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The Fourth International Symposium on Genetic Disorders of the Ras/MAPK Pathway

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Abstract

The RASopathies are a group of disorders due to variations of genes associated with the Ras/MAPK pathway. Some of the RASopathies include neurofibromatosis type 1 (NF1), Noonan syndrome, Noonan syndrome with multiple lentigines, cardiofaciocutaneous (CFC) syndrome, Costello syndrome, Legius syndrome, and capillary malformation–arteriovenous malformation (CM-AVM) syndrome. In combination, the RASopathies are a frequent group of genetic disorders. This report summarizes the proceedings of the 4th International Symposium on Genetic Disorders of the Ras/MAPK pathway and highlights gaps in the field.

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Keywords

RASopathy; Ras/MAPK; cancer; rare disorders; clinical trials; experimental models

INTRODUCTION

Alterations of the Ras/mitogen activated protein kinase (MAPK) transduction pathway impact proliferation and differentiation of many cell types and lead to an array of phenotypes [De Luca et al., 2012]. A number of genetic syndromes due to mutations of genes of the Ras/MAPK pathway have been collectively termed RASopathies [Rauen, 2013]. In aggregate, the RASopathies represent one of the largest groups of genetic disorders, with an estimated prevalence of approximately 1 in 1,000. Some of the RASopathies include neurofibromatosis type 1 (NF1), Noonan syndrome (NS),Noonan syndrome with multiple lentigines (NSML), cardiofaciocutaneous (CFC) syndrome, Costello syndrome (CS), Legius syndrome, and capillary malformation–arteriovenous malformation (CM–AVM) syndrome. Although each RASopathy disorder presents with a distinct constellation of clinical problems, there is significant phenotypic overlap.

In order to better understand the molecular bases of the RASopathies and translate information across syndromes for better medical management and development of new therapies, a biannual symposium on RASopathies was initiated in 2009. This report summarizes the proceedings of the 4th International Symposium on Genetic Disorders of the Ras/MAPK pathway held in July, 2015 in Seattle, Washington and highlights gaps in the field. The symposium included a broad array of trainees, clinicians, scientists, industry, advocates, funding agencies, and patients/families. The symposium is unique in its approach as it is held in conjunction with multiple advocacy meetings for RASopathy family/patient support groups. A poster session of submitted scientific abstracts and invited posters for representative parent/patient support groups was held to open the symposium allowing for interaction and discussion between the lay community and the scientific community. Details of specific sessions are provided below.

PROCEEDINGS

Keynote Presentation

The *keynote speech* by Frank McCormick provided a review of Ras signaling pathways and protein interactions of the *NF1* product neurofibromin, which are required for Ras activity [McCormick, 2015]. He described a key role of SPRED proteins: neurofibromin binds directly to SPRED proteins, resulting in translocation to the plasma membrane, an essential interaction 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. He highlighted that a detailed understanding of SPRED binding partners may lead to identification of novel therapeutic approaches. He emphasized the importance of neurofibromin as a major negative regulator of Ras, and that *NF1* is frequently mutated in a variety of human cancers. *KRAS* is significantly more potent than *HRAS* in tumorigenesis models, and he described the developing understanding of

differences between cells transformed by *KRAS* and by *HRAS*: cells transformed by *KRAS*, but not by *HRAS*, cause a stem-like phenotype. An interaction of *KRAS* (but not *HRAS* or *NRAS*) and calmodulin modulates tumor formation through inhibition of calmodulin kinase activity. Disruption of this interaction by the natural product prostratin suppresses tumor growth in preclinical models [Wang et al., 2015]. These findings and the existence of a new effector pathway specific for *KRAS*, therefore, offer new opportunities for therapeutic interventions.

RASopathy Phenotypes (Shared and Discordant)

In this session, speakers were encouraged to discuss the main shared and discordant clinical features among RASopathies and provide potential measurable endpoints for clinical trials.

Bruce Korf discussed cancer predisposition in RASopathies. NF1 demonstrates a ≈10% average lifetime risk of malignant peripheral nerve sheath tumour (MPNST), as well as increased risk of other tumors, such as gliomas, rhabdomyosarcomas, and juvenile myelomonocytic leukemia (JMML). For the other RASopathies, the overall malignancy risk is about ten-fold higher than the general population [Kratz et al., 2015], and highest for embryonal rhabdomyosarcoma (ERMS) in CS and JMML in NS. Tumor studies have demonstrated loss of heterozygosity in NF1 neurofibromas, but more complex changes in MPNST and ERMS. He also described experiments in which an *NF1* stop mutation mouse model has been created and is being used to test nonsense read-through drugs as a possible therapeutic strategy.

Mechanisms underlying cognitive deficits in the RASopathies were discussed by Ype Elgersma. Mouse models suggest that the cognitive deficits in the different pathway disorders may have unique underlying mechanisms not easily predicted from knowledge of perturbations in the pathway. For example, the perturbations in hyperpolarization-activated cyclic nucleotide-gated (HCN) channel currents observed in the *NF1* mouse model were not observed in the *HRAS* mouse model. Treatment trials in mice demonstrated different responses to medication, with statins rescuing cognitive deficits in both NF1 and NS (not confirmed by placebo controlled clinical trials in NF1 individuals), but having no effects on cognitive performance in adult CS mice. In addition, MEK inhibitors rescued the plasticity phenotype of CS mice but failed to rescue the cognitive deficits. A review of treatment trials [van der Vaart et al., 2016] in genetic cognitive disorders has shown very limited efficacy, and there is need for larger scale studies, international collaboration and improved reporting and design, particularly with predefinition of outcome measures.

Kathryn Chatfield provided a detailed description of cardiac manifestations in the RASopathy syndromes with discussion of targeted therapies. Noonan syndrome (NS) is the most common syndromic cause of congenital heart disease. In a large retrospective cohort study [Prendiville et al., 2014], cardiovascular involvement occurred in 81% of individuals affected by NS, most commonly pulmonary stenosis and atrial septal defects, with cardiac hypertrophy occurring in 16%. There was a significant association between *PTPN11* mutations and both pulmonary stenosis and atrial septal defect, whereas *RAF1* mutations were associated with hypertrophic cardiomyopathy (HCM). NS associated pulmonary stenosis was significantly more likely to require re-intervention after percutaneous balloon

valvuloplasty. Hypertrophic cardiomyopathy of infantile onset was more likely to regress (17%). Predictors of poor outlook include a mixed cardiomyopathy picture, onset less than 1 year, and congestive cardiac failure at presentation. Although supraventricular arrhythmia was first associated with CS, with or without congenital heart disease or HCM, it has been reported in patients with mutations in *RAF1*, *KRAS*, and *PTPN11*, and may be refractory to treatment, and less likely to resolve with age than in CS. Clinical trials with MEK inhibitors are in development to evaluate the potential benefit on HCM in individuals with NS.

Advocate Panel

The Advocates' Panel, comprising Judy Doyle for CFC syndrome, Angelica Thomas for CS, Rosemary Anderson for NF1, Michelle Ellis for NS, and Tammy Bowers for NSML, advocated for research to address chronic pain in each of the syndromes. Similarities included joint pain, headaches, and abdominal pain—though there was a spectrum of severity and presentation. Lack of knowledge on the etiology and treatment of pain was a concern. Future studies characterizing pain in RASopathies are needed, and may not only improve patient management, but also lead to the development of pain as a meaningful endpoint in clinical trials.

Preclinical Studies

The session on preclinical studies aimed at describing the RASopathies models currently in use in preclinical drug discovery and testing.

The session was opened by Katherine Rauen, who presented her lab's efforts to evaluate the impact of Ras pathway activation in skeletal muscle development. She reviewed results on muscle biopsies from patients, demonstrating specific myopathic abnormalities, including muscle fiber size and variability of type-2 fiber (fast twitch) predominance, that are consistent with the muscle weakness and hypotonia observed in young patients with CS and CFC. She described the development of a skeletal myogenic culture assay that allows assessment of specific Ras/MAPK mutations and evaluation of pathway inhibitors.

Bruce Gelb described the use of a *Drosophila* NS (*RAF1* mutation) model to perform a high-throughput screen of a large (>14,000) chemical compound library. He explained the advantages of screening with a "whole organism" model rather than cultured cells to assess the role of drugs intended to modulate—rather than shutdown—the Ras pathway. The screen identified compounds capable of rescuing this NS fly's lethality that will be further tested in iPS cells and mouse models.

Karlyne Reilly generated mouse MPNST cell lines and used them together with established human lines in high-throughput drug treatment assays. She emphasized the need to precisely evaluate dose responses in several cell lines to analyze heterogeneous responses of tumor cells. She proposed a stepwise drug screening strategy, where responses to candidate pathway inhibitors is progressively tested in an increasing number of cell lines, before being prioritized for in vivo work in murine tumor models.

Regulation of alternative splicing was proposed by Brage Andresen as a novel therapeutic strategy to modulate Ras overexpression. He described the molecular characterization of an

unusual germline *HRAS* mutation, which revealed the presence of an Exonic Splicing Enhancer (ESE) necessary for efficient splicing. The c.35_36GC>TG (p.G12V) mutation destroys the ESE motif, leading to exon skipping (and reduced mutant protein production), explaining the patient's attenuated CS phenotype. Using splices-witching antisense oligonucleotides targeting crucial ESE sites, he showed how splicing can be modulated to knock-out activity of the resulting Ras protein and demonstrated the use of splice-switching antisense oligonucleotides as therapeutic agents in cancer cell lines carrying *HRAS* mutations.

Florent Elefteriou considered treatment for short stature, a phenotype commonly associated with RASopathies. Although the use of growth hormone therapy may be of concern in cancer-prone children, it has been used in these patients with satisfactory results. The etiology of RASopathies-associated short stature remains poorly characterized but can be studied in NS and CFC mouse models as they exhibit this phenotype. He discussed the efficacy of BMN111 (Biomarin), a RAF1-inhibitor currently in clinical trial for the treatment of achondroplasia (FGFR3), in promoting growth in an NF1 mouse model, and proposed that it may also be beneficial for other Ras-associated disorders.

The increasing number of RASopathies models under study attests of the complex pathology associated with these disorders. Evaluation of therapeutic approaches requires well-characterized biological models. Using these parallel lines of research will contribute to disentangling the complexity and specificity of Ras signaling in different cell-types and tissues, a step necessary to the long-term goal of fine-tuning, and modulating the Ras pathway in clinical practice.

Clinical Trials in RASopathies: Design, Endpoints, and Results

This session highlighted ongoing clinical trials efforts and reviewed successes, challenges, and pitfalls.

Brigitte Widemann described results from the NCI phase I trial of selumetinib, a specific MEK inhibitor, in children with NF1 and inoperable plexiform neurofibromas [Widemann et al., 2014b]. Plexiform neurofibromas are benign tumors, which occur in up to 50% of individuals with NF1. Many are diagnosed at young age and grow the fastest in young patients. These tumors can cause substantial morbidity including pain, motor dysfunction, and airway compromise. The Ras/Raf/MAPK pathway is active in plexiform neurofibromas and in a genetically engineered mouse model of NF1 neurofibromas a MEK inhibitor resulted in substantial tumor shrinkage for the first time, supporting clinical trials with MEK inhibitors directed at plexiform neurofibromas [Jessen et al., 2013]. The maximum tolerated dose of selumetinib in this trial was 60% of the recommended dose for adults with solid cancers. At this dose selumetinib was well tolerated and all dose-limiting toxicities were reversible. Plexiform neurofibroma volume decreases were observed in more than 50% of the patients and associated with clinical improvement in some patients. This is the first clinical trial describing shrinkage of large plexiform neurofibromas in a substantial number of patients. Based on these promising findings a phase II trial is in development, which has the primary objective to confirm the response rate using volumetric MRI analysis, and as key secondary objectives to prospectively evaluate the effect of selumetinib on pain, quality of

life, and functional outcomes depending on the location of the PN. She emphasized the need for validated outcome measures in NF and the challenge of selecting appropriate patient reported and functional outcome measures given the variable locations of plexiform neurofibromas. She highlighted that recommendations from the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) international working group proved very helpful for the selection of outcome measures and suggested that similar working groups might be beneficial for development of treatments for RASopathies [Plotkin et al., 2013].

The Ras/MAPK signaling cascade is involved in neuronal plasticity and long-term potentiation. 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 [Costa and Silva, 2002]. Karin Walsh described her ancillary pilot trial of the effect of Ras/MAPK inhibitors on neurocognitive function in NF1. 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, adolescents, and young adults with NF1. Patients with NF1 enrolling on trials targeting the Ras/MAPK pathway for tumor manifestations have the opportunity to enroll on this ancillary study, which uses a novel, computerized assessment approach (Cogstate) [Costa and Silva, 2002] to examine change in cognitive function over the first 12 weeks of treatment. This pilot trial maximizes the opportunity for understanding the relationship between regulation of the Ras/MAPK pathway and cognitive functions from multiple ongoing treatment trials using a novel evaluative approach.

Amy Roberts described Therapeutics for Rare and Neglected Diseases (TRND)—funded research on NSML. The TRND program supports the pre-clinical development of therapeutic candidates intended to treat rare or neglected disorders, with the goal of enabling Investigational New Drug applications. In addition, because of the lack of published natural history data, TRND is conducting a multi-center retrospective analysis of individuals with NSML to analyze genotype, overall phenotype, medical history, and cardiac features as detailed by ECG, echocardiogram, cardiac MRI, and/or exercise testing.

NSML is a rare genetic disease affecting only about 200 patients worldwide. Nearly all cases of NSML result from mutations in the *PTPN11* gene. In the heart, the most common manifestation is hypertrophic cardiomyopathy [Gelb et al., 2015]. There is no effective standard treatment for NSML patients who have HCM and end-stage heart failure can lead to early death. In animal models of NSML, the mTOR inhibitor sirolimus can prevent and reverse HCM. Additional preclinical studies with similar agents are ongoing and may provide the basis for future clinical trials targeting HCM in patients with NSML.

Clinical trials for rare genetic syndromes present multiple challenges including the ability to enroll sufficient patient numbers in a timely manner. In addition, the varied clinical presentation of these conditions may require investigators to design multiple independent trials for patient subgroups with different clinical presentations. For example, several clinical trials may be developed to evaluate the activity of an experimental agent in NF2-related vestibular schwannomas, meningiomas, and ependymomas. Scott Plotkin presented a

collaborative effort with Rebecca Betensky *(Harvard TH Chan School of Public Health, Boston, MA)* to develop a novel statistical clinical trial design with individualized endpoint analysis in trials for NF. Patients with a heterogeneous disease and with patient-specific manifestations and associated endpoints for clinical trials will be considered for this design. For example, this design would be applicable to patients with NF1 and plexiform neurofibromas with different locations and associated morbidities: plexiform neurofibroma of the orbit causing vision loss, of the airway causing respiratory compromise, of motor nerves causing motor weakness, or plexiform neurofibromas causing pain. The design initially recruits all patient subtypes, each with its own, preselected endpoint. At an interim futility analysis, subgroups in which the treatment appears ineffective are discontinued, and recruitment continues with the remaining subgroups. Preliminary findings demonstrate that when a relatively rare subgroup responds to treatment, use of the proposed design can increase the overall power of the trial, though at the unavoidable expense of lengthening the time of the trial. The design also demonstrates superior power relative to the alternative of conducting separate trials for each patient subtype or using a composite endpoint for unselected patients. The proposed individualized endpoint design, in conjunction with an interim analysis for futility, may hold promise in some clinical scenarios.

In summary, this session described substantial advances required for conduct of meaningful clinical trials for RASopathies including the value of: i) longitudinal natural history data; ii) relevant preclinical models to screen for active agents; iii) novel trial designs and applicable endpoints; and iv) international working groups with the goal to establish consensus in clinical trials.

Collaborations, Industry, and Funding

The goal of this session was to discuss mechanisms of collaboration with advocacy groups, academia, industry, and government agencies, which can accelerate the translation of promising preclinical findings into the clinic.

To set the stage, Michael Fisher provided an overview of agents targeting Ras/MAPK in clinical development and highlighted specific considerations for RASopathies. Somatic mutations in the MAPK pathway genes occur in greater than 30% of sporadic malignancies, and have been identified in many of the genes implicated in RASopathy syndromes, including *NF1, PTPN11, KRAS, HRAS, BRAF*, and *MEK* Efforts to target the MAPK pathway genes with single agent inhibitors have had mixed success and include the use of the farnesyl transferase inhibitor tipifarnib [Widemann et al., 2014a] and the antisense 20-mer oligodeoxy-nucleotide targeted to *HRAS* [Alberts et al., 2004]. There are three FDA approved BRAF inhibitors: Sorafenib, which also targets other receptor tyrosine kinases, and vemurafenib and dabrafenib, which are FDA approved for V600E melanoma. Although mutations in *MEK* are rare in cancer, they represent an attractive target for Ras/MPAK driven cancers, as ERK 1/2 are the only known targets of MEK 1/2. Early experience with the MEK inhibitor selumetinib for NF1-assoicated plexiform neurofibromas is promising (NCT01362803) [Widemann et al., 2014b]. More recently, clinical trials of inhibitors of ERK have begun (NCT01781429, NCT02296242). Although MAPK pathway targeting is promising, challenges remain. Identification of the appropriate inhibitor will depend on the

specific molecular abnormality and tumor type. Thus far, many of the agents appear to be cytostatic; therefore, single target therapies may be unlikely to permanently eradicate tumors. The development of resistance to single agent therapy is common and multiple mechanisms have been described including paradoxical activation of other MAPK pathway proteins [Gibney et al., 2013], upregulation of growth factor or growth factor receptors, and activation of parallel signaling pathways (e.g., PI3 K pathway). In order to increase efficacy and overcome resistance, future therapy will likely be combinatorial and focused on targeting multiple MAPK pathway members, or utilizing MAPK pathway inhibitors along with cytotoxic chemotherapy and/or inhibitors of the PI3 K pathway, transcription (e.g., cell cycle inhibitors), or translation.

The NCI intramural research program has a long track record of studying rare tumors. Recognizing the potential to better leverage the expertise of NCI intramural investigators to more effectively translate potential new therapies for rare tumors, the NCI Rare Tumors Initiative was launched in 2013. Abby Sandler explained that the main focus of the initiative is to foster collaborations of laboratory-based investigators with clinical investigators to more effectively and efficiently translate basic findings to the clinic. In addition, the initiative emphasizes the need for state of the art comprehensive analyses of biopsy materials or other tissue collected during clinical trials by basic investigators to better inform future clinical trials. She highlighted the first two pilot projects of the initiative: i) a phase II study of a γ–secretase inhibitor in adults with desmoid tumors and/or aggressive fibromatosis. The NCI had not previously conducted trials specifically for desmoid tumors. With help from the Desmoid Tumor Research Foundation, enrollment on this trial was completed within less than 1 year, highlighting the benefits of close collaboration with advocacy groups. ii) A pilot study of clinical, imaging, and genomic studies in patients with NF1 and tumors considered at risk for malignant transformation. NCI support for the genomic and imaging studies allowed for optimal analysis of clinical information and tumor samples in the small number of patients studied. The initiative plans to identify and support additional collaborations of basic and clinical researches in rare tumors with unmet need for effective therapies.

As an example of the efforts of advocacy groups towards the identification of effective therapies, Annette Bakker presented the work and business model of the Children’s Tumor Foundation (CTF). The CTF works to find cures for the approximately 1 in 3,000 people living with NF, which encompasses three distinct genetic disorders: NF1, NF2, and schwannomatosis. NF is associated with multiple clinical manifestations and different challenges in the development of effective therapies. She described that the traditional model of funding research by passively providing grants to promising scientists was not resulting in an expedient development of effective treatments. The CTF thus completely revised the funding model 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. While CTF continues to provide grants to promising scientists for interesting research, the foundation was rebuilt to fill the gaps to develop a fast and efficient translation of basic discovery into clinical benefit. All of the “investment” choices are made with the main goal to set in motion a process that promises to get treatments into the hands of their patients quickly. She highlighted several of the novel approaches taken by the CTF.

For example, recognizing the need for biomarkers in NF, the CTF invested in building a state of the art biobank, which will be accessible to the entire research community. An NF patient registry was developed to identify patients suitable for future clinical trials with specific NF manifestations. The CTF has created Synodos as a funding mechanism of highly collaborative and committed research teams with broad expertise to accelerate identifying active therapies for NF.

This session concluded with a panel discussion with representatives from four pharmaceutical companies. Industry representatives emphasized that the rarity of a disease alone would not preclude from the development of an agent by a company. Several companies have embarked in clinical trials for very rare diseases. However, companies emphasized the critical need of a comprehensive understanding of the target population to consider clinical evaluation of a novel agent for rare tumors, including knowledge of: i) the incidence and natural history of the rare disease and its manifestations; ii) the number of patients potentially available for a clinical trial; and iii) meaningful trial endpoints that can be reproducibly measured. The identification of a target for therapy and a strong scientific rationale for evaluation of an agent in the target population were considered as the most critical requirement. The availability of patient registries and strong collaborations of academia, patient advocacy groups, industry, and regulatory agencies were also considered critical to the success of clinical trials in very rare diseases. Patient engagement also has a critical role in research. Funding opportunities that involve patients as partners in research at every stage was discussed.

Young Investigator Abstracts

From the abstracts submitted by young investigators, three finalists were selected for platform presentation.

Shin-ichi Inoue reported on developmental defects and therapeutic interventions in a *BRAF* Q241R/+ knock-in mouse model, reflecting the most common *BRAF* p.Q241R mutation seen in patients with CFC syndrome. These mice showed embryonic or neonatal lethality mostly owing to liver necrosis and edema, as well as craniofacial abnormalities. Prenatal treatment with a MEK inhibitor (PD0325901) rescued the embryonic lethality. Combination treatment with the MEK inhibitor and a histone 3 demethylase inhibitor (GSK-J4) further increased the rescue, suggesting that epigenetic modulations, as well as ERK pathway inhibition may be potential therapeutic strategies in the treatment of RASopathies.

Elizabeth Pierpont studied children with NF1 and NS in order to compare social function in these two common RASopathies. She analyzed 21 individuals with NF1, 21 with NS and 20 unaffected siblings, and found lower overall social competence in the individuals with a RASopathy with no difference in social competency between NS and NF1. In children with NF1, problems with social-pragmatic language ability and externalizing behaviors were the strongest predictors of social skills, whereas social motivation and attention deficit hyperactivity disorder symptoms were most associated with social competence in those with NS.

Katherine Robbins reported on a cohort of 118 patients with CS and their *HRAS* mutation spectrum. Samples from embryonal rhabdomyosarcoma (ERMS) of seven individuals were studied at the molecular, transcriptional and cytogenetic levels, and compared to sporadic nonsyndromic tumors. All seven individuals carried a paternally derived HRAS mutation affecting G12, and their ERMS was characterized by a complete loss of the wild-type maternally derived G12 allele. Complete uniparental disomy with loss of the maternal chromosome 11 was present in all but one CS ERMS. Complete loss of heterozygosity was also observed in many sporadic tumors with and without RAS mutations, indicating that loss of heterozygosity in itself is an important driver for this cancer. Katherine Robbins received the Young Investigator Award for her work.

These studies on patient samples and matched tumor samples, mouse models and potential pharmacological interventions, and the effect of RASopathies on social functioning, highlight the pleiotropic effects of RASopathies, as well as the numerous potential endpoints for therapeutic strategies aimed at improving patient lives.

Engaging Families

This symposium included methods to build mutual understanding between researchers, clinicians, industry members and families. The poster session, open to families and professionals, included posters of patients and researchers. The patients' posters elucidated phenotypes of CFC, CS, NF1, NS, and NSML and provided a window into their lives. Researchers were able to educate family members on the latest scientific findings in patient-centric terms. Along with the poster session and the Advocates' Panel, researchers shared findings from the symposium with the CS, CFC, NS, and NF1 families in break-out sessions, providing an opportunity for the families to better understand the progress of research toward understanding and increasing the quality of their lives.

RASopathy Cousins

In the RASopathy Cousins session, disorders caused by mutations in genes in pathways related to the RAS pathway were discussed. In the first presentation, Kim Keppler-Noreuil described the recent advances in the segmental overgrowth disorders, in which somatic activating mutations in the phosphatidylinositol-3-kinase/AKT/mTOR pathway, particularly *PIK3CA* mutations, have been found to be causative. These include 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). Researchers recently met at the National Institutes of Health (NIH) to create consensus guidelines for the diagnosis and treatment of individuals with these entities, designated as "*PIK3CA* -Related Overgrowth Spectrum (PROS)" [Keppler-Noreuil et al., 2015]. A nonrandomized, phase II pilot trial with the mTOR inhibitor sirolimus is being conducted at NIH, in patients aged ≥3 and ≤65 years, with a confirmed somatic *PIK3CA* mutation and progressive overgrowth. The objectives of the trial include determination of the effect size of sirolimus therapy in reducing pathological overgrowth in PROS by evaluating the use of objective therapeutic

endpoints for measuring serial changes in growth. These include volumetric MRI analysis, and dual-energy X-ray absorptiometry for body composition (fat, muscle, and skeletal tissues), along with physical exam measurements of overgrown body parts. In addition, quality of life measures in pre- and post-treatment periods are being assessed. These results will inform a future randomized control trial with a potential drugs directly targeting the PI3K pathway.

Pinar Bayrak-Toydemir discussed the *RASA1*-related disorders. The *RASA1*-related disorders are characterized by capillary malformations, which can be associated with arteriovenous malformations, arteriovenous fistulas, or Parkes Weber syndrome. She described the clinical characteristics of the patients with *RASA1* mutations and the possible complications including hemorrhage, neurological consequences and congestive heart failure. She discussed the clinical and molecular findings in 50 unrelated cases with *RASA1* mutations. Of the patients with mutations in *RASA1*, 74% had multifocal capillary malformations and 50% had arteriovenous malformations or arteriovenous fistulas. *RASA1* sequencing should be considered for patients with multifocal capillary malformations.

David Neal Franz discussed the therapeutic options for tuberous sclerosis complex, including the mTOR inhibitors sirolimus, and everolimus. The current FDA approved tuberous sclerosis-related indications for the mTOR inhibitors include subependymal giant cell astrocytoma, renal angiomyolipoma, and lymphangioleiomyomatosis. Off-label use of topical sirolimus has also been used for the treatment of facial angiofibromas. He also discussed clinical trials using mTOR inhibitors in tuberous sclerosis, focusing on long-term safety and efficacy. He reported the medications were well tolerated and side effects decrease with long-term use.

Antonio Hardan gave a presentation on the *PTEN* Project. This project focused on treatment of the neuro-cognitive deficits in patients with *PTEN* mutations. He described a new study, which will be a 6-month randomized, double-blind, multi-site study to evaluate the safety and efficacy of everolimus in 40 children with *PTEN* mutations. The study will focus on the effect of everolimus on working memory and processing speed deficits, overall global clinical functioning, autism symptoms, and adaptive abilities.

In summary, the “RASopathy cousins” session focused on recent advances in the diagnosis, evaluation and emerging therapies for the *PIK3CA*-related overgrowth spectrum, the *RASA1*-related disorders, tuberous sclerosis complex, and *PTEN*-related disorders. The mTOR inhibitors are currently in clinical trials for a wide range of complications in these disorders ranging from overgrowth of tissue and tumors to treatment of cognitive function. Future studies are needed to determine the safety and efficacy of these agents for these disorders. Additional future studies may focus on more targeted agents to treat these conditions.

CONCLUSIONS

The 4th International Symposium on Genetic Disorders of the Ras/MAPK pathway provided a forum for discussion between scientists, families, and industry about the state of the field

of RASopathies and gaps in knowledge. The symposium was essential to form a framework for future research, translational applications directed towards therapy, and best clinical practices for the RASopathies.

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