The Clinical Roya Kabuki Program – Year 4 Report

We are pleased to report that the partnership between Olaf Bodamer, MD, PhD, and Emanuela Gussoni, PhD, continues to thrive, and that their work continues to focus on understanding the underlying mechanisms of Kabuki syndrome, discovering treatments and building an international databank and registry. Dr. Gussoni is a world-renowned scientist in skeletal muscle biology with a passion for translational research. Together with Dr. Bodamer, she is determined to answer the many questions about this illness.

Our teams aim to define the individual presentation of Kabuki—the clinical phenotype and its natural history—and pave the way for clinical trials that test the efficacy of novel drugs and treatments. We know there is enormous variation in symptoms, which range from mild growth delays and facial characteristics to devastating impacts on the kidneys, heart, skeleton and brain. We also know that the key concepts used to identify the mechanisms for Kabuki syndrome will likely be applicable to a broader range of neurodevelopmental disorders. Our hope is that the care strategies we identify can be scaled to other illnesses.

The Clinical Roya Kabuki Program

Our multidisciplinary team of "Kabuki Champions" remains steadfast in its commitment to treating, managing and innovating care for children and adults with Kabuki syndrome. The following experts and departments are an integral part of the work we do every day in the Roya Kabuki Clinic at Boston Children's Hospital:

• Laura Mansfield, MD, Cardiology

• Matthew Harper, DDS, Dentistry

• Jessica Kremen, MD, Endocrinology

• Margaret Kenna, MD, MPH, FACS, FAAP, Otolaryngology

• Samuel Nurko, MD, MPH, Gastroenterology/Motility

• Christina Yee, MD, PhD, Immunology

• Benjamin Goodlett, PhD, Neuropsychology

• Anne Fulton, MD, Ophthalmology

• Travis Matheney, MD, MLA, Orthopedic Surgery,

• Amy Pasternak, PT, DPT, PCS, Physical Therapy and Occupational Therapy Services

Dr. Bodamer and genetic counselor Asma Rashid continue to conduct phone interviews and pre-visit needs assessments with all new families seeking care, which is an important initial step that helps guide and define the patient experience. This work also helps us coordinate care among our

many providers and allows an opportunity to review families' needs and expectations. For the last two years, The Roya Kabuki Clinic has worked closely with Boston

For the last two years, The Roya Kabuki Clinic has worked closely with Boston Children's Integrated Care Program, led by Richard Antonelli, MD, MS, to adopt collaborative toolsets that allow us to communicate action items defined at a patient encounter to other care teams along with a timeline of next steps. This work, along with conducting both provider and patient surveys, has generated invaluable insight and allowed our team to raise the standard of care for our patients. While we all had hoped to return to a more in-person clinical model last year, the majority of our outpatient staff continue to work remotely. The Roya Kabuki Clinic adapted to a virtual care model through the provision of telehealth services, which has had the unforeseen benefit of increasing our out-of-state and international referrals.

Patient recruitment

The newest version of our study protocol was approved by Boston Children's Institutional Review Board (IRB) on December 27, 2021. The study protocol and subsequent amendments have also been made available using a HIPAA-compliant electronic consent platform to broaden the reach and ease of enrollment, and increase future recruitment

Total index patients consented to research

- 143 Total participants consented to research
- 388 Families in genetic clinic
- 73 Families in neuropsychology clinic 47

• 143 index patients have been consented to the research protocol since September 2017

• 388 individuals, including parents, siblings, and other family members have been consented to the research protocol since September 2017

• 199 individuals have provided blood samples, with 76 of those being index patients

• 306 individuals have provided urine samples, with 111 index patients

• 18 patients with Kabuki syndrome have provided tissue samples, and we have collected muscle tissue biopsies from four patients

• 47 index patients have had neuropsychological evaluations through this research study

• 11 index patients have had neuropsychological evaluations and consented to share data with Takeda

Clinical research

Natural History Study

To date, 85 patients with genetically confirmed Kabuki syndrome are enrolled in the Natural History Study. Cell lines have been established for 34 of these patients in the lab. Another 35 patients with a Kabuki syndrome phenotype are enrolled who either lack genetic testing or carry a variant of uncertain significance in either Kabuki gene. This Natural History Study for Kabuki syndrome is unique as it seeks to understand the natural evolution of phenotypes affecting different organ systems (brain, muscle, immune system and others) across the entire age spectrum in order to identify clinical trial endpoints and to assure clinical trial readiness. In the coming months, we plan to submit the study to the FDA for multi-site funding consideration.

Phenotype and biorepositories

The Kabuki syndrome phenotype and biorepositories based on a REDCap database continues to expand. To date, clinical information on 143 patients has been uploaded. We have collected 76 blood samples and 111 urine samples from index patients. These biorepositories are a central resource for collaborative clinical research among different institutions

Patient registry

We continue to work diligently with the patient advocacy group All Things Kabuki to initiate a patient registry (e.g., National Organization for Rare Disorders [NORD], IAMRARE Registry Program) with Dr. Bodamer serving as principal investigator. The addition of a patient registry to our current data collection efforts will increase the breadth and depth of what is currently known within the Kabuki syndrome community. A registry of this sort will provide our internal and collaborating researchers with keen insights on a greater number of patients, allow them to learn more about patient-driven treatment needs and goals, identify clinical trial ends and advocate for future grant funding to increase research opportunities. The creation of the registry was delayed due to the pandemic, but it is now planned to launch soon. The patient registry committee includes Drs. Bodamer and Gussoni, as well as Siddharth Banka, MBBS, MRCPCH, PhD, University of Manchester, UK; Margaret Adam, MD, Seattle Children's Hospital; and Hans Bjornsson, MD, PhD, University of Iceland

Laboratory research

Biomarker for Kabuki syndrome

The identification of a biomarker (metabolites, peptides/proteins, methylation and gene expression pattern) specific to Kabuki syndrome is critical for clinical trial readiness and would be used to evaluate therapeutic efficacy and establish clinical trial endpoints. In our search for such a biomarker, we have completed the study of RNA sequencing, chromatin immunoprecipitation sequencing (ChIP-Seq), immune-signature profiling and metabolomics in a large number of patients with genetically proven Kabuki syndrome. Bioinformatics analyses are currently being performed by Lucy Jungsook, PhD, who joined the Roya Kabuki Program as biostatistician in January 2021. Lucy has identified robust expression profiles in Kabuki syndrome as outcome measures for clinical trial readiness. A manuscript on these findings is now being prepared for submission

Muscle phenotype in Kabuki syndrome

To better understand how hypotonia develops in patients with Kabuki syndrome and what tissues are affected (muscle or nervous tissue—or both), we have obtained and studied skeletal muscle samples of five KMT2D patients. Studies on tissue sections have shown presence of areas of normal muscle fibers interspersed with abnormal areas containing accumulated fibrotic and fat tissues. Analyses of muscle stem cell function were also performed on the samples. Muscle stem cell differentiation appears to occur normally, with muscle cells differentiating into myofibers in vitro. One limitation of these studies is the low number of samples and the intrinsic variability in age, gender and muscle sample obtained. Thus, additional human samples and matched controls will need to be analyzed in future studies.

To overcome the variability seen with human samples, we have analyzed muscle tissues from mice with a heterozygote kmt2d mutation, a model that genetically resembles the human condition. Control mice of the same age and sex were analyzed in parallel and both male and female cohorts were studied. Histological analyses on muscle tissue showed that both young male and female mice with the mutant gene have significantly smaller myofibers compared to control mice. In vitro muscle stem cell differentiation studies were also completed and showed that kmt2d muscle cells differentiate less efficiently into mature myofibers than control cells. Further, kmt2d mouse muscles regenerate less efficiently in vivo than control muscle. Interestingly, when muscle stem cells from control and kmt2d mouse muscle were transplanted in a non-Kabuki environment, muscle differentiation was similar for both strains and muscle stem from cells engrafted with similar efficiency

Haplotyping of Kabuki alleles

The majority of KMT2D and KDM6A mutations arise spontaneously, meaning they are not inherited from either parent. It may be important to understand whether these spontaneous mutations occur on the maternal or paternal gene copy, however. We have developed and validated a novel experimental protocol that allows us to sequence long stretches of genetic information at high quality and to identify on which of the parentally inherited alleles the mutation appears.

Small molecule screen for therapeutic development

We have developed and validated cell lines, which will report the activity of the KMT2D or KDM6A promoter respectively by the use of a fluorescent signal. These reporter cell lines are used to screen small molecule libraries for compounds capable of increasing the promoter activity and thus increasing KMT2D or KDM6A gene expression. Although an increase of gene expression will boost both the normal and mutated allele, we believe that the increased expression of the normal gene copy can compensate for the loss of function caused by the mutated allele in Kabuki syndrome patients. High throughput screening of compounds started late last year and is ongoing.

Kmt2d mouse model

We have expanded the mouse colony for the KMT2D model in accordance with the program's needs. In the coming years, we anticipate to expand the mouse colony to include KDM6A mouse models to test our hypothesis across the entire molecular spectrum of Kabuki syndrome.

Caenorhabditis elegans (C. elegans) model

We have made great strides in our C. elegans research in the last year. Most importantly, we overcame significant research hurdles that were preventing us from making full use of the models, and built updated versions that will allow us to rapidly screen for small molecules and compounds that can boost residual KMT2D. To do so, we performed rigorous transcriptomic analysis of our previous models' response to reduced KMT2D and KDM6A. This allowed us to identify 2,737 "responder" genes whose expression is highly correlated with the levels of KMT2D and KDM6A. We then used these genes to engineer new C. elegans strains where the animals carrying the responder genes exhibited a strong green fluorescence. In preliminary data, we find that these strains show a striking on/off switch in fluorescence when KMT2D is only mildly reduced to 75% of its normal levels, within the range that might be seen in patients (Fig 1). Importantly, these new strains will allow quantitative fluorescence measurements that enable automated screening, and allow us to detect drugs that modestly boost activity of residual KMT2D—perhaps leading us to the most promising candidates for clinical trials.

Online resources

Roya Kabuki Program website.

Boston Children's International Kabuki website.