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The 8th International RASopathies Symposium: Expanding research and care practice through global collaboration and advocacy

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Abstract

Germline pathogenic variants in the RAS/mitogen-activated protein kinase (MAPK) signaling pathway are the molecular cause of RASopathies, a group of clinically overlapping genetic syndromes. RASopathies constitute a wide clinical spectrum characterized by distinct facial features, short stature, predisposition to cancer, and variable anomalies in nearly all the major body systems. With increasing global recognition of these conditions, the 8th International RASopathies Symposium spotlighted global perspectives on clinical care and research, including strategies for building international collaborations and developing diverse patient cohorts in anticipation of interventional trials. This biannual meeting, organized by RASopathies Network, was held in a hybrid virtual/in-person format. The agenda featured emerging discoveries and case findings as well as progress in preclinical and therapeutic pipelines. Stakeholders including basic scientists, clinician-scientists, practitioners, industry representatives, patients, and family advocates gathered to discuss cutting edge science, recognize current gaps in knowledge, and hear from people with RASopathies about the experience of daily living. Presentations by RASopathy self-advocates and early-stage investigators were featured throughout the program to encourage a sustainable, diverse, long-term research and advocacy partnership focused on improving health and bringing treatments to people with RASopathies.

Keywords

cardio-facio-cutaneus syndrome; Costello syndrome; neurofibromatosis; Noonan syndrome; Legius syndrome; RASopathy; signaling; therapeutics

1 Introduction

The RAS/mitogen-activated protein kinase (MAPK) signaling pathway is a highly conserved intracellular cascade that plays a pivotal role in the regulation of numerous fundamental cellular processes, including cell growth, proliferation, differentiation, and survival, both during development and adult life. This pathway is predominantly initiated and mediated by

a series of protein kinases and is profoundly dependent on the activation of RAS proteins. The RAS/MAPK signaling cascade, operating with remarkable precision, enables cells to respond to external signals and adapt to changes in their environment.

Dysregulation of the RAS/MAPK pathway through germline pathogenic variants results in RASopathies, a wide spectrum of similar, yet distinct developmental syndromes (Tidyman & Rauen, 2009). RASopathies encompass a group of rare but impactful conditions, with notable examples including Noonan syndrome (NS), neurofibromatosis type 1 (NF1), cardio-facio-cutaneous syndrome (CFC) and Costello syndrome (CS), among others. These disorders exhibit a broad range of clinical manifestations, spanning from growth abnormalities, cardiovascular disease, and cognitive impairment to an elevated susceptibility to tumors. While the diverse clinical spectrum of RASopathies highlights the critical role of precise RAS/MAPK signaling in human development, the underlying molecular commonalities are now increasingly exploited in the search of therapeutic targets.

The 8th International RASopathies Symposium, held in hybrid format in Denver on July 21–23, 2023, united 217 registered participants including RASopathy patients, their families, clinicians, basic scientists, advocates, trainees, and members from industry. The goal was to provide an open forum for sharing the latest research results, exchanging ideas, and expanding research and care practice through global collaboration and advocacy. Given the exciting recent developments in the therapeutic arena, it has become increasingly clear that only more global sharing of resources will provide enough power for meaningful therapeutic studies. In this review, we share the proceedings of the meeting and hope to attract early career investigators, principal investigators and all other stakeholders to this fascinating field.

2 Self-advocacy Panel

Modeling the importance of teamwork among medical specialists, researchers, and those affected by RASopathies, the symposium opened with a session of presentations by RASopathy parent- and self-advocates. Rather than just recounting the burden of daily living with a RASopathy, the speakers were encouraged to share how they are thriving despite their conditions. Genevieve (“Gigi”) Sevilla, an advocate with *BRAF*-associated CFC syndrome, used her speech communication device to share key parts of her life story thus far. She was joined by her mother Christine in sharing her pursuits, which included being 2018 Miss Amazing Illinois Teen Queen, a model and social media talent, a disability advocate, a nature conservationist, and a human rights activist motivated by her love of people. Next, Ryan Sheedy shared how his journey as primary caregiver for his son with Costello syndrome led him to develop an app, MyMejo.com, for parents to manage complex medical information and to safely store and share the information with whomever they choose. The session concluded with a presentation by April Anschutz who shared her self-advocacy journey from childhood to adulthood. She emphasized the need for adult RASopathy research by sharing adult-onset NS-related medical problems that she and fellow individuals with NS have experienced, as well as under-appreciated complications and experiences arising from adult doctors’ lack of knowledge. Recognizing that research

results may not come in time to help her and her peers within their lifetime, she informed the attendees that there are adults restless and ready to assist researchers in advancing progress.

3 Clinical Session: Awareness of clinical indications & recent progress

The first talk in a session discussing clinical indications, moderated by Karen Gripp, MD, was presented by Benjamin Briggs, MD, who focused on hematological indications in RASopathies. Dr. Briggs outlined the lack of a consistent definition for a bleeding disorder and the suggested laboratory evaluation in individuals with RASopathies. It remains uncertain whether abnormal laboratory study results or reported bleeding symptoms should define a bleeding disorder in RASopathies. Laboratory findings can be milder than symptoms would suggest, and more than one defect is often found, raising the possibility that perhaps the aggregate impact explains the bleeding phenotype. Thrombelastography measures all aspects of coagulation as a single assay, but it was not more effective in detecting a bleeding phenotype (Bruno et al., 2023). This lack of a definitive laboratory test to clearly identify bleeding risks in RASopathies underscores the need for a detailed history of symptoms which could evolve over time. The current clinical approach should include comprehensive investigation of the clinical bleeding history and a laboratory evaluation in consultation with a hematologist.

Elliot Stieglitz, MD, reported on his work targeting the RAS/MAPK pathway in juvenile myelomonocytic leukemia (JMML) with trametinib. The aggressive myeloproliferative disorder of childhood JMML is characterized by abnormal signaling through the RAS pathway, driven by initiating pathogenic variants in different genes. Trametinib functions by inhibiting MEK1/2 downstream of RAS signaling. A phase 2 clinical trial with trametinib for relapsed or refractory JMML showed an objective response in 4/9 patients. While trametinib controls the disease well in responders, it does not eradicate it and symptoms of JMML can occur upon stopping the medication. Trametinib will be used in an upcoming trial in combination with a second drug, azacitidine, which is FDA approved for newly diagnosed JMML. These novel strategies hold promise for avoiding the need for hemopoietic stem cell transplantation.

A presentation on cell cycle defects that underlie NS-associated cardiomyopathy was presented by Cordula Wolf, MD. While NS-associated hypertrophic cardiomyopathy shares some findings with hypertrophic cardiomyopathy unrelated to NS and caused by pathogenic variants in sarcomeric proteins, there are notable clinical differences. In patients with NS, cardiac hypertrophy and dysfunction do not progress in a subset of patients, in contrast to the expected universal progression in individuals without NS. This difference in clinical course suggests differences in the disease mechanism. Data from whole-transcriptome analysis of NS myocardium and from induced pluripotent stem cells with a pathogenic *PTPN11* variant suggested cell cycle defects leading to increased cardiomyocyte proliferation as a potential driver (Meier et al., 2022).

Dagmar Tiemens, a PhD candidate and parent advocate, studied the needs of patients with NS and their family members and found that coagulation problems, cardiac issues and feeding problems are of highest concern (Tiemens et al., 2023). She discussed work toward

a systematic approach to feeding difficulties, GERD and vomiting in NS spectrum disorders. While coagulation issues and cardiac problems are actively investigated as reflected in the prior presentations in this session, feeding difficulties are less well studied. She advocated that the mechanisms underlying the feeding difficulties, gastroesophageal reflux, and frequent emesis in young individuals with RASopathies should be studied in order to provide therapeutic suggestions benefitting patients with NS and related RASopathies and guide RASopathy-specific clinical management.

MEK inhibitor (MEKi) treatment effects and quality of life in diverse patients with NF1 tumors were the focus of a presentation by Christopher Moertel, MD. He reviewed the experience of using MEKi therapies in individuals with NF1-associated neoplasia. Selumetinib, trametinib and mirdametinib have shown benefit in this patient population. However, side effects on the skin, the gastrointestinal tract, the heart and the eyes are recognized. Experience gained over the last years through using these medications in the NF1 population can inform use and potential side effects in RASopathy patients. In this context it is important to consider dosage, as the most serious side effects occurred with higher doses, whereas the suggested dosing in individuals with RASopathy-related cardiomyopathy may be lower than for malignancies.

Fieke Draaisma reported on hypertrophic neuropathy as a possible cause of pain in children with NS and related disorders. A review of pediatric patients with NS or related RASopathies and leg pain identified four individuals, three with NS and one with NS with multiple lentigines (NSML). High-resolution nerve ultrasound showed multifocal hypertrophic neuropathy in all four. These results are consistent with literature reports on hypertrophic neuropathy in individuals with RASopathies. While MRI is typically used to assess the extent of nerve enlargement, high resolution ultrasound may be a practical alternative. Further research may increase our understanding of this relatively rare complication and its most effective treatment.

4 Neurology Session: Neurodevelopmental focus - from brain to behavior

Elizabeth (Rene) Pierpont, PhD moderated a session focused on progress in neurodevelopmental and neurological studies of RASopathies. This session showcased work spanning the spectrum of clinical and translational brain science, comprising presentations focused on preclinical models, neuroimaging, correlations between genotype and phenotype, and promising interventions to support social well-being and mental health. In the first presentation, Tamar Green, MD, reviewed a study exploring neuroimaging patterns associated with NS and discussed the use of topological data analysis to investigate the correlation patterns of surface area, cortical thickness and brain volume between children with NS and controls. Dr. Green described results showing distinct neuroimaging patterns associated with *PTPN11* and *SOS1* gene variants and elucidated the predictive value of these patterns for neurocognitive abilities. Dr. Green's talk continued with the presentation of data related to neuropsychiatric evaluation of children with NS. Comparisons of children with NS to typically developing children found increased symptoms of attention deficit hyperactivity disorder, autism spectrum disorder and oppositional defiant disorder (Naylor et al., 2023). Conclusions noted the need for investigation into treatment modalities and next steps for

studies connecting genetic variants with brain structure analysis and neurocognitive and neuropsychiatric outcomes.

A collaborative presentation by Ellen Wingbermühle, PhD, and Renée Roelofs, PhD, discussed work on genotype-phenotype correlations in cognitive processes among individuals with RAS/MAPK variants along the NS spectrum and initial results from a telehealth trial of a social cognitive intervention for adults with NS. Dr. Wingbermühle described findings regarding behavioral features, emotional characteristics, and cognitive strengths and weaknesses of individuals with NS across the lifespan. Although overall intellectual abilities and some behavioral features showed associations with particular RASopathy gene variants, a lack of specific cognitive impairments was noted in most study participants (Wingbermuhle et al., 2022). Results of this work underscore the importance of accounting for general cognitive abilities when interpreting neuropsychological test performance across domains. Dr. Roelofs shared about treatment options for social and behavioral concerns among individuals with NS and related conditions. She discussed results from a pilot trial of online social cognitive training for Dutch adult patients. Considerations for the feasibility of developing and implementing therapeutic interventions tailored to individuals with NS were discussed.

Bonnie Klein-Tasman, PhD, spoke about a novel approach to addressing elevated rates of social difficulties among teens with NF1. She described Danielle Glad, PhD's dissertation study that used a telehealth version of the Program for Education and Enrichment of Relational Skills (PEERS®), an evidence-based intervention designed to improve social functioning in teens with neurodevelopmental disabilities (Laugeson et al., 2012). Adolescents with NF1 who had social skills difficulties and their caregivers participated in virtual 14-week PEERS® intervention sessions, with administration of adolescent and caregiver-report study measures assessing social outcomes pre- and post-treatment and after a follow-up period. Dr. Klein-Tasman shared results and insights from this pilot open trial indicating improvement across multiple aspects of caregiver-reported social function and friendship skills following the intervention. As one of the first investigations offering a treatment approach to support social skills among adolescents with NF1, this study offers a bridge toward the goal of offering evidence-based, tailored mental health care for individuals with RASopathies.

Sarah Borrie, PhD, presented research on social behavioral phenotypes in a RASopathy mouse model. This talk focused on the assessment of social communication and learning behaviors in a Spred1−/− mouse model of Legius syndrome. Dr. Borrie shared research showing that dysregulation of RAS/MAPK signaling induced by loss of Spred1 resulted in atypical nesting behaviors, social interactions, and associative learning in these mice (Borrie, Horner, et al., 2021; Borrie, Plasschaert, et al., 2021). Differences in communication via ultrasonic vocalizations were seen both in early postnatal stages and in adult mice. Among adult *Spred1*−/− mice treated with a MEKi, deficits in social and nesting behaviors (but not learning impairments) were reduced. These results underscore the potential for animal models exhibiting social and communication deficits to inform understanding of the effects of RAS/MAPK dysregulation on brain and behavior, and to test relevant therapeutic approaches.

Completing the session, Sattar Khoshkhoo, MD presented an ESI-selected abstract discussing results from a multi-institutional study utilizing high-coverage whole-exome sequencing (WES) to detect somatic variants activating RAS/MAPK signaling in hippocampal brain tissue of surgically-treated patients with intractable focal epilepsy. Pathogenic somatic RAS/MAPK variants were detected by WES in a subset of patients with mesial temporal lobe epilepsy (MTLE) but were not identified in neurotypical controls (Khoshkhoo et al., 2023). Follow-up gene-panel sequencing on remaining cases also identified additional pathogenic somatic RAS/MAPK variants in a further subset of patients with MTLE. Dr. Khoshkhoo discussed the results of immunohistochemical studies of hippocampal tissue harboring these pathogenic somatic variants which confirmed RAS/MAPK overactivation. He concluded that MTLE with sporadic, drug-resistant disease may result from somatic variants activating RAS/MAPK signaling, and reflected on potential implications of this work for the understanding and treatment of focal epilepsy in sporadic MTLE and in patients with germline RASopathies.

5 Poster Session: Investigators and families

The symposium poster session featured a combination of RASopathy advocate posters and scientific abstracts from research teams. Advocate posters showcased the unique stories of 10 individuals with RASopathies, presented by caregivers and self-advocates, highlighting each advocates' experiences with photos and narratives. Participants shared their most challenging medical, neurocognitive and rehabilitation concerns affecting quality of life, as well as medical issues and treatment avenues that they would like to see addressed in research and clinical care. Research teams presented scientific abstracts, including 20 submissions from early-stage investigators, covering a wide array of basic and clinical research across all major RASopathies.

6 Physiology Session: Causes and impacts of disordered metabolism and energy homeostasis

Gregor Andelfinger, MD, moderated a session that focused on recent advances in often-overlooked metabolic aspects of RASopathies. Opening the session was Maria Kontaridis, PhD, who presented novel work using inducible pluripotent stem cells (iPSCs) to delineate molecular mechanisms that cause gastrointestinal difficulties in RASopathy patients. To this end, her group used iPSCs to generate intestinal organoids replicating the intestinal pathophysiology of *PTPN11* mutations for both NS and NSML alleles (Y63C and Q510E, respectively). Comparing these two lines with a wildtype line, they found that differences started as early as endoderm differentiation and persisted throughout organoid development. Main differences included NS organoids having smaller (but normal number of) goblet cells with decreased mucin production, whereas NSML mutant organoids had the opposite phenotype of goblet cell hyperplasia, and increased mucin production. Similar findings were obtained in NS and NSML mouse models. These findings highlight a potential role of perturbation of the intestinal barrier in RASopathies, in an allele-dependent fashion.

The next speaker, Rodrigue Rossignol, PhD, focused on the role of mitochondrial homeostasis in CS mouse models. Using an unbiased mass spectrometry approach across

several wildtype tissues/cells versus those with an *HRAS* p.G12S mutation, they found mitochondrial components to be strongly dysregulated. This was corroborated by direct measurements of oxidative phosphorylation, and attributed to LKB1-mediated AMPK inhibition (Dard et al., 2022). Dr. Rossignol concluded by showing a rescue of varying efficiency according to the model, and measured parameters using a bifunctional molecule targeting mitochondrial biogenesis/fueling as well as mitochondrial proteostasis.

In a similar approach, Ion Cirstea, PhD, presented results on adipose tissue atrophy in CS, in an attempt to address questions surrounding nutritional problems and weight gain in CS. In the *Hras G12V* mouse model, they reported premature aging-like features such as reduced lifespan, bone loss (Nandi et al., 2022), and lipofuscin accumulation in organs (e.g., liver). The reduced weight gain throughout the life-cycle correlated with structural and metabolic anomalies of white adipose tissue. Decreased adipogenesis could be directly linked to downregulation of known regulators of this process. Adipose tissue reduction and browning was enhanced by stressors. A possible clinical link was suggested for the antihypertensive medication of the angiotensin and beta-receptor pathways, with their blockers having a potential to enhance lipolytic effects of white adipose tissue in RASopathy patients. The conclusions therefore called for further studies of CS as a mildly cachectic wasting syndrome.

In a joint presentation from Chiara Leoni, MD, and Elisabetta Flex, PhD, the audience heard about bone homeostasis and metabolic profiling in CS. From a clinical standpoint, the presented compilation of data points towards significantly decreased levels of 25-OH vitamin D and bone mineral density in CS versus normal controls. While oral vitamin D supplementation was able to correct blood levels, there was no concomitant increase in bone density, suggesting additional, unknown mechanism for dysregulation of this parameter in CS patients. In parallel, mild hypoglycemia and hypercholesterolemia without endocrine anomalies or impairment of fatty acid observation could be observed. Similarly, CS patient fibroblasts did not exhibit any deficits of mitochondrial oxidative phosphorylation, but an acceleration of glycolysis and intracellular lipid droplets, which was independent of PI3K/AKT signalling. This finding was attributed to HRAS-driven generation of reactive oxygen species, and ensuing membrane translocation of the glucose transporter GLUT4.

As a selected early career investigator, Nadia Merchant, MD, presented on the use of vosoritide to improve growth in children with NS. Vosoritide is a synthetic analog of C-type natriuretic peptide, which binds to natriuretic peptide receptor-B on chondrocytes leading to increased chondrocyte proliferation and differentiation via its inhibition of the ERK 1/2-MAPK pathway. It has been recently approved by the FDA in 2021 for treatment of achondroplasia.

Taken together, this session shed light on several clinically relevant aspects of RASopathies, with translational studies offering first avenues towards potential novel treatment approaches. Another important point emanating from the discussions of this session was the role of cell culture conditions to assess certain cellular phenotypes. Dr. Rossignol pointed out that his studies were carried out in medium replicating physiological glucose levels, whereas most studies in the field use DMEM, which contains 1.0 – 4.5 g/l of

glucose, depending on formulation (up to 7-fold higher). There was consensus that some of the *in vitro* findings on the role of metabolism in RASopathies may depend heavily on physiological versus standard culture conditions.

7 Molecular Session: Advances in drug discovery

Pau Castel, PhD, moderated a session focused on recent advances in preclinical models of RASopathies that are used to identify and assess potential novel and repurposed therapies for these disorders. The session began with the work of Daochun Sun, PhD, who presented his research using bioinformatic integration of data obtained from different open-source datasets (namely transcriptomics and targeted drug screens deposited at NF Data Portal) using a customized algorithm developed as part of the 2021 Hack4Rare data mining competition organized by the Children's Tumor Foundation. Dr. Sun presented a series of conserved gene regulation networks and that were identified as potential therapeutic vulnerabilities for NF1 and explained how these bioinformatic approaches could be expanded to other RASopathies.

Dr. Castel discussed his recent work related to *RIT1*-mutant NS, including the development of preclinical models and the mechanisms underlying regulation of RIT1 and downstream signaling. Using biochemical approaches and mouse models, Dr. Castel provided mechanistic insights into the activation of the RAS/MAPK pathway by *RIT1* pathogenic variants and discussed the rationale for the use of trametinib in order to treat cardiac phenotypes in this patient population.

Bruce Gelb, MD, described a preclinical platform based on genetically engineered fruit flies (*Drosophila melanogaster*) carrying the most common genetic variants associated with RASopathies. Using this experimental model, Dr. Gelb presented the candidate molecules identified from drug screenings carried out to rescue the phenotypes in the different RASopathy Drosophila avatars. In addition, validation and characterization of the mechanism of action of these inhibitors was undertaken in human induced pluripotent stem cell (iPSC)-derived cardiomyocytes and mouse models of RASopathies.

Clifford Liu, an MD/PhD candidate, described progress in developing iPSC differentiation strategies to recapitulate the process of human valvulogenesis *in vitro*. Using iPSC-derived endocardial cells, Liu showed that these cells can be further differentiated into valvular interstitial cells, through an endothelial-to-mesenchymal transition (EndMT). Through CRISPR/Cas9 editing, Liu engineered iPSC with several pathogenic variants commonly found in individuals with NS and applied the differentiation protocol described above. While these cells exhibited increased differentiation into endocardial cells, they displayed defective EndMT, and the molecular defects were analyzed using single cell RNA sequencing. Overall, this session provided unique mechanistic insights and preclinical models of RASopathies that will accelerate the path to the development of novel therapies.

8 Discussion Panel: Current treatment landscape and new therapeutic developments

Moderated by Anton Bennett, PhD, the panel consisted of Forest White, PhD, Gregor Andelfinger, MD, FRCPC and Mark Kieran, MD, PhD. The session kicked-off with a talk from Dr. White who discussed a variety of different proteomic approaches that could be applied to decipher the signaling changes that occur in the RASopathies. One example utilized an ATP analog-sensitive version of ERK that allowed for the identification of substrates phosphorylated by ERK in cells and tissues driven by enhanced RAS signaling. By combining this strategy with immobilized metal affinity chromatography, it was shown that one can identify a number of downstream ERK substrates. It was also shown that ERK2 substrates are differentially phosphorylated depending upon the nature of the RAS mutation. From a therapeutic perspective, the phosphoproteomic approaches also enable a non-biased assessment of the actions of drugs on the RAS/MAPK pathway. Dr. White highlighted how inhibition of BRAF using vemurafenib increases signaling through the Src family of kinases that could promote cell survival. The take home message here was that inhibiting the RAS/MAPK pathway may exhibit initial benefits but caution should be applied as “escape” signaling mechanisms can be acquired. To this end, one could consider multi-modal therapies that target the primary pathway (e.g., RAS/MAPK) in addition to other ancillary “escape” pathways (e.g., Src family of kinases). Dr. White presented data showing that *in vitro* and *in vivo* inhibition of both BRAF and Src family of kinases is more effective in preventing the growth of cancer cells than either inhibition of BRAF or Src family kinases alone. Finally, Dr. White presented data demonstrating proof-of-principle that phosphoproteomic approaches could be used to directly assess the extent of signaling pathway activation and suggested in follow-up questions that such approaches could be directly applied for use in the assessment of pathway activation in RASopathy patients. In conclusion, Dr. White’s presentation illustrated how state-of-the-art phosphoproteomic approaches could be leveraged to assess the complexities of RASopathy pathway activation to assess drug action, identify novel targets and possibly understand genotype-phenotype relationships.

Dr. Andelfinger presented a short talk focused on new therapeutic developments, specifically an update on compassionate use of MEKi and mTOR inhibition for RASopathy-associated cardiac disease. Dr. Andelfinger began by reiterating the importance of early intervention in patients diagnosed with RASopathy-associated hypertrophic cardiomyopathy. He focused his discussion on a compassionate use trial with low dose re-purposed cancer drugs, trametinib and everolimus/sirolimus, for the treatment of progressive RASopathy-associated hypertrophic cardiomyopathy. The study was conducted throughout 22 institutions across Europe and North-America and included 37 patients. Of these patients, 27 had NS, six NSML and four with either CS or CFC syndrome. The majority of the patients received trametinib, and a smaller cohort were treated with the mTOR inhibitor or a combination of both drugs. He showed that as compared with historical controls, trametinib and/or mTOR inhibitor treatment strongly reduced the outcomes on mortality, the need for surgery or transplantation. As a composite endpoint (mortality/surgery/transplantation) trametinib and/or mTOR inhibitor treatment was highly significant. Clinical parameters such as left

ventricular mass were greatly improved in these patients. In addition, he showed that children below 6 months benefited the most from these treatments. Dr. Andelfinger noted that cutaneous side effects are common in patients treated with trametinib and hair thinning can occur. These encouraging results raised important questions such as: What is the optimal dose and time window for treatment? Is treatment effect sustainable long-term? Which RASopathies are amenable to MEKi/mTORi? What are the side-effects of long-term MEKi/mTORi in infants and children? Which manifestations of RASopathies are amenable to MEKi/mTORi?

The discussion panel fielded questions that related to the clinical management of RASopathy patients treated with trametinib and/or mTOR inhibitors as it relates to the length of treatment and other developmental issues given that these patients are very young. The panel responded that it was too early to know and that further trials need to be done. However, there is evidence that in some RASopathies, one can wean off the drug without re-emergence of clinical symptoms suggesting that this might not necessarily be a life-long therapy. As a follow-up from the response that more trials are needed to obtain this information, a question was posed regarding how drug companies can be incentivized to support clinical trials for RASopathy patients. Dr. Kieran responded that this is a very difficult problem to attract drug companies to develop drugs for the RASopathies due to the high cost of drug development. A solution that he suggested was to re-purpose drugs which can dramatically reduce the cost. It was noted that the approach of drugs aiming to kill cancer cells driven by RASopathy mutations differs from the strategies used to “fix” heart cells in cardiomyopathies. Dr. White responded that this problem was largely going to be solved by assessing the dose required to tune down the signal back to normal levels. A question about endpoint was raised that related to younger and older children and the use of historical data. It was commented that there is no option for “placebo” trials in the younger children as the mortality risk is too high, therefore trial design must incorporate historical data. For older children with a lower mortality risk, a placebo trial arm may be considered.

Overall, the sentiment emerging from the panel discussion was the importance of considering re-purposed drugs targeting the most severe clinical presentation of the RASopathies, namely hypertrophic cardiomyopathy. The goal will be to gain success in this area. Indeed, encouraging data are emerging that the re-purposing of cancer drugs to treat RASopathy-related hypertrophic cardiomyopathy can be successful. In this way, the cost of drug development can be reduced and drug companies can be enticed into the RASopathy space. Finally, there is a need for more clinical trials to get a better understanding of the long-term effects of these potential treatments.

9 Genetic Session: Genotype-phenotype correlations

A session focused on genotype-phenotype associations of the RASopathies was moderated by Katherine (Kate) Rauen, MD, PhD. Vanessa Fear, PhD, from the University of Western Australia addressed the prospects of utilizing expression signatures of RASopathies to better inform diagnosis and treatment. Focusing on the *BRAF* gene, the team used CRISPR gene editing and inducible pluripotent stem cell disease modeling to derive neural and cardiac tissue from genetically engineered stem cells. *BRAF* was chosen to explore the molecular

signatures of a *BRAF*-related CFC gene variant, a *BRAF* variant which has been reported in the literature with a more "Noonan-like" phenotype, and for comparison, a *BRAF* variant of uncertain significance. Work is ongoing to identify changes in neural differentiation in these cells. Cardiac cell differentiation to form cardiomyocytes and determine functional changes in cellular and molecular pathways is underway.

Megan Frone, MS, CGC, from the National Institutes of Health provided updates on the NCI RASopathies Natural History Study and preliminary Genome-First results. The NCI RASopathy study aims to collect a large prospective longitudinal cohort of pediatric and adult individuals with clinical or molecular RASopathy diagnoses to study the prevalence of cancer; to assess the spectrum of benign and malignant clinical findings; to describe novel phenotypes associated with germline RAS/MAPK pathway variation; and to create a biospecimen repository for use in subsequent translational research projects. To assess cancer incidence associated with germline pathogenic or likely pathogenic RASopathy variants in an unascertained adult population, a genotype-first approach was taken to analyze exome sequencing and phenotypic data from electronic health records from Mount Sinai's BioMe, the UK Biobank and the Geisinger MyCode Community Health Initiative. RASopathy genes associated with NS were the most prevalent in this combined cohort followed by CFC and Legius syndrome associated genes. With the exception of carriers of the *SPRED1* variants, cancer incidence for NS and CFC variant carriers were similar to non-carriers in this cohort.

Karolin Kleemann, a PhD candidate and ESI Awardee, discussed myocardial tissue engineering to study hypertrophic cardiomyopathy in a $RIT1^{F82L/+}$-associated NS *in vitro* disease model. The gain-of-function mutation $RIT1^{F82L/+}$ was investigated by employing induced pluripotent stem cells (iPSCs), derived from the patient to generate 3D bioartificial cardiac tissues. CRISPR/Cas9 gene-corrected iPSCs served as isogenic controls. In addition to characterizing the distinct (electro-)physiological phenotype, they investigated changes on morphological, histological, ultrastructural and molecular levels in the engineered myocardium. The MEK inhibitor trametinib was tested in a reverse translational approach (Andelfinger et al., 2019). The results recapitulated the clinical observation of beneficial effects of trametinib which rescued the phenotype on HCM in $RIT1^{F82L/+}$-associated NS and supported the therapeutic potential of targeting the hyperactivated RAS/MAPK pathway.

10 Keynote Presentation: Building and maintaining multinational rare disease networks/collaborations

Martin Zenker, MD, director of the Institute of Human Genetics at the University Hospital of Magdeburg and investigator with over 20 years of experience in RASopathies, presented a keynote address sharing insights into assembling and directing large collaborations and partner networks. Dr. Zenker first reviewed achievements of past multilateral RASopathies research consortia, which enabled substantial progress in gene discovery and the mechanistic understanding of RASopathies as pathway disorders with common pathophysiology. He noted that despite these achievements, the networks involved in these discoveries often did not have the structures and composition needed to efficiently translate these discoveries

into clinical applications. Dr. Zenker explained that translational research efforts may be limited by a lack of several key resources. These resources include comprehensive patient registries, genotype-stratified natural history data, infrastructure for executing clinical trials, pharmaceutical partners, and involvement of patient advocacy groups. In terms of developing more effective collaboration approaches, Dr. Zenker emphasized the need to improve the structure of RASopathy research networks to include greater coordination across specialized centers, enabling larger cohorts and the collection of standardized data elements. He reviewed critical next steps for collaborative networks, highlighting the importance of valid, long-term clinical data sets from large patient cohorts stratified by genotype. He discussed needs in the areas of drug development and described current efforts toward harmonization of guidelines for patient management. Dr. Zenker ended with perspectives on how patient and family advocates can help collaborative efforts to advance research in RASopathies. He suggested that these groups play a key role in scientific and clinical developments by increasing communication channels, transferring patient data and resources, sharing clinical observations and medical needs, and supporting research through fundraising and advocacy.

11 Discussion Panel: Global perspectives and collaborations

The final panel discussion moderated by Pilar Magoulas, MS, CGC was conducted to stimulate dialogue regarding ways to advance global perspectives and collaborations. As discussed in the keynote address, given the rapid advancement of targeted therapeutic and treatment strategies for RASopathies on the horizon, there is a pressing need for effective global collaboration amongst families, advocacy organizations, researchers, and clinicians. The panelists included Rene Pierpont, PhD, Carlos Prada, MD, Emma Burkitt-Wright, MBChB, PhD and Paul Kruszka, MD. The session was loosely divided into four topics with questions for the panelists within each section. The topics included: syndrome commonalities across ethnicities; international collaboration in practice, standards, guidelines, and updates for RASopathies diagnosis and treatment; establishing collaboration among advocacy networks; and diversity and access in rare disease clinical research.

The panelists began by highlighting key similarities and differences within the same syndrome across various nationalities and ethnicities. Dr. Kruszka had previously reported several common features in individuals with NS across different ethnicities, including facial characteristics (e.g., hypertelorism, low-set ears) and short stature (Kruszka et al., 2017). Differences included the presence of ptosis and webbed neck. Given the shared facial gestalt in individuals with NS, the utility of facial recognition software as a tool in patients with suspected RASopathies was discussed. Panelists noted that clinical diagnosis of a RASopathy with the assistance of facial recognition software may be particularly useful in limited resource settings with decreased access to genetic testing. In addition, facial recognition software could potentially have a role in validating or refuting genomic variants identified in RASopathy genes to aid in interpretation.

Given extensive experience collaborating with clinicians and researchers nationally and internationally, the panelists shared their perspectives on barriers to increased international

collaboration within the RASopathies. These barriers include logistical and regulatory challenges, such as sharing biological specimens across countries; not having common data elements that may make sharing clinical information difficult; limited access to medical records from other countries; and language barriers with interpretation of records. It was noted that clinicians and researchers can improve and foster increased collaboration in RASopathy research by selecting patient-reported outcomes that have been or can be translated into other languages. In addition, the role of syndrome registries or databases can help bridge the gap in understanding certain clinical aspects and the natural history of the RASopathies. Establishing an international registry for individuals with RASopathies presents several challenges. Registries often rely on patient-reported data, which may not be validated by medical records and/or be of questionable quality. Other challenges include the lack of standardized data items and HPO (Human Phenotype Ontology) terms; the (in)ability to provide incentives for physician/clinician-reported data; the need for strategies for collecting follow-up data; and the infrastructure needed to store and share large amounts of data in a secure and sustainable manner.

Session panelists expressed various ways that they had successfully navigated barriers to international collaboration, such as with their ability to extrapolate their experiences from practicing clinical genetics in different countries, which, in turn, promotes new partnerships while cultivating existing ones. In addition, the panelists favorably endorsed sharing their work at international meetings, consortia, and consensus groups. The ability to connect via online platforms can reduce the financial, logistical, and time constraints that occur when attempting to meet as a large group in person, while still providing the beneficial effects of global collaboration and participation.

The third major topic of the panel session centered around the roles that advocacy organizations can play in increasing and promoting collaboration for RASopathy-related research. Panelists spoke of the importance of the individual syndrome organizations, as well as the larger rare disease networks, facilitating connections between families, clinicians, and researchers. Some organizations may have staff or personnel whose responsibilities include recruitment, promotion, and dissemination of research studies to their membership. However, this can present challenges for smaller organizations who may not have dedicated staff or effort allocated to these endeavors. Leadership from different RASopathy advocacy organizations participated in the discussion and validated that while this engagement is mutually beneficial for all parties, it requires dedication and motivation from all sides for it to be implemented successfully.

Advocacy organizations are taking proactive steps to include diversity initiatives in their strategic planning. Increasing diversity and access in rare disease clinical research is not unique to the RASopathies but was highlighted as a priority among the panelists and conference participants. They suggested various strategies to assist in this effort including offering study participation in multiple languages (building this into the budget), advocating for interpreters and other resources, and finding different methods to facilitate connections (e.g., recruitment through clinics, advocacy groups, and conferences). In addition, building trust with underrepresented groups and historically marginalized communities is essential to encourage and nurture collaborative relationships with families and researchers. Specific

methods to obtain and share diverse perspectives might include hosting webinars, listening sessions, and focus groups. It was also noted that these methods could increase stakeholder motivation to develop, support, and participate in research initiatives.

National or international patient registries, consensus guidelines for management and treatment, and increasing access to research for rare disorders in diverse populations requires careful planning, organization, and effort. Panelists highlighted the need for grants and other funding sources to support these ambitious endeavors. In looking towards the future and as we move towards increased understanding of the molecular mechanisms, pathophysiology, and natural history of RASopathies, global initiatives that foster increased collaboration will be a fundamental guiding force.

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