the high homology between *HRAS* and *KRAS* and because *in silico* prediction showed that *KRAS* exon 2 has a weak 3' splice site, we speculated that splicing of *KRAS* exon 2 is also vulnerable and dependent on a finely tuned balance between positive and negative splicing regulatory elements (SREs).

The goal of this study was to develop Splice Shifting Oligonucleotides (SSO)s, which can mediate exclusion of the mutated *KRAS* exon 2 during splicing by blocking access to positive SREs.

We performed a SSO walk covering the entire exon 2 and identified several efficient SSOs capable of inducing *KRAS* exon 2 skipping in both pancreatic and lung cancer cells. This leads to reduced growth/proliferation and cell death.

We used the SSO initially identified to be most efficient in cell culture for preliminary experiments *in vivo* in Xenograft mouse models of Mia PaCa-2-derived pancreatic carcinoma. This showed some exon 2 skipping, but emphasized that improved targeting/more efficient SSOs are needed.

In order to optimize SSO efficiency we have identified a more efficient SRE to be targeted and further improved SSO design by attaching a tail sequence containing an ESS (exon splicing silencing) motif to the SSOs. The ESS motif attracts the splicing inhibitory factors hnRNP F/H and hnRNP A1 resulting in more efficient exon 2 skipping. Recent results employing the tailed SSOs indicate that they are at least fourfold more efficient than our initial designs. Moreover, using combinations of SSOs targeting different SREs, we observe an additive or synergistic effect and consequently more pronounced exon 2 skipping.

We hope that our SSO based approach can be further developed into a future potential therapy.

Bone mineral density in Costello syndrome: two years follow up of vitamin D treatment

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Introduction: Costello syndrome (CS) is a multisystemic disorder caused by activating germline mutations in the HRAS proto-oncogene. Facial dysmorphisms, failure to thrive, heart defects, cognitive impairment and musculoskeletal anomalies are distinctive manifestations of CS. In 2014, we described decreased bone density in a group of individuals affected by Costello syndrome associated with low serum 25-hydroxy (OH) vitamin D concentrations. Herein we report data on the effect of 25OH vitamin D concentrations on bone mineral density during vitamin D supplementation over 2 years.

Materials and methods: Seven individuals with clinical and molecular diagnosis of Costello syndrome were recruited at the Center for Rare Diseases, Catholic University (Rome, Italy). All participants underwent blood sample and 24 hour urine collection for biochemical bone markers (e.g. calcium, phosphorus, magnesium, 25OH vitamin D and parathyroid hormone). Dual-energy X-ray absorptiometry (DXA) was utilized to assess bone mineral density (BMD g/cm^2), in particular subtotal BMD (S-BMD, whole body less head), lumbar, femoral neck and femur BMD (L-BMD, FN-BMD and F-BMD respectively) in all subjects. Vitamin D supplementation was administering orally, once monthly; cholecalciferol doses ranged between 25.000 to 50.000 IU monthly (21.6 to 61.7 IU/kg/day). Using paired t-test, biochemical and densitometry data after one and two years treatment with vitamin D supplementation (cholecalciferol) were compared with those obtained before treatment. Significant values were set at p=0.05.

Results: Herein we reported the values referring to the seven individual who completed 2 years follow-up. At the baseline all Costello individuals showed low serum 25OH vitamin D concentration (mean value ±SD: 15.86±7.58 ng/ml); S-BMD, L-BMD, FN-BMD and F-BMD were all decreased compared to the control group, with L-BMD parameter more severely affected. During the follow up, all individuals showed significant improvement of serum 25OH vitamin D concentration respectively after one (34.70±5.66 ng/ml; p= 0.0011) and two years (31.46±7.37 ng/ml; p=0.0004) treatment with oral cholecalciferol supplementation. No significant changes in other biochemical parameters were detected (e.g. blood calcium, phosphorus, magnesium, parathyroid hormone, urine calcium, phosphorus and urine tubular phosphate reabsorption). DXA scan results showed a slight improvement in S-BMD (p=0.15 after 1 year treatment and p=0.18 after

two years treatment); increased L-BMD reaching the statistical significance both after one (p=0.006) and two years (p=0.02) of vitamin D supplementation was observed; other parameters (FN-BMD and F-BMD) were almost stable.

Discussion: Costello syndrome is a rare condition belonging to the RAS-MAP chinase pathway related diseases. Other RASopathies presents skeletal deformities, osteoporosis, and with impaired bone metabolism with lack of specific therapies. Our data confirm that osteopenia and vitamin D insufficiency is a common finding of Costello syndrome and that vitamin D supplementation improves 25OH vitamin D concentrations. The evidence of the significant improvement of L-BMD suggests a possible use of oral vitamin D supplementation as a therapy to improve BMD and prevent fractures in individuals with Costello syndrome.

Essential Roles of Cbl Family Proteins in Maintaining Global Proteome Homeostasis

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CBL is one of the genes whose mutations are found in patients with Noonan syndrome. A large body of studies firmly established that Cbl family E3 ubiquitin ligases, Cbl, Cbl-b and Cbl-c/Cbl-3 in mammals, were required to limit the magnitude and duration of active tyrosine kinase-mediated signal transduction. Physiological significance of this regulatory mechanism is well portrayed in hematological pathologies of patients with *CBL* mutations, which promote aberrant expansion of myeloid lineage cells. Unexpectedly, acute loss of all three Cbl family members in primary mouse mammary epithelial cells (MECs) induced rapid cell death. Transcriptome analysis revealed characteristic changes associated with the unfolded protein response (UPR), indicating disruption of protein homeostasis.

Intracellular environment is highly crowded with macromolecules. Therefore, protein synthesis and degradation must be tightly regulated to prevent protein aggregation. In the complete absence of Cbl family proteins, we found EGFR and c-Src, well-characterized Cbl substrates, partitioned into RIPA (mild detergent)-insoluble fractions. We envision that accumulation of insoluble protein aggregates disrupted global protein homeostasis and triggered the UPR-induced cell death in MECs.

Previous studies in Cbl family proteins focused primarily on identifying Cbl substrates and trying to reconstruct biochemical pathways through ever-expanding protein-protein interaction networks. The present study uncovers an additional layer of regulation through the UPR, and may provide novel insights into various enigmatic phenotypes associated with *CBL* mutations.

Analysis of Clinical Indications, Ordering Behavior, and Genetic Testing Results for Rasopathies Genes and Panels

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Rasopathies, also known as Noonan Spectrum Disorders (NSD) are caused by genetic defects that impact the RAS-mitogen-activated protein kinase (MAPK) intracellular signaling pathway. They are pediatric disorders that share a common spectrum of symptoms, including congenital heart defects, distinctive craniofacial features, cutaneous abnormalities affecting skin and hair, and developmental tumors, both benign and cancers. The most common NSD is Noonan syndrome, with a prevalence of 1:1000–1:2500 births.

Because NSDs present with a phenotypic spectrum, testing for individual candidate genes can become an iterative time-consuming process. Multi-gene genetic tests have become increasingly available and our laboratory offers both a customizable panel and a variety of pre-curated panels for NSDs. We use NGS for read-through variant and copy-number variant analysis for detecting variants. A score-based system developed at Invitae Corporation is used in interpretation of variants for reporting with a 5-tier classification and a clinical assessment for individual variants and clinical cases.

Analysis of orders received to date at Invitae Corporation for genes and/or panel testing was evaluated for clinical indication(s) provided by the ordering clinicians. This analysis was used to understand the relationship between clinical indication(s) and genes/panels tested as well as the value of panel-based genetic testing for NSD. For example, most clinicians have ordered the Noonan spectrum disorders panel of 12 genes including BRAF1, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, SHOC2, SOS1, and SPRED1, but in 41% of the orders NF1 was also selected for testing. In 5% of the tests ordered, the clinical indication was hypertrophic cardiomyopathy (HCM), and testing included the 12 NSD panel genes and 16 genes in the Invitae HCM panel. For the majority of the tests ordered (92%), the clinical indication phenotypes provided include short stature, facial dysmorphism, heart defects and other features that fit the wide spectrum of NSD. In 34.6% of cases, a likely-pathogenic or pathogenic variant was identified, and in 9% of the cases, a variant of unknown significance was identified. A detailed analysis of clinical indications, genes tested, and variants identified will be presented.

Natural History Study of Hypertrophic Cardiomyopathy in NS-ML

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Noonan syndrome with multiple lentigines (NS-ML) is caused by mutations in the RAS/MAPK pathway genes. Many of the phenotypic abnormalities in NS-ML and the other RASopathies are quite similar and include cardiac anomalies. The most common cardiac manifestation in patients with NS-ML is hypertrophic cardiomyopathy (HCM) with an estimated prevalence of 70%. There is no existing treatment for HCM patients with NS-ML and many patients die early on from end-stage heart failure. The HCM exhibited in an NS-ML mouse model can be prevented or cured by treatment with rapamycin. This model suggests that rapamycin or a rapamycin analog could potentially be used to treat children with NS-MLassociated HCM. There is no published natural history study that details the expected course, severity, morbidity, or mortality of NS-ML-associated HCM. Similarly, it is not known if there are genotype-phenotype correlations with regard to HCM severity or natural history. The collection and review of these data will provide a better understanding of how HCM manifests and progresses in individuals with NS-ML and will identify the most appropriate candidates and endpoints for a future treatment trial. We have begun a multi-center international natural history retrospective cohort study to review serial echocardiograms and clinical outcome data for upwards of 40 patients with NSML. There are currently 7 patients fully consented for the study in the United States and additional patients to be consented in Italy. Entry criteria include patients of any age with a diagnosis of HCM and NS-ML (to be confirmed by molecular genetic testing), who is willing to provide appropriate medical records. Recruitment of patients with this rare disease is ongoing. We are approaching patients of cardiologists (with the physician's permission), and via advertising in patient foundation websites. In an initial review of the data, the charts of five subjects at a single institution were examined to pilot data procurement and echo review strategies. Of the five subjects, currently three have

known mutations in PTPN11 (1 T468M; 2 Y279C). Two of these subjects and an additional subject have family history of either gene-positive NS-ML or members with similar appearance and HCM. Four of the five subjects were diagnosed with hypertrophy, typically early asymmetric septal hypertrophy or a definitive diagnosis of HCM within the first year of life. Among these five subjects, who range in current age from 3 years to 24 years old, there has been no negative cardiac events/deaths or significant interventions. One subject was placed on a beta-blocker for the first year of life due to an episode of supraventricular tachycardia. Another has had both non-sustained ventricular tachycardia and blunted blood pressure response during an exercise test, but opted against ICD implantation. All subjects developed the diagnosis of HCM at some point in childhood, along with variable levels of pulmonary valve stenosis, branch pulmonary artery stenosis, or left ventricular outflow tract obstruction. Two subjects had progressive thickening of their ventricular septum through adolescence. One subject had significant septal hypertrophy in infancy, which improved into the toddler years. The other two subjects had stable septal thickness from infancy through the school-aged years. Chart review revealed significant variability in assessment of, criteria for, and monitoring of HCM when comparing written reports to echo images, necessitating centralized review and measurement of echocardiographic data at the primary study site. Data procurement will be ongoing between now and the meeting. Up to date information will be analyzed and presented.

Case Study of a Large Three-Generation Family with Variable Noonan Syndrome Phenotypes

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Noonan syndrome (NS), which occurs in approximately 1:1000–1:2500 individuals, is characterized by variable presentation of a wide range of phenotypes, including short stature, congenital heart defects, distinctive facial features, skeletal defects, a broad or webbed neck, coagulation deficiencies, and mild cognitive impairment. Individuals with NS also have an increased risk of developing various types of cancer, often with early onset. Because of ascertainment bias and variable expressivity often with subtle features, many affected adults are diagnosed only after the birth of a more noticeably affected infant.

Here we present the clinical and molecular findings of a large three-generation family with variable Noonan syndrome phenotypes. The proband is a 12-year-old female of normal intellect with short stature, pectus excavatum, cafe au lait spots, joint pain, and a history of atrial septal defect and mild pulmonary stenosis noted shortly after birth that resolved spontaneously. She is one of seven children born to her mother and father. The mother and maternal grandmother are generally healthy but both have short stature. The grandmother also appears to have subtle facial features of NS and a history of osteoarthritis. Three of six proband siblings have heart murmurs, and one of these individuals also has mild learning difficulties and cafe au lait macules. Other obvious features of NS were not noted for these individuals. Finally, two maternal half first cousins of the proband had histories of childhood cancers.

Sequence analysis of the PTPN11 gene revealed a missense variant in exon 3, c.209A>G (p.Lys70Arg), in the proband and her mother. This variant has not been reported in general-population databases but has been documented to segregate with disease in one family with Noonan syndrome features. Sequence analysis of other family members is in progress to establish segregation of this variant with disease. The findings in this report illustrate the importance of testing families with variable expressivity to expand the clinical and molecular spectrums of NS.

Effects of Lovastatin on Neurobehavioral Function in Neurofibromatosis I

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Studies in the *Nf1* mouse model demonstrate that the HMG-CoA reductase inhibitor Lovastatin, which acts as a potent inhibitor of p21Ras activity, can reverse the biochemical, electrophysiological and cognitive deficits observed in the mouse. In this study, we explored the efficacy of Lovastatin treatment for cognitive deficits of individuals with Neurofibromatosis Type 1 (NF1).

Methods: A prospective, double-blind, placebo-controlled, randomized14-week clinical trial was conducted in children and adults with NF1 between January 2010 and February 2013. Neuropsychological/ behavioral testing and fMRI studies were performed on 44 individuals with NF1 (21 randomly assigned to Lovastatin, 23 to placebo). Outcome measures were assessed at baseline and after 14 weeks of treatment. The primary outcome measures included one neuropsychological test and one behavioral measure that were analogous to statin-responsive tests in *Nf1* mice (tests measuring visual-spatial memory and attention). An fMRI spatial capacity working memory (SCAP) task was also performed and we investigated pre-post treatment neural activity in 10 regions of interest (ROIs).

Results: Neuropsychological/behavioral testing: There was a significant difference in trajectories over time (i.e. significant group x time interaction) between the two treatment groups for the Hopkins Verbal Learning Task (HVLT; ($F(1,33)=6.37$, $p=.02$) and Letter-Number Sequencing (LNS; $F(1,30)=20.93$, $p<.01$), in both cases with differential improvement in the statin-treated group. For the CBCL Young Adult Self Report the Internalizing score showed a difference in trajectories between the two treatment groups ($F(1,19)=5.09$, $p=.03$), with greater improvement seen in the statin group. There were no significant treatment effects (i.e., no group x time interaction) for neural activity during SCAP performance in any of the ROI's investigated. To determine if the observed treatment-associated changes in neurocognition were associated with changes in functional neuroimaging signal, we calculated Pearson correlations between change scores for these variables: Changes in the HVLT were significantly associated with changes in neural activity in the left parietal ROI ($r=.51$, $p=.04$), while changes in LNS performance were associated with changes in neural activity in the left frontal eye fields ($r=-.61$, $p=.01$) and right Brodmann Area 10 ($r=-.59$, $p=.02$). Lovastatin was well tolerated and there were no significant adverse events.

Conclusions: Compared to the placebo group, NF1 subjects taking Lovastatin showed improvement in verbal learning, working memory and attention tasks which mirror findings seen in the NF1 mouse model. Our findings suggest that Lovastatin, through modulation of the ras-MAPK pathway, ameliorates some of the abnormal cortical networks involved in NF1 cognitive dysfunction.

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HRAS codon 12 and 13 mutations in Costello syndrome and cancer

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Costello syndrome (CS) is a rare genetic disorder caused by heterozygous *de novo* germline mutations affecting the codon for glycine 12 or 13 of the *HRAS* oncogene. These mutations results in a constitutively active Ras protein and constant stimulation of proliferation and growth, ultimately leading to CS or cancer. p.G12V mutations in *HRAS* have the highest transforming activity and a high frequency in cancers, but are very rare in CS. All reported CS patients with p.G12V mutations (c.35G>T; c.35_36delinsTT; c.35_36delinsTA), have had a severe, early lethal, phenotype. A 12-year-old patient with an attenuated CS phenotype revealed, to our surprise, a new germline p.G12V mutation, c.35_36delinsTG. We showed that this mutation results in pronounced exon 2 skipping and consequently little oncogenic mutant protein, explaining the attenuated phenotype and suggesting that exon 2 splicing is vulnerable to mutations.

In this study we have characterized the molecular mechanism underlying exon 2 skipping. We used minigene transfection experiments to show that *HRAS* exon 2 holds an intrinsically weak 3'-splice site, which makes the exon vulnerable. Deletion of nucleotides c.32-37 caused exon 2 skipping, indicating presence of an ESE in this region. Optimization of the weak 3' splice site corrected splicing from the mutants confirming vulnerability of exon 2. Testing in different splicing reporter minigenes showed that ESE strength is increased by the c.35G>T (p.G12V) mutation and other mutations with strong transforming potential, whereas c.35_36delinsTG inactivated splicing. RNA affinity purification, ITRAQ labeling followed by MS/MS, showed that c.35_36delinsTG increases binding of hnRNPF/H splicing inhibitory proteins and reduces binding of the SRSF2 splicing stimulatory SR protein, whereas c.35G>T increased binding of several splicing stimulatory SR proteins consistent with the observed effect on splicing. Replacement in the *HRAS* minigene of the wild type sequence with hnRNPF/H binding ESS motifs confirmed that binding of hnRNPF/H results in exon 2 skipping. Knock down of SRSF2 caused skipping of *HRAS* exon 2 suggesting that binding of SRSF2 to an ESE is important for exon 2 inclusion. The presence of a critical ESE harboring position c.35 was confirmed by transfection of splice shifting antisense oligonucleotides (SSOs), causing skipping of exon 2 from wildtype and mutant minigenes as well as from the endogenous *HRAS* gene in T24 and HepG2 cancer cells. SSO mediated skipping of exon 2 caused reduced growth and proliferation of cancer cells and significant cell death.

Costello syndrome and somatic cancer phenotypes are a result of the transforming potential of the mutant *HRAS* protein, but can also be impacted by the efficiency of exon 2 inclusion. This has potential implications for our understanding of the correlation between genotype and phenotype in diseases caused by *HRAS* mutations, and for development of new therapeutic approaches such as splice shifting oligonucleotides.

4th International RASopathies Symposium

Speaker's Biographies

Rosemary Anderson is vice-president of the NF Network and president of NF Michigan, based in Grand Rapids. She has been involved with this group since 1985 when it was founded as the NF Support Group of West Michigan. Rosemary acts as patient advocate for the NF Clinic at Helen DeVos Children's Hospital. NF Michigan holds awareness/community events around the state, sponsors a family camp each summer, and operates a medical travel fund for Michigan residents.

Brage Storstein Andresen is professor of Human Molecular Genetics at the Department of Biochemistry and Molecular Biology, University of Southern Denmark. His laboratory focuses on the molecular genetics and the molecular pathology of human disease with a special emphasis on regulation of normal and aberrant alternative pre-mRNA splicing. They use splice shifting oligonucleotides (SSOs) to modulate splicing aiming at development of new therapies for inherited diseases as well as cancer.

Annette Bakker, PhD, is President and Chief Scientific Officer of Children's Tumor Foundation in New York City. Before joining CTF she conducted research and developed programs in pharmaceutical and biotech companies in Europe. At CTF she was previously an award winning Research Manager and Senior VP; CTF's mission consists of support and encouragement for R&D, families, clinical centers, and public awareness of NF1, NF2, and associated conditions.

Pinar Bayrak-Toydemir, MD, PhD, is Medical Director of ARUP Laboratories as well as Associate Professor in the Pathology Department at the University of Utah in Salt Lake City. She is a board-certified Medical Geneticist with a wide range of contributions including academic duties, 31 diagnostic tests developed at ARUP Laboratories, and 59 peer-reviewed publications. Her work includes studies, analysis and test development associated with RASA1.

Tammy Bowers is an advocate for advanced technology in medical care. She is Founder and CEO of Lionheart, a technology-focused company selected to work with Microsoft Accelerator. She regularly makes technology-related advocacy presentations at conferences and hospitals and is also a strong promoter of parent involvement with medical care; her son Lion has Noonan Syndrome with Multiple Lentigenes.

Emma Burkitt Wright, MRCP, PhD, is a Locum Consultant in Clinical Genetics and Honorary Clinical Fellow at the University of Manchester Centre for Genomic Medicine. She has a continuing interest in Ras-MAPK and B-Raf genes as applied to CFC syndrome. She has clinical experience with patients with Costello, CFC, Noonan, and NF1; and her PhD research included massivly parallel sequencing techniques for diagnosis of the Rasopathies.

Kathryn Chatfield, MD, PhD, is an Instructor of Pediatrics in the Cardiology Section at the University of Colorado, Denver Anschutz Medical Campus. In addition to academic duties, she is a pediatric cardiologist specializing in cardiomyopathy including, among others, applications to Costello and Noonan syndromes. Her currently funded research focuses on improving outcomes for children with cardiomyopathy.

Judy Doyle is the Parent Advisor Coordinator at Akron Children's Hospital in Ohio and is a board member for CFC International. She is team leader, trainer, parent lead, or member of many hospital- and community-associated committees and is also associated with fund-raising to promote research into CFC. Her son Jack has CFC and is 16 years old.

Florent Elefteriou, PhD, is Associate Professor at Vanderbilt University and Director of the Vanderbilt Center for Bone Biology. His research program focuses on investigating the biological mechanisms that control bone development, remodeling, repair and cancer metastasis, with the aim to develop new therapeutic strategies to prevent or treat skeletal diseases. For ten years, his laboratory has studied the formation of skeletal features associated with NF1; they recently identified molecular targets of neurofibromin signaling that are now being used for the design of novel targeted therapeutic strategies to improve bone mass, quality, strength and bone repair in individuals with NF1.

Ype Elgersma, PhD, is Professor of Molecular Neuroscience at Erasmus MC University Medical center in Rotterdam, and Director of the ENCORE clincal expertise center. His research interest is the molecular and cellular basis of neurodevelopmental disorders, and use of this knowledge to develop treatments. Mice with analogous mutations are studied at the molecular, cellular, and behavioral level. The ENCORE center was developed to bring research studies into contact with the clinical community and ENCORE is the Dutch national referral center for NF1, CFC, Costello, and other syndromes.

Michelle Ellis has NS, is a Self Advocate, and is a founding member of RASopathies Network UK and Noonan Syndrome UK. Michelle has been a conference volunteer and presenter, as well as a family support group representative, and has worked with Genetic Alliance UK and Gentic Disorders UK. Michelle has passed a message of knowledge-based empowerment on to many parents, individuals with NS, and associated doctors.

Michael J. Fisher, MD, is Associate Professor of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, and Director, Children's Hospital of Philadelphia Affiliate Clinic of the Children's Tumor Foundation NF Clinic Network. He has served as a member of the Research Advisory Board for the Children's Tumor Foundation and has also served on NF research grant panels and review boards. In addition to academic duties and other research interests, he has an extensive record of publication and leadership associated with medical aspects of neurofibromatosis.

David N. Franz, MD, is Professor of Pediatrics and Neurology, University of Cincinnati, as well as an Attending Pediatric Neurologist at Children's Hospital Medical Center and University Hospital, in Cincinnati, Ohio. He has a long-standing record of leadership and publication, including extensive work on tuberous scelerosis and comparisons to NF1.

Bruce Gelb, MD, is Gogel Family Professor of Child Health and Development and Professor of Pediatrics and of Genetics and Genomic Sciences at Mount Sinai School of Medicine in New York. His research has focused on identifying the genetic causes of congenital heart defects and then elaborating disease pathogenesis. Much of the group's work for the past decade has focused on Noonan syndrome and related disorders, which are associated with hypertrophic cardiomyopathy and congenital heart defects such as pulmonic stenosis. The group identified *PTPN11* as the first gene for the trait and have identified several others for these RASopathies.

Anne Goriely, PhD, is Associate Professor of Human Genetics and WIMM Senior Research Fellow at the Weatherall Institute of Molecular Medicine, University of Oxford. Her main research interests lie in elucidating processes of spontaneous mutation. She analyzes specific *de novo* point mutations in paternal sex cells; these studies have resulted in proposed RAS-associated mechanisms for paternal age effects in Apert syndrome, achrondroplasia, and Costello syndrome.

Karen W. Gripp, MD, is Chief of the Division of Medical Genetics, A. I. duPont Hospital for Children in Wilmington, Delaware and is Professor of Pediatrics, Jefferson Medical College, Philadelphia, Pennsylvania. In 2014 she received the Nemours Physician Excellence Award for Scholarship. Dr. Gripp has an exceptional record of clinical research and service to the Costello syndrome and RASopathies communities.

Rick Guidotti, an award-winning former fashion photographer, has spent the past fifteen years working internationally with advocacy organizations and other institutions to effect a sea-change in societal attitudes towards individuals living with genetic difference. This work has been published in Elle, GQ, People, the American Journal of Medical Genetics, The Lancet, Spirituality and Health, the Washington Post, Atlantic Monthly and Life Magazine, and others. Rick is the founder and director of Positive Exposure, an innovative arts, education and advocacy organization working with individuals living with genetic difference.

Antonio Hardan, MD, is Professor of Psychiatry and Behavioral Sciences, Child and Adolescent Psychiatry at the Stanford University Medical Center. In addition to academic duties, his research focuses on the neurobiology of autism, neuroimaging in individuals with autism, as well as psychopharmacological treatment of children and adults with autism and/or developmental disorders.

Marguerite A. Hutchinson, JD, is Senior Director, Business Development for Plexxikon Inc. in Berkeley, CA. She leads cross-functional teams in the origination and execution of collaborations with industry and academics, and is a key contributor to corporate and strategy development, while supervising legal matters for the company. Prior to Plexxikon, Marguerite worked for the UCSF Cancer Center as an Industry Contracts Officer, providing business development and legal support for strategic relationships in basic and clinical research.

Kim M. Keppler-Noreuil, MD, is Senior Staff Clinician, NHGRI/ NIH, Genetics Disease Research Branch, Human Development Section. She has provided a long history of review and classification for the Iowa and national professional communities on congenital and inherited disorders. Current research interests include studies of OEIS complex/cloacal exstrophy, Dandy-Walker malformation, and hydrocephalus. Additional research interests associated with the NIH include natural history, clinical characterization, genetic studies, and therapeutic interventions of overgrowth disorders, including Proteus syndrome and PIK3CA-Related Overgrowth Spectrum, Bardet-Biedl syndrome, and other rare genetic disorders, including Jeune syndrome.

Bronwyn Kerr, MBBS, FRACP, MD, is a consultant clinical geneticist who has worked in Manchester in the UK since 1995. Her principal research interest has been Costello syndrome, and more recently, other disorders of the RAS/MAPK pathway. She is a member of the Medical Advisory Boards of the International Costello syndrome support group, the Association Française des syndromes de Costello and CFC, and the Costello Family Support Network. She has a number of key publications in this area, and is frequently consulted by national and international colleagues for advice on diagnosis and management in this group of disorders. A particular interest is developing an evidence base for management of rare disorders.

Bruce R. Korf, MD, PhD, is Wayne H. and Sara Crews Finley Chair in Medical Genetics, Professor and Chair of the Department of Genetics, Director of the Heflin Center for Genomic Sciences at UAB, and Co-Director of the UAB-HudsonAlpha Center for Genomic Medicine. His major research interests are molecular diagnosis of genetic disorders and the natural history, genetics, and treatment of neurofibromatosis and serves as principal investigator of the Department of Defense funded Neurofibromatosis Clinical Trials Consortium. He is co-author of Human Genetics and Genomics (medical student textbook, now in fourth edition), Medical Genetics at a Glance (medical student textbook, now in third edition), Emery and Rimoin's Principles and Practice of Medical Genetics (now in sixth edition), and Current Protocols in Human Genetics.

Chiara Leoni, MD, is at the Center for Rare Diseases, Department of Pediatrics, Università Cattolica del Sacro Cuore, Rome, Italy. Throughout her education and training, Dr Leoni has always been interested in birth defect syndromes and the care of children with special care needs, in particular individuals affected by RASopthies. Since 2007 she collaborates with Dr Zampino at the Center for Rare Diseases (Catholic University in Rome) for the diagnosis and clinical management of children with genetic conditions. Dr Leoni's research focus has been in congenital malformation, primarily RASopathies, in collaboration with Dr Zampino and Dr Tartaglia.

Ram Mandalam, PhD, is the President and CEO of Cellerant Therapeutics Inc., a clinical stage biotechnology company developing novel cell-based and antibody therapies for cancer treatment and blood-related disorders. Prior to joining Cellerant in 2005, he was the Executive Director of Product Development at Geron Corporation, a biopharmaceutical company in Menlo Park, CA. His responsibility at Geron included strategic planning and implementation of development and manufacturing of cellular products for regenerative medicine, oncology and drug discovery applications. Dr. Mandalam served as the Director of Developmental Research at Aastrom Biosciences managing research and development programs involving *ex vivo* expansion of human primary cells for cell therapy applications.

Andre Marozsan, PhD, is R&D Director, Biological Discovery / Translational Research at Alexion Pharmaceuticals in Cheshire, CT. He leads a department of 17 scientists by setting priorities, providing scientific input, and obtaining resources to develop and deliver drug/therapy candidates for further development. He also coordinates external research relationship, determines strategies in the Preclinical Pharmacology department, and recently led an internal effort to develop training curricula for Alexion industrial scientists.

Frank McCormick, PhD, FRS, is Professor Emeritus, Helen Diller Family Comprehensive Cancer Center, UC San Francisco. His research projects have made a number of important contributions to knowledge, role, and behavior of Ras-associated biology. He co-founded ONYX pharmaceuticals to effectively find therapies for Ras cancers. He directed the UCSF Comprehensive Cancer Center and has recently taken a leadership role in the new National Ras Program.

Rene Pierpont, PhD, is a Pediatric Neuropsychology Fellow at the University of Minnesota Medical School. Her research focuses on neurobehavioral development in children and adolescents with genetic syndromes including Noonan syndrome, CFC syndrome, neurofibromatosis type 1, Down syndrome, and fragile X syndrome. She has published several papers examining language, cognition, memory, attention and adaptive behavior skills in individuals with CFC and Noonan syndrome and has won several grants and awards for her work on the RASopathies. Dr. Pierpont aims to improve identification and treatment of learning and behavioral difficulties among individuals with RASopathies.

Scott R. Plotkin, MD, PhD, is Assistant Professor of Neurology at Harvard Medical School, Assistant Neurologist and Director of the Neurofibromatosis Clinc at Massachusetts General Hospital, and is the Director of the MGH-DFCI-BWH Neuro-Oncology Fellowship Program. His clinical research group is interested in characterizing the phenotype of patients with tumor suppressor syndromes such as neurofibromatosis (NF), and perform whole-body MRI scans in patients to measure the distribution and volume of internal nerve sheath tumors in patients with NF1, NF2, and schwannomatosis. A second clinical research interest is in developing new therapies for patients with NF, which includes designing and executing clinical trials for these diseases using small molecule inhibitors of growth factor pathways.

Katherine (Kate) Rauen, MD, PhD, is Professor in the Department of Pediatrics, Division of Genomic Medicine at the UC Davis, Chief of Genomic Medicine, and currently the Albert Holmes Rowe Endowed Chair in Human Genetics. She is internationally known for her pioneering work in the early application of microarray technology in clinical genetics and as a leader and major contributor to the understanding of the "RASopathies", the Ras/MAPK pathway genetics syndromes. Her research program involves the clinical and basic science study of cancer syndromes with effort to identify underlying genetic abnormalities affecting common developmental and cancer pathways. Dr. Rauen led the research team, including the CFC International Family Support Group, that discovered the genetic cause of cardio-facio-cutaneous syndrome.

Karlyne M. Reilly, PhD, is Associate Scientist and Section Head, Genetic Modifiers of Tumorigenesis Section, Rare Tumors Initiative, Office of the Director, Center for Cancer Research, Intramural Research Program, National Cancer Institute, Bethesda, MD. In addition to a research history associated with rare tumors and their development, as well as with cancer development, Dr. Reilly has a significant record of activity and service related to neurofibromatosis. She co-holds a patent on a drug, Schweinfurthin, potentially therapeutic for NF1.

Amy Roberts, MD, is Director of the Cardiovascular Genetics Research Program at Boston Children's Hospital and Associate Professor of Pediatrics at Harvard Medical School. Dr Roberts is a medical geneticist with a clinical and research focus on cardiovascular genetics. Her particular areas of expertise are gene discovery, genotype phenotype correlations, and the management/treatment of a group the Rasopathies. Dr Roberts' research has led to the discovery of several genes that cause Noonan syndrome.

Abby Sandler, PhD, works at the National Cancer Institute (NCI). She splits her time between the Center for Cancer Research Office of the Director, as Special Assistant for the Rare Tumors Initiative (RTI), and the NCI Office of the Director, as Executive Secretary for the President's Cancer Panel. The RTI was launched in early 2013 to increase and facilitate collaborations between basic and clinical investigators studying rare tumors within the NCI's Intramural Research Program. The President's Cancer Panel is a Federally-chartered advisory committee that reports annually to the President on impediments to the execution of the National Cancer Program.

Lisa Schill, Vice President of RASopathies Network USA, works as a Patient and Family Advocacy Consultant specializing in connecting caregivers, researchers, support organizations and families. She spearheads collaboration with other non-profits to raise awareness of rare diseases. Currently, she also works as a Meetup Advocacy Ambassador for Global Genes facilitating rare disease efforts for aHUS (Atypical Hemolytic-Uremic Syndrome). She is co-investigator and Chairs the Advocates' Advisory Committee for the 2015 4th International RASopathies Symposium.

Lisa Schoyer is Founder and President of RASopathies Network USA. She is Principal Investigator on the NIH grant supporting the 2015 4th International RASopathies Symposium. As parent advocate of a child with Costello syndrome, she has a long-standing and passionate interest the syndrome's biomolecular overlap, based on the RAS pathway, to other syndromes including Noonan syndrome, CFC, and NF1. She has the ongoing role of convening this biennial scientific meeting held concurrently with associated family conferences, based on long experience collaborating with physician-scientists on previous related scientific meetings.

Suzanne Schrandt, JD, is the Deputy Director of Patient Engagement at the Patient-Centered Outcomes Research Institute (PCORI). She is responsible for supporting the Director of Patient Engagement in creating networks and engaging patients across the nation to provide broad-based input on the development and execution of PCORI's research. Schrandt has been involved in patient education and advocacy since being diagnosed with a form of rheumatoid arthritis as a teenager. For more than 15 years, she has advocated on behalf of children and adults with arthritis and has been engaged in numerous patient and provider education initiatives aimed at increasing early diagnosis and appropriate, patient-centered management of chronic disease.

Dawn Siegel, MD, is Associate Professor, Department of Dermatology and Pediatrics, University of Wisconsin, and Co-Director, Neurofibromatosis and RASopathy Center, Children's Hospital of Wisconsin. She is also the Director of the Vascular Genetics Translational Research and Gene Discovery Program, Dermatology, Medical College of Wisconsin. In addition to academic duties she maintains an active research presence in among others, PHACE syndrome and NF1.

Alison Silva is EVP, COO of Synlogic, a synthetic biology company pioneering therapeutic development of engineered microbes to address areas of unmet medical need. She is also the Principal and Co-founder of The Orphan Group, a specialty consulting company focused on assisting companies with their orphan drug development strategy, implementation and lifecycle product management. In addition, she is a board member of Critical Outcome Technologies, Inc., a TSX-listed company with a lead drug candidate in clinical development for gynecological malignancies utilizing a small molecule activator of misfolded mutant p53 protein.

Patroula Smpokou, MD, is a Pediatrician and Clinical Genetecist at Children's National Health System in Washington, DC. Her interests are in disorders of the Ras/MAPK pathway including NF1, Noonan syndrome, and related disorders. She runs the Noonan-Spectrum Disorders clinic in the Division of Genetics and Metabolism.

David A. Stevenson, MD, is an Associate Professor of Pediatrics in the Division of Medical Genetics at Stanford University. His research focuses on genetic modifiers and the musculoskeletal features of the RASopathies. He is the co-chair of the Costello Syndrome Family Support Network's Professional Advisory Committee and serves on the Medical Advisory Board for Cardiofaciocutaneous (CFC) syndrome International. He is actively involved in diagnosing and treating individuals with disorders of the RAS/MAPK pathway, and is on the RASopathies Network USA's Scientific Advisory Board.

Angelica (Angel) Thomas is the mother of Westin Thomas, who has Costello syndrome. Angelica became a board member of the Costello Syndrome Family Network (CSFN) in 2013. She reaches out to families of newly diagnosed children with CS to welcome them and answer questions about Costello syndrome. She links families to doctors and researchers as needed. She also assists with various fundraising campaigns and is active in the online community. Angelica is also a member of the U.R. Our Hope organization that supports children with rare and undiagnosed disorders, and is active in many other local support organizations.

Karin Scheetz Walsh, PsyD, is Pediatric Neuropsychologist, Children's National Health System, Division of Neuropsychology, Washington, DC, as well as Assistant Professor, Department of Neurology, Psychiatry, and Pediatrics, George Washington University Medical School, Washington, DC. In addition to academic duties, she has served on and chaired review boards related to the Children's Oncology Group and NF1, among others. She is PI on a new Children's Tumor Foundation grant to assess the impact of Ras/MAPK signaling pathway-targeted therapies on neurocognitive functioning in children and adults with NF1. She also has developed the Neuro-Oncology and Neurofibromatosis Neuropsychological Research training program, which was designed to attract students at all levels (undergraduate through graduate) that are interested in a career in pediatric clinical research with these populations.

Brigitte C. Widemann, MD, is Head of the Pharmacology and Experimental Therapeutics Section at the NCI Pediatric Oncology Branch. Her research program focuses on clinical development of new agents for the treatment of refractory childhood cancers (leukemias and solid tumors) and genetic tumor predisposition syndromes, in particular NF1, hereditary medullary thyroid carcinoma, and NF2. The overlap of NF1 with other RASopathies makes her work applicable to other Ras pathway disorders. She plans to use her experience developing trial designs and endpoints to assist the translation of basic research findings to the clinic.

Life with a RASopathy

CFC Syndrome

Graham Randall, 7 years old
Colorado Springs, Colorado

Likes to:
- Swing at the park
- Collect crayons
- Play with his siblings and stuffed dogs
- Take his stuffed animals for rides in his shoppoing cart
- Read letter and number books
- Surf youtube on the iPad, favorites are garages breaking, electrical poles exploding, making things with crayons and funniest home videos

Education:
Graham attends elementary school with his friends and siblings. He walks and talks. His newest discovery is jumping and galloping.

When we first knew Graham had CFC Syndrome:
When Graham was born the neonatologists knew Graham had some type of Syndrome. Initially Graham was thought to have possibly had Noonan Syndrome but tests came back negative. After a conversation with DR. Noonan and blood tests, it was confirmed that Graham (at 7 months old) had CFC Syndrome.

How we get help for him, and who his supports are:
Graham has an extensive team of medical professionals who see him on a regular basis. He also has a team of teachers, therapists and para professionals who work with him at school and privately. His family is his primary support network.

What bothers my family about living with CFC Syndrome:
CFC Syndrome is difficult to live with due to the many unknowns. There is a certain amount of anxiety when wondering if certain milestones will be meet, what additional medical needs will arise and how independent Graham will become. Of corse, more importantly, is the concern for Graham to feel accepted by his peers and to feel a sense of contentment and purpose in his life.

What our family likes about living with CFC Syndrome:
At this stage in Graham's life he's like a rock star! People are naturally drawn to him. The kids at school gather around Him for high-fives. It seems as though everyone knows him and includes him. The attention he has received because of his CFC Syndrome has been very positive so far!
Graham's disability also helps us, as a family, keep things in perspective, slow down, and remember what's really important.

What we would tell younger children with CFC syndrome or parents or doctors:
To parents: Learn to appreciate and enjoy your CFC child for who they are and all of their unique gifts and talents. Try not to dwell on what your child isn't or won't become. Everyone's journey is different but no less important. So many lives are touched and changed for good by the unassuming and loving spirits of these people.
Allow yourself to find joy in each of their accomplishments and don't compare them to anyone else. Throw out the clocks and calendars and let them grow and develop on their own time. How exciting it is to watch them surprise you time and time again.
To doctors: Please don't forget that these people are someone's son, daughter, brother or sister. Be sensitive to how you present information and make sure to treat them like people and not a science experiment.

How to include Graham better:
Graham could be included more by making sure that he is not excluded in activities that are difficult for him such as eating, climbing and running. Inclusion can be increased by having a few kids stay behind with him and moving at his speed or using devices that would help him keep up with the group. It would also be nice if his opinion was frequently asked so he could offer his input.

What we hope for Graham and what our dreams are for him:
We are striving to give Graham every opportunity to reach his own potential and to become as independent as possible. Often times Graham must be challenged and pushed to realize that he is capable of accomplishing more. As a parent, it can be painful to watch him struggle but very satisfying to see him succeed.
We hope that Graham will find a trade that he really enjoys and work hard to become skilled in that area. We hope he will feel pleased in the many things he has accomplished and will accomplish in his life. He brings a tremendous amount of joy and perspective to our family.

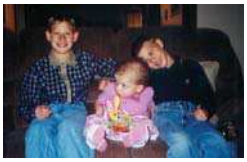
Life with a RASopathy

CFC Syndrome

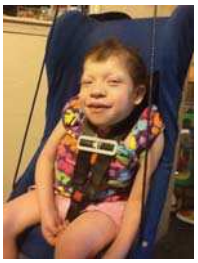
Megan Nicole Ankeny, 21
Albuquerque, New Mexico
by her mother, Maria Ankeny

Enjoys:
- The outdoors
- Going for walks
- Sitting in her swing
- Playing with our dog Baloo
- Playing on her iPad
- Water – baths and swimming








Education/Activities:
- School full time, with one more year at Albuquerque Public Schools
- Receives Physical Therapy at home. She rolls, scouts and pivots on the floor.

When we first knew Megan had CFC Syndrome:
Megan was diagnosed with CFC between the ages of 2-3 years old, after her genetics doctor went to a medical conference and saw a picture of a child that look just like Megan and the medical history sounded just like Megan.

How Megan get help when she need it, and who her supports are:
My husband and I are devoted to Megan and all her needs. Due to Megan's developmental delays she is 100% dependent on us. We are very persistent when something is going on with Megan. We have video taped her during difficult times and taken the video to doctors to let them see first hand what she is like when she stays up all night screaming & crying. We have a wonderful support group in our family and friends.

What bothers us about living with CFC Syndrome:
The lack of information our local doctors have, as Megan is the only child in New Mexico with CFC Syndrome. Since Megan is unable to communicate with us, it can be very frustrating and challenging when something is bothering her. It is like having an infant for 21 years.

What we like about living with CFC Syndrome:
The love & joy Megan has brought to our family is endless. Seeing her smile, hearing her laughter is honestly the most rewarding and inspiring thing a person can experience. She has taught us what priorities really are and, most of all, unconditional patience and love.

What we would tell younger children with CFC syndrome or parents or doctors:
Anything is possible, don't let anyone tell you what your child can or cannot do. If they take away your hopes and dreams, it will take away the possibilities for your child. Don't compare your special angel to others, they are all the same in some ways and very different in others.

How others can include Megan better:
- Accept Megan for who she is and never underestimate her, she will surprise you. When you think Megan isn't paying attention give her time alone. Although she may not do what you want her to do at that minute, she will usually do it on her own if you're not pushing her.
- Megan's motto is "when it's her idea it's a great idea, when it's your idea, it's a bad idea."

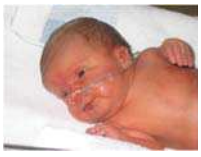
What we want in life for Megan and what our dreams are for her life:
My hopes and dreams for Megan are for her to be healthy and happy, to always be surrounded by people who love her and will put her first and give her the best quality of life.

Life with a RASopathy

Costello Syndrome

Daisy Rose Nimmo, 10 years old
London, United Kingdom

Likes to:
- Going to school when I am able
- Playing with my friends
- Drawing
- When my parents and siblings read to me
- Chill with my iPad
- Visiting the children's hospice which allows me time to be a big girl and do stuff on my own terms
- (would love to do more activities but it's diffucult to find nurses who can care for my needs when my mum and dad are not around)









Education:
I attend the MSI (multi-sensory impairment) unit at Linden Lodge School in Wimbledon 2 days a week. Because of my complex medical needs I can only manage 2 days in school but I also attend a wonderful respite centre called The Children's Trust which is in Surrey two days a week.

When I first knew I had CS Syndrome:
I am Daisy. I have never known anything different in my life. I was born early and have probably spent half my life in hospital. I rely on my parents, siblings and caregivers to speak and advocate for me and to make decisions for me. I just want to be a little girl but I am used to all the tubes that are part of me, I cannot remember a time without them because there has never been a time without them.

How I get help, and who my supports are:
Because of my complex needs I need a lot of help. I have intestinal failure and now need Intravenous Nutrition (TPN) 24 hours a day via a double lumen hickman line. , I also have an ileostomy, a jejenostomy, a gastrostomy and a mitrofanoff stoma with a catheter into my bladder. I have multifocal epilepsy as well as a visual impairment. I communicate mainly through sign language but I understand a huge amount more than I can say. My caregivers are mainly my parents - they are my voice. They manage all my IV drips and infusions and make sure I am safe, especially when I have a seizure. They also make sure that I get everything I need and that everyone, especially doctors and nurses, understand what I want and need.
I have some great nurse carers who help me have independent time away from mummy and daddy as it's important to me to get out and about and be a little girl. Most of all I love my big sister and big brothers, they make sure that I just get to have fun, we look at funny videos on YouTube together, sing along to Frozen and my big brother makes sure that my ipad has the best games on it.

What bothers my family about living with Costello Syndrome:
Now I am older I am more aware of what I want and what I don't want. I don't want to spend time in hospital away from my friends and family. I hate having to go for hospital appointments and I do not like some of the painful interventions that have to be done at home.. I get frustrated with my body because there is a lot I want to do like walking and swimming but I can't do these things any more because my body doesn't do the things it was once able to do. This makes me sad but my my parents always focus on the things I can do and make sure I am included in everything.

What I like about living with CS Syndrome:
Having such complex needs means my parents want to make sure I have the most fun possible when I am not in hospital or feeling poorly. I have been able to experience a lot of fun things and meet a lot of amazing people that maybe I would not have been able to do if I did not have Costello Syndrome. I love my school because I have made great friends there and when I have to go to hospital I love visits from Singing Hands, my favourite singing group.

What I would tell younger children with Costello syndrome or parents or doctors:
I have probably got a lot more going on with me that most other children with this syndrome but this still has not stopped me from having a great life. Even though life is tougher as I have got older and my body is not doing the things it should I am still a happy person, I still enjoy visiting places in our new wheelchair van, I get to school when I can and I spend lots of time with my family and friends. My parents would say that their life is very different to the one they thought they would have but they have learned to manage my care and we all have fun. They think of me as Daisy, not a syndrome. I know it was tough for them when I was first born but now I think they find dealing with my teenage siblings tougher than managing my medical care some days! I know my mum would want doctors to know that I am Daisy, I am not a diagnosis, and although I cannot speak very well I can understand a huge amount and I have an opinion on what you are saying. Include me in your conversations please.

How to include me better:
Please see me, I'm a 10 year old girl and in many ways I am not different to other 10 year olds. I'm obsessed with Frozen, love the colour pink, spend far too much time on my IPad and love shopping, going to the cinema and hanging out with my siblings and friends. I just need a little more help to do these things. Don't talk down to me but please take your time and check that I understand. Be patient and be confident with me and I will be your friend for life (I have my favourites though, bring me a Princess Elsa t shirt and you can be top of my friends list!)

What we hope for Daisy and what our dreams are for him:
I don't know what the future will hold for me, I have taught my parents to live in the now. It's all about what we are doing tomorrow or maybe next week. If you ask me I'll tell you that I'd like to help my favourite singing group Singing Hands by handing out stickers to all the children at the end of a session. If you ask my parents they will say they just want me to be happy and comfortable and to have as full a life as possible, no different to what they want for my brothers and sister I guess.

Life with a RASopathy

Costello Syndrome

Kelsi Moore, 21
Birmingham, Alabama
By Kelsi Moore and her mom, Tammy Moore

Education/profession:
- Part-time job at a local restaurant
- Volunteer work at the nonprofit that her mom works for
- Attends Unless U (a program with a collegiate atmosphere for young people with disAbilities)

Hobbies:
- Art
- Competitive cheerleading

When I first knew I had Costello Syndrome:
When I was little.

How I get help when I need it, and who my supports are:
Kelsi: I just ask for help!

Mom: Kelsi has become quite independent, although she does need supports and gets the majority of her supports from her parents. She has had a job and has been using public transportation for almost 3 years. She has benefited from her participation in self-advocate training at the Association for Supporting Employment First and People First conferences and at Full Life Ahead Family Weekends. She is on a waiting list of over 3500 people for Alabama's Medicaid Waiver which would give her additional supports and services that she would benefit from.

What bothers me about living with Costello Syndrome:
It doesn't bother me!

What I like about living with Costello Syndrome:
I love it! I am me! And I get to see my friends at the conferences!

What I would tell younger children with Costello syndrome or parents or doctors:
Everybody should talk to ME and talk to my mom and dad when they need to.

How others can include me better:
Kelsi: Be a good friend!

Mom: Talk to HER and get to know her. She can answer most questions for herself and has her own opinions. Just give her a chance and you will see what a funny, caring person she is… Appreciate her uniqueness like she enjoys the uniqueness of everyone she meets.

What I want in life and what my dreams are for my life:
Kelsi: I want my own apartment at my mom and dad's house like Jill and Amanda. I want more hours at work. I want to go to Disney World. Maybe get married one day. Maybe have kids.

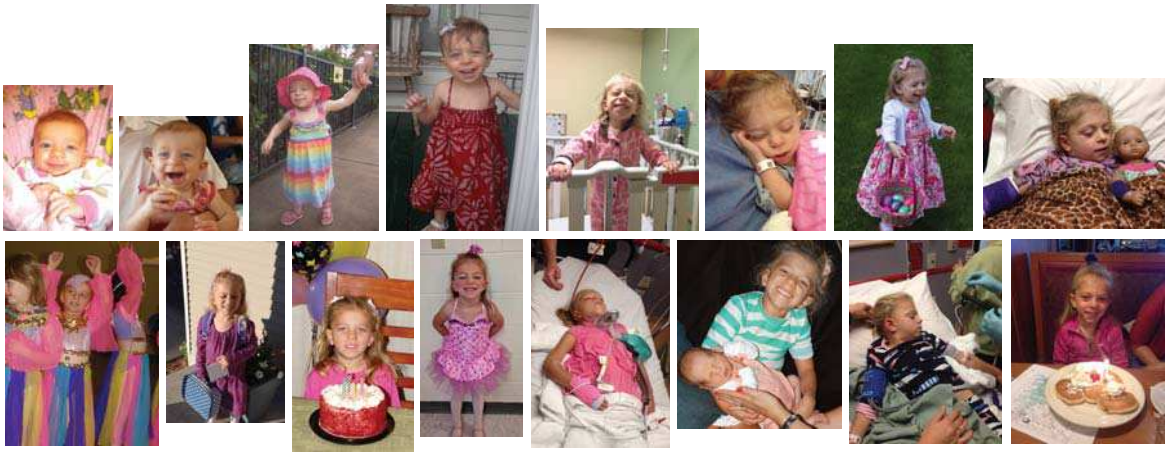
Mom: My dreams for her are what she dreams for. I want her to be as independent as possible and live a full life. My biggest hope is that she will be able to continue on with her dreams with the supports she may need and be safe, healthy and happy after her dad and I are gone.

Life with a RASopathy

NF type 1

Lauren Marie Geier, 5 years old
Verona, Wisconsin

Hobbies:
- Swimming
- Ballet
- Tennis
- Taking care of my babies



Education:
Starting kindergarten in fall 2015

When I first knew I had Neurofibrotosis type 1:
My parents found out I had NF when I was 18 months old but only recently am I starting to understand more about NF.

How I get help, and who my supports are:
I am currently receiving great care from the NF clinic at UW Children's Hospital in Madison, WI. I also have met some amazing "NF Fighters" through the NF Network.

What bothers me about living with Neurofibromatosis type 1:
I have to go to the hospital a lot for MRIs and spinal taps and I don't like getting shots!

What I like about living with Neurofibromatosis type 1:
I like the friends I have made that also have NF. My friend Olivia is one of my best friends and she also has NF.

What I would tell younger children with Neurofibromatosis type 1 or parents or doctors:
I would tell other kids to be brave, I would tell doctors that I hope they find a treatment or a cure because NF is no fun, and I would tell parents to love your kids with NF because we all struggle to fit in outside of the home.

How to include me better:
Please don't judge me on how I look or act sometimes, some days are hard for me and I just don't know why! Please be patient with me, sometimes I don't know what I need.

What I want in life and what my dreams for my life:
I want to grow up and be a doctor and a mommy just like my mommy.

Life with a RASopathy

NF type 1

Sloan O'Dell Mayer, 24
Newberry, South Carolina

Hobbies/Pleasures:
- Spending time with my family, including my niece and my nephew
- Turkey hunting with my father, which allows my father and me to spend quality time together, with nature and with God

Education:
2015: Bachelors of Science in Social Work, University of South Carolina
Married to Nathan Mayer

When I first knew I had NF1:
I was diagnosed when I was 19 years old by a local dermatologist, Dr. Dina Grice. After my diagnosis, there were a lot of emotions my family and I experienced. I had only been married to Nathan for eight months. He and I thought we were going to have a different 'newlywed' year together. However, it consisted of many doctor visits, trips to Duke University Hospital, hospital stays and tests.

How I get help when I need it, and who my supports are:
I have never seen the love and compassion of a family unit more than I have with my own. I have witnessed the true test of faith and dedication by not only my parents and siblings, but most importantly by my husband; he is truly a blessing from God.

What bothers my family about living with NF1:
I could sit here and tell you nothing bothers me about having NF, but I would be lying. When I was told that I have NF when I was 19, I couldn't grasp the diagnosis. I sat in the doctor's office with my sister, totally confused. How is it that I have already had one major brain surgery to remove an astrocytoma off of my cerebellum and now I'm looking at the possibility of more tumors? I thought that was a one time thing? Boy was I wrong. It bothers me that I can't be 'prepared' for the next tumor. It bothers me that I won't have a 'healthy child' with my husband. It bothers me that my mother has to watch me go through the things I have to go through.

What our family likes about living with NF1:
A year ago, I could not answer this question. I was still trying to figure out exactly what my purpose in life was. As a preacher's daughter, I have experienced, on more occasions than most, life. Everyone's days are numbered, regardless what your beliefs are. This life that we are living now is just temporary. My father has stated it best, "Why shouldn't Sloan have neurofibromatosis?" Your question asks, "What I like about living with NF?" Neurofibromatosis is just temporary. If you do not keep the faith and believe in a cure, then there is nothing to like about it.

What we would tell younger children with NF1 or parents or doctors:
I have learned through my diagnosis that it is very important to advocate for yourself. No one knows your body better than you do. Only you can tell when something doesn't feel right and inform your physicians. It's important to also keep a healthy relationship with your medical team. Emailing or calling my physicians at Duke University Hospital any time with concerns has been invaluable.

How others can include me better:
I don't honestly believe that I could be included any more in my activities than I already am. Now, as a child, it was tough. Physically I was not athletic and had headaches on a daily basis. If there was one thing I would want people to understand better, it's that just because you have a tumor, doesn't mean you have cancer. I cannot explain how many people in my home town honestly believe I have cancer.

What I want in life and what my dreams are for my life:
What I want in my life is a cure. I understand that my case is very manageable at this point in my life. We all know though, NF can change overnight. I don't want other parents to have to experience what my parents have gone through. I want to know that all children with NF get to go out on their 'first date' and have the chance to attend college - like I did. I have neurofibromatosis, neurofibromatosis does not have me.

Life with a RASopathy

Noonan Syndrome

Ezra Parker, 2 years old
Corvalis, Oregon

Likes to:
- Play with his big brother
- Watch Mickey Mouse Clubhouse
- Throw balls

When we first knew Ezra had Noonan Syndrome:
Ezra was diagnosed with Noonan Syndrome at 6 months old, two months after he was diagnosed with Juvenile Myelomonocytic Leukemia (JMML).

How we get help for him, and who his supports are:
Ezra's parents and primary pediatrician have been instrumental in getting all the referrals he needs to manage his symptoms. He has seen/sees a number of specialists at Doernbecher Children's Hospital including: hematology/oncology, gastroenterology, urology, genetics, feeding clinic, audiology, and ophthalmology. He also receives early intervention services through his county

What bothers my family about living with Noonan Syndrome:
Ezra's biggest bother living with Noonan Syndrome was when he had JMML for the better part of his first year of life. It made his spleen dangerously enlarged and caused him to vomit forcefully for months. It made him even slower to grow and gain weight and required countless blood draws, two bone marrow biopsies, and a g-tube placement to administer two months of oral chemo. Today Ezra struggles to communicate with a limited vocabulary which makes him very frustrated at times.

What our family likes about living with Noonan Syndrome:
Ezra likes the attention he gets for his exceptionally cute face and big, gorgeous blue eyes. He also likes that he gets to wear his favorite clothes longer than typical kids because it takes him much longer to outgrow them.

What we would tell younger children with Noonan syndrome or parents or doctors:
Ezra would like to tell other kids living with Noonan Syndrome and their parents that they're not alone! The NS support groups have been such a blessing to his family and they are available to everyone as an outlet for solidarity in triumphs and trials. He would like doctors to know more about Noonan Syndrome! So far, Ezra is many of his doctors' first patient with NS and he really appreciates the doctors who take extra time to learn more so they can better care for him.

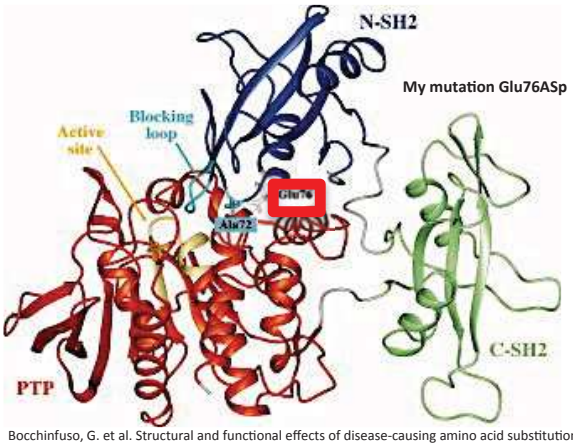
How to include Ezra better:
Others can include Ezra better by not making assumptions about his age and capabilities based on his size. They can look for creative ways to communicate with him when he isn't able to say what he wants.

What we to have in Ezra's life and what our dreams are for him:
Ezra's parents think that his dream is to get to do everything his big brother gets to do. He wants to have the same opportunities as everyone else. He wants everyone to know more about the RASopathies and other rare diseases.

Life with a RASopathy

Noonan Syndrome

Judith van de Meerakker, 38
Utrecht, The Netherlands



Bocchinfuso, G. et al. Structural and functional effects of disease-causing amino acid substitutions affecting residues Ala72 and Glu76 of the protein tyrosine phosphatase SHP-2. Proteins 2007; 66(4):963-74.

Education/profession:
- Almost finished my PhD in Genetic aspects of congenital heart defects
- Currently working in the quality system at the department of DNA-Diagnostics, AMC, Amsterdam, The Netherlands

When I first knew I had Noonan Syndrome:
I first met the person who later diagnosed me, in 2006. She was a clinical geneticist from Newcastle upon Tyne. At that time, I told her I was born with a congenital heart defect (which is why I am working on a PhD addressing the genetic aspects of congenital heart defects). Then I went to a conference in 2007 where I saw her again, and where she apparently had been observing me. After the conference, she suggested that I may have Noonan syndrome because of my heart defect and low set ears. Back in the Netherlands I went to the department of Clinical Genetics to ask for a genetic test for Noonan syndrome. Although I didn't meet the diagnostic criteria at that time, I got tested anyway. Within six weeks the test came back indicating a mutation in the PTPN11 gene. The clinical geneticist in the Netherlands was in shock. This was in September of 2007, I was 31 years old. (Only a couple of weeks later I had the chance to meet Dr Jacqueline Noonan herself at a meeting of the Dutch Noonan syndrome foundation.) In 2008 I worked for three months in Newcastle upon Tyne as part of a European collaboration project - for the same clinical geneticist that gave me the diagnosis. Before my diagnosis I was a researcher, now I am also a person with Noonan Syndrome, I have to find a balance between the two.

Hobbies:
- Reading
- Hiking
- Scuba Diving
- Visiting Concerts
- Hanging out with friends
- Travelling







How I get help when I need it, and who my supports are:
I was born with ASD, VSD and a leaking mitral valve. I had three open heart surgeries before the age of 3. When I was little it was my mother who made sure I got the help I needed. Because this was a struggle for her, she taught me to get the help I need even if it means switching doctors. Nowadays I am capable of getting the help I need myself, with the help of my GP. She really is great and takes me seriously in the things I say and need. She also provides me with a good set of medical specialists.

My support is still my mom of course and I have a good set of friends. I also have a wonderful boyfriend who takes care of me when I don't feel well and is just there when I need him.

What bothers me about living with Noonan Syndrome:
Not much really. I was born with a congenital heart defect that I had to deal with my whole life. I am used to take care of myself and I have boundaries different from "normal" people. Compared to a lot of other people with the syndrome, my problems are not that big. The only thing that I sometimes get tired of is that because I have a syndrome, every now I experience a new problem or I have to get some new findings checked if they also apply to me.
I don't know what the rest of my life will bring, but on the other hand who does?

What I like about living with Noonan Syndrome:
It makes me different and unique from the rest of the world and it has given me a bubbly personality. It has also given me the motivation to continue doing research.

What I would tell younger children with Noonan syndrome or parents or doctors:
The most important thing that I want to tell everyone is to look at what you or your child/patient CAN do and not what you or your child/patient cannot do. Don't let other people tell you what you cannot do, but try it yourself. Enjoy life with all the things you can do and look for people that like you the way you are and appreciate the extra joy you will bring into their lives.

How others can include me better:
Respect me for being a human being and not being perfect and you will get a real valid friendship back.

What I want in life and what my dreams are for my life:
I will finish my PhD this fall. My ultimate goal is to do research about the Noonan syndrome and RASopathies. To find cures and to improve the understanding of the syndromes, not only for doctors but also for the people living with it on a daily basis.

Life with a RASopathy

NSML/LS

Landen Lion Bowers, 5 years old
Lehi, Utah

Likes to: Play with puzzles, especially ones that are of maps

Education: in Preschool












When we first knew Landen had Noonan Syndrome with Multiple Lentigenes: 3 months old (started testing at/near birth)

What we would tell younger children with NSML or parents or doctors:
Landen always tells people that getting your blood drawn isn't a big deal and doesn't really hurt...plus you get a sucker afterward

How we get help for him, and who his supports are:
His family (dad, mom, 1 older brother and 2 older sisters)

How to include Landon better:
He is always included...although he doesn't quite run as fast...he works hard.

What bothers our family about living with NSML:
Nothing he loves life...although he would probably like it if his knees didn't hurt so much.

What our family likes about living with NSML:
His new heart and his spots

What we to have in Landon's life and what our dreams are for him:
Being a mailman, police offer and a doctor are all on his list.

Life with a RASopathy

NSML/LS

Sophie Konopka, 14 years old
Swanzey, New Hampshire

Hobbies:
- Crocheting
- Sewing
- Playing theclarinet
- Video games, especially Mineraft

Education: Finishing 8th Grade

When I first knew I had Noonan Syndrome with Multiple Lentigenes:
My parents told me in July 2011. (but I was diagnosed in July 2010 via genetic testing).

How I get help, and who my supports are:
I ask my parents for help. and who my supports are: My family and friends and sometimes my doctors.

What bothers me most about living with NSML:
The constant pain and it's hard to focus on stuff.

What I like about living with NSML:
I like my freckles ☺

What we would tell younger children with NSML or parents or doctors:
There are things that can help you deal with all the pain.

How to include Sophie better:
I am lucky to be included in many things. I don't really feel left out.

What I want in life and what my dreams are for my life:
I want to have a good career -either as a doctor or an animator because both jobs have the ability to help people -to help them feel better or to help them feel happy.

4th International RASopathies Symposium

Planning Committees

Scientific Advisory Board

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Karen Gripp, MD, Nemours/duPont Hospital for Children, Wilmington, DE

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Chair: Lisa Schill, RASopathies Network USA

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Ilse Levins, CFC International

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Costello syndrome:

Colin Stone, International Costello Syndrome Support Group (ICSSG)

Tammy Moore, Costello Syndrome Family Network (CSFN)

Neurofibromatosis type 1:

Kim Bischoff, NF Network

Annette Bakker, PhD, Children's Tumor Foundation (CTF)

Noonan syndrome:

Michelle Ellis, Noonan UK

Ann Yurcek, Noonan Syndrome Foundation

Noonan syndrome with multiple lentigenes (NSML)/LEOPARD syndrome:

Tammy Bowers

Investigators

Lisa Schoyer, President, RASopathies Network USA, Principal Investigator

Lisa Schill, Vice President, RASopathies Network USA, Co-Investgator

4th International RASopathies Symposium

Notes

4th International RASopathies Symposium

Notes

THANK YOU TO OUR PARTNERING ORGANIZATIONS

CFC International
Cardio-Facio-Cutaneous Syndrome

CHILDREN'S TUMOR FOUNDATION
ENDING NF THROUGH RESEARCH

ICSSG
International Costello syndrome Support group

NEUROFIBROMATOSIS NF NETWORK

Costello Syndrome
FAMILY NETWORK

NOONAN SYNDROME FOUNDATION

Noonan UK

SAVE THE DATE

Fifth International RASopathies Symposium

Hosted by:

RASopathies Network USA

RASopathiesNet

Connect ~ Collaborate ~ Cure

Co-chairs:

Dr. Frank McCormick

and

Dr. Katherine A. Rauen

Renaissance Orlando at SeaWorld
July 28-31, 2017

Tentative Itinerary:

Friday 28th....poster/reception night

Saturday 29th and Sunday 30th am....scientific meetings

Sunday 30th pm....post-symposium family meetings

Monday 31st....meet the experts and researchers

Please visit https://rasopathiesnet.org for furture information