Smith-Kingsmore Syndrome Gap and Landscape Analysis

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Scope of Work: The goal of this document is to provide actionable recommendations to the Smith-Kingsmore Syndrome Foundation about how best to direct their research efforts for Smith-Kingsmore Syndrome. To this end, we have interviewed relevant key opinion leaders, reviewed published material and public databases, reviewed foundation provided materials, and integrated learnings from other related fields and disorders to inform our recommendations. The activities we are suggesting should be considered alongside the organizational priorities, available funding, and bandwidth or resources needed to pursue these activities, and in the context of scientific understanding of SKS at the time of this report. Smith-Kingsmore syndrome is a disease that affects multiple physiological systems including seizure activity and sleep. Priorities may shift over time as biological and medical understanding of the disease progresses and as therapeutic technologies mature. We recommend revisiting strategic plans and community priorities yearly to ensure your foundation's goals are aligned with your research activities. Due to the length of the document several sections repeat content since we assume the reader might skip around and we do not want key information to be missed. The intended audience for this document is the Smith-Kingsmore Syndrome Foundation board members and their constituents. The writing style aims to be accessible to patients and families that are familiar with Smith-Kingsmore syndrome.

Priority Recommendations

1. Governance & Community Structure: Recommendations were made to the SKS Board on ways to optimize their organizational structure. These recommendations remain with the Board.
2. Tool Development — *Building the foundation for discovery*

We recommend further investment into the use of current tools and to better understand their translational utility, as well as development of new tools, especially cell lines.

- **Flies:** Support ongoing fly model studies to characterize seizure, sleep, and blood–brain barrier phenotypes. Flies should be prioritized as a cost-effective platform for high-throughput drug repurposing screens.
- **Mice:** Continue characterizing existing SKS mouse models (ΔR1480–C1483, E1799K). Early determination of phenotypes will guide whether additional mouse models, including mosaic models, are needed.
- **Cell lines:** Invest in building an accessible cell line library carrying representative SKS mutations (e.g., E1799K, mosaic, hypoactive variants) to better understand the molecular impact of these mutations and to develop a screenable phenotype
- **Registry and clinical tools:** Refresh the SKS registry to capture longitudinal outcomes and off-label treatment responses; co-develop a consensus clinical severity scale to standardize measurement across care and trials.

3. Research & Therapeutic Opportunities — *Turning biology into treatments*

We recommend focusing on therapeutic strategies that are feasible in the near term, while laying groundwork for longer-term approaches.

- **Basic biology:** Define how SKS-associated mTOR mutations alter specific functions of mTOR
- **Clinical understanding and research:** Refine clinical stratification of patients (haploinsufficient, mosaic, activating mutations) to inform prognosis and therapeutic pairing.
- **Repurposing:** Launch small-molecule repurposing screens in fly and/or cell models
- **Targeted Therapies:** Initiate antisense oligonucleotide (ASO) development for common mutations such as E1799K

Recommendations for Research

The main goal of this document is to provide concrete recommendations to the SKS Foundation for how to best direct their research efforts. To this end, we have reviewed published literature, interviewed stakeholders (including researchers, clinicians, and families) and integrated information from other related fields and disorders to inform our recommendations. All recommendations are based on what is known as of October 2024.

A significant part of the review process involves identifying key gaps in biological understanding. While there is much research underway on mTOR, there is a substantial gap in knowledge about SKS and the specific effects of SKS-related mutations on mTOR function. We prioritize recommendations based upon the potential for impact and the potential for success. The activities we suggest should be considered alongside the organizational priorities, available funding, and bandwidth or resources available to the SKS Foundation to pursue these activities. Priorities will continue to shift over time as scientific understanding of the disease increases and as therapeutic development advances. We recommend revisiting strategic plans and community priorities annually or biannually to ensure the Foundation's goals are aligned with ongoing and future activities with the Medical and Scientific Advisory group.

Following our review of published literature and discussions with researchers, clinicians, and families, several key areas of scientific understanding stand out as critical for future therapeutic development. These can be further subdivided into research and therapeutic development.

Smith-Kingsmore Syndrome Overview

Smith-Kingsmore syndrome (SKS) is a rare genetic autosomal dominant disorder in which the presence of one mutated *MTOR* allele (or one copy) leads to a range of clinical symptoms including macrocephaly, developmental delay, intellectual disability, hypotonia, autism spectrum disorder, seizures, behavioral problems, sleep disturbances, and more. SKS was first described in 2013 following exome sequencing of a one year old patient presenting with megalocephaly and intractable seizures (*1*).

Following the identification of the initial patient, a standard diagnosis of SKS has been defined by the existence of a heterozygous (or a single copy) activating (causing increased activity) de novo mutation (a mutation that occurred by chance and was not inherited from a parent) in the *MTOR* gene. However, since this initial case, it has become clear that this type of mutation is not the only mutation in *MTOR* to cause neurodevelopmental disorder. There is also an emerging hypothesis that a subset of patients may harbor *inactivating* mutations in **mTOR**. This possibility warrants careful consideration in the context of therapeutic development and evaluation, as such variants could elicit responses distinct from those associated with the more commonly observed *activating* mutations. Additionally, another subset of patients has been identified with a completely different inheritance pattern called somatic mosaicism (*2*). Mosaic mutations are mutations that occur during cell division in embryogenesis (or during the development of the fetus) and are therefore found in only a subset of cells.

There may be important phenotypic and clinical implications to these different mutations, or subgrouping of mutations. Understanding the specific genetic mutation and how it leads to pathogenicity is important for scientific understanding and for developing effective therapeutics. There are examples of other monogenic disorders with a similar situation, some of whom remain unified and others who have separated into different groups due in part to competing research priorities.

Clinical Understanding and Diagnosis of SKS

Smith-Kingsmore Syndrome Diagnosis and Recurrence Risk

Key Recommendations

- **Advocate for inclusion of *MTOR* on genetic diagnostic panels** including: seizure, macrocephaly, microcephaly and overgrowth panels.
- **Develop recurrence-risk materials for the SKS Foundation website** and create clinician/counselor resources that highlight germline mosaicism and documented cases of multiple siblings with SKS despite unaffected parents
- **Keep track of number of families with more than one individual affected by SKS** to strengthen understanding of recurrence and prevalence, while acknowledging that the numbers may be lower than reality since some families choose not to have additional children.

The diagnosis of patients with SKS continues to increase as access to genetic testing broadens. Since the discovery of Smith-Kingsmore syndrome in 2013, the patient population has grown to almost 350 worldwide (as of October 2024, https://smithkingsmore.org/). Diagnosis continues to be more prevalent in regions where there is easier and cheaper access to genetic testing.

As the prevalence of genetic testing increases, patients are also receiving diagnoses at younger ages, as well as some older patients (although this is still very rare). *MTOR* is often included on panels for genetic causes of overgrowth syndromes. However, these panels will likely not catch patients with supposed haploinsufficiency of mTOR which often leads to microcephaly instead of macrocephaly. Individuals with more severe symptoms, especially seizures, may get diagnosed at younger ages, however many epilepsy gene panels still do not include the *MTOR* gene.

***Advocating for inclusion of MTOR on seizure and newborn panels may help patients get earlier diagnoses and allow for earlier treatment*.** This advocacy usually includes:

- Sending genetic diagnostic companies an update on SKS and published research showing the association between *MTOR* and seizures etc., noting the expected under diagnosis rate.

- Including a cited cover letter (citations - published literature with clinical associations), signed by the Foundation and co-signors from the clinical community, genetic counselors, and research community.
- It is also possible to add letters of support from the research and clinical communities.

As the age of diagnosis gets younger, families in the SKS community have a particular need for better information on recurrence risk. Within the community, there are families who have a child diagnosed with SKS due to *de novo* mutations in *MTOR* who go on to have another child with the same *de novo* mutation. These are unlikely to be the result of truly de novo mutations and are likely caused by mosaic carriage of the *MTOR* mutation in either parent's eggs or sperm (germline mosaicism). In these cases, the recurrence risk of a second SKS child, is higher than the typically cited ~1% risk of recurrence of a de novo mutation.

This is also something that is not unique to SKS and has been documented in other neurodevelopmental disorders (*3*, *4*). However, the presence of families who've had more than one child because of germline mosaicism is something that clinical geneticists and genetic counselors should be more aware of, and that parents may want to consider in terms of future family planning.

We recommend developing specific content on SKS recurrence risk on the SKS Foundation website, as well as resources for clinicians and genetic counselors, to highlight cases of multiple children from the same family having SKS when neither parent is a somatic carrier of an mTOR mutation. We also recommend the foundation **keeps track of the number of families with multiple SKS affected individuals** so the foundation can build greater understanding of recurrence and prevalence in the community, keeping in mind that this number may be under representative since SKS families may decide against having another child.

SKS Symptoms and Severity – Patient Experience

Key Recommendations

- A critical revamping of the SKS patient registry. This includes reducing barriers of entry, refining what data is collected, and developing a plan to reengage caregivers over time
- Support a formal Natural History Study that would collect a complimentary clinical dataset on a subset of SKS patients
- A consensus-endorsed clinical severity measure to evaluate SKS patients and support future clinical trials
- Create/maintain a cohesive reference document outlining known symptoms and treatments that have had some impact for patients

SKS patients clinically present with a broad spectrum of symptoms and severity. The way SKS presents may look very different even between patients who have the same mutation, and siblings who both have SKS. Most known SKS patients experience intellectual disability, sleep disturbances, disrupted temperature homeostasis, and macrocephaly while some patients also experience seizures to varying degrees of severity (*5*–*7*). Other common symptoms include autism, hypotonia, anxiety, communication challenges (limited or non-verbal), aggression (particularly as approaching/during puberty), hyperphagia, gastro-intestinal challenges, and developmental/motor delays and disabilities (https://smithkingsmore.org/).

Overall, there is not a recognized genotype/phenotype correlation in SKS patients (*5*). Heterogeneity in the spectrum of SKS symptoms and severity may be a result of the specific *MTOR* mutation but the data so far does not show clear genotype- phenotype correlations in the SKS community in a way that can be clearly defined (See Section on Genotype-Phenotype correlations for further discussion). This uncertainty underscores the need for accurate data collection and clinical studies to better understand potential genotype/phenotype relationships and their possible implications for prognosis and therapeutic development.

The Smith Kingsmore Syndrome Foundation has created a patient registry and is actively collecting data from patients and their families with SKS. As of October 2024, there had been a recent update to the registry (by the centralized registry company CoRDs) that caused the site to require participants to complete hard copies of the forms instead of online versions. This has since been rectified but there was likely a drop off in the number of people who submitted their data.

Odylia would recommend revamping specific questions for clarity and pertinence following discussions with the Medical and Scientific Advisory group (See Advisory Board Section). This includes reducing barriers of entry, refining what data is collected, and developing a plan to reengage caregivers overtime to prioritize participation in order to gain longitudinal data. Because there is a change in symptoms, symptom severity, and drugs taken across time for

patients, it is recommended that the Foundation encourage frequent updating of data, following up from the annual 'nudge' from the CoRDS system. Having this information empowers the Foundation to provide critical information and resources to the community and provides researchers and clinicians a more comprehensive understanding of disease progression for future research and treatment. Ultimately registry data collection needs to be a balanced approach to avoid 'registry fatigue.' ***Input should be sought from the Advisory group and ultimately approved by The Foundation or a Patient Advisory Board if one is formed separately to ensure the time commitment needed from caregivers is appropriate.***

Registry data collection can also be enhanced through complimentary clinical data collection efforts. **A natural history study detailing both the genotype and symptoms over time** for patients is critical for understanding the disease, determining clinically relevant endpoints, and informing clinical outcomes. Clinical experts in the Medical and Scientific Advisory group could assist in re-shaping registry data collection and advising on feasibility of starting clinical data collection.

Additionally, **a consensus-endorsed clinical severity measure** is needed to evaluate SKS patients and to support future clinical trials. It's recommended that the Foundation work with the Medical & Scientific Advisory group to establish a cohesive clinical evaluation system for SKS that can track the diversity of symptoms and their severity over time, to aid in disease understanding, symptom management, caregiver education, therapeutic development, and clinical trial design.

As more caregiver/patient reported data, natural history and an SKS clinical evaluation data is collected, we recommend the Foundation engage the Advisory group to create **a living reference document** for patients, families and clinicians that outlines known symptoms and treatments that have had some therapeutic effects for patients.

Genetics of Smith Kingsmore Syndrome

Key recommendations:

- Encourage a better understanding of clinical stratifications in SKS through grants (clinical research and cell lines)
- Compile basic summary information including mutations found in the SKS community, inheritance pattern (mosaic, somatic), severity of key symptoms (seizures, macrocephaly, etc.)
- The Foundation should treat the question of which mTOR-related disorders to represent as an ongoing strategic discussion, since these choices will guide future use of resources and research priorities

Background

There are many different types of mutations that can impact DNA (missense mutations, nonsense mutations, frameshift mutations, indels and copy number variations) and therefore expression and function of genes. Some mutations have little to no impact on function, while others can be life threatening.

Missense mutations occur when a single base pair in the DNA sequence is changed which results in a different amino acid being incorporated into the protein. A nonsense mutation is similar to a missense mutation in that only one base pair is changed, but instead of changing the amino acid the base pair change results in a premature stop signal which causes an early termination of the protein (or shortened protein).

A frameshift mutation happens when a base is either added or removed from the DNA code. This shifts the entire 'message' of the DNA code following this mutation and typically, this leads to several inappropriate amino acids to be added from that point followed by an early termination of the protein. An indel is similar to a frameshift mutation but the mutation itself is larger than a single base pair.

Finally, a copy number variation involves entire sections of DNA that are either deleted or duplicated. This can include multiple genes. Due to the non-perfect nature of cell division and embryogenesis, everyone has de novo mutations (*8*). Oftentimes these mutations have little to no impact on the life of an individual. However, if these mutations disrupt the function of particular genes, they can have significant implications, as is the case for many people with *MTOR* mutations.

Predictive models used to classify variants

Missense mutations are classified as pathogenic, likely pathogenic, variant of unknown significance (VUS), or benign mainly via predictive algorithms and machine learning tools. Currently, for SKS and other diseases, clinicians and genetic counselors use industry standard machine learning tools such as *Alpha fold 3* (Google Deep Mind) (*9*) to determine the likely pathogenicity of the mutation, but this does not indicate the effect on mTOR function.

The starting point for genotype-phenotype correlations is usually genetic and clinical data. Clinicians look at the mutations or where they are located in the protein and the severity, age-at-onset, number of different symptoms, specific types of symptoms, or symptom intensity. The machine learning tools also take into account the structure of the protein, including functional domains and known folding patterns, nodes of interaction, the type of amino acid substitution, etc. Eventually pairing this information with in vitro or in vivo activity measures of mTOR might further elucidate genotype- phenotype understanding in SKS.

mTOR mutations and phenotype classification

While there may not be conclusive genotype-phenotype correlations for SKS patients, there are other important conclusions that can be made. It is clear that the genetic background of patients, environmental factors, hormone influences, and stochastic/random events that occur during development can be important in the presentation of symptoms. This is apparent because even across patients with the same mutations, the disease presents differently. This is common across monogenetic neurodevelopmental disorders and indicates that there are additional factors, other than just the specific mutation, that have an influence on the disease progression (*10*).

Broadly, when looking at the entire SKS patient population, patients can be stratified by symptoms and severity of symptoms (Table 1). The easiest to bracket are those patients with loss-of-function mutations (or deletions). The mechanism of action for these patients is a loss of mTOR function and these patients present with drastically different clinical phenotypes. The second group are those patients with mosaic mTOR mutations. The majority of these patients have severe symptoms including earlier diagnosis, earlier and more frequent seizures, greater intellectual disability (ID), and behavioral phenotypes, and distinct clinical phenotypes from the loss of function group. It is also believed that these specific mutations lead to greater mTOR activation than the non-mosaic patients. A key question remaining in the field is whether there is a clear stratification of these mutations, and the same *MTOR* mutations that exist in mosaic patients also exist in SKS patients that are not mosaic, and vice versa. It is possible that the most severe *MTOR* mutations are not compatible with life in a non-mosaic, heterozygous patient, but are able to exist in a mosaic patient because not every cell in the body carries these severe mutations. The majority of patients who have been diagnosed do not fall into either the haploinsufficient SKS or the mosaic SKS categories. Although these patients all fall into what could be referred to as 'classical SKS', they have a wide range of symptoms and symptom severity.

Table 1. Stratification of SKS patients into mutation type and inheritance pattern categories

SKS stratification	Genetic cause	Specific biological or phenotypes/ symptoms	Critical questions
Haploinsufficient SKS	mTOR haploinsufficiency	Microcephaly, haploinsufficient or mildly inactivating mutations	• Do all these patients have nonsense or deletion mTOR mutations?
Mosaic SKS	Mosaic activating mTOR mutation	More severe symptoms	• Are the mutations found in these patients specific only to mosaic SKS patients?

Classical SKS	Activating mTOR mutation		• Are there differences in the epigenetic mechanisms of disease for this group? • If there is more than one patient with the same mutation, how do their symptoms vary? Is this consistent over time?

Going forward, the SKS Foundation could drive forward greater knowledge of how mTOR mutations drive symptoms and possible clinical stratifications by **strategically investing in grants (clinical research and cell lines)** that are targeted at understanding the underlying biology of different mutations.

Additionally, the Foundation is well placed to **compile basic summary information including mutations found in the SKS community, inheritance pattern (mosaic, somatic), and severity of key symptoms** (seizures, macrocephaly, etc.). This will aid understanding of the current prevalence of each mutation, the common inheritance pattern(s) and how mutations correlate with severe symptoms and convey that externally for various purposes. This information will be of importance both to the community (one of the first questions typically asked after diagnosis is whether there are other individuals with the same mutation), and to clinicians and researchers in the SKS and mTOR field.

Because the understanding of mTOR biology and its clinical spectrum will continue to evolve, the Foundation's position will need to remain flexible to adapt to new evidence and community needs. While there is still a relatively small SKS patient population, there is benefit in using the umbrella term of Smith-Kingsmore syndrome to define all patients with a neurodevelopmental disorder caused by mTOR mutations. The Foundation should revisit on an ongoing basis how broadly it represents mTOR-related disorders, as this decision will directly shape the allocation of resources and the direction of research efforts. Questions include whether to include both hypo- and hyperactive mutations, whether to encompass mosaic mutations, and whether to extend representation to patients with focal cortical dysplasia caused by MTOR mutations.

Exploring the Clinical Landscape

Current Treatment

Key Recommendations

- Continue utilizing the registry to **understand what treatments are being used by patients and if or how well they are working**. This may be useful if subgroups of SKS respond differently to different treatments.
- **Re-survey the clinical landscape at a least once a year** to identify new clinical trials relevant to mTOR activity, other diseases that result in altered mTOR function, novel seizure medications, new formulations of mTOR inhibitors or endpoints that are relevant to SKS patients (seizure treatment, aggression, sleep, etc.)
- **Explore repurposing opportunities** by reviewing existing drugs with potential off-label benefit for SKS symptoms or investing in broader drug repurposing studies
- **Develop clinical protocols for off-label use for drugs like sirolimus,** codifying/standardizing its current off-label use within the community
- **Establish processes for observation and tracking off-label use,** using the SKS registry or other forms of reporting to capture outcomes across patients

Current treatment for patients with SKS revolves around management of symptoms. Patients present with different symptoms and they are generally treated with a range of pharmacological and behavioral interventions.

At present, there is no clear consensus among patients regarding which interventions reliably alleviate specific symptoms. Additionally, there is anecdotal evidence in the patient community that even when treatments provide initial benefit, their efficacy may diminish over time within the same individual. **The Foundation should continue to use the patient registry to understand what treatments are being used by patients and if/how well they are working.**

Clinical Trials

Current Clinical Trials relevant to Smith-Kingsmore Syndrome

While there are no current clinical trials specific to SKS, there are ongoing trials that could impact treatment for SKS patients. **It is best practice to re-survey the clinical landscape at least once a year** to identify new clinical trials (or the results of clinical trials) testing new mTOR modulators or focus on endpoints that are relevant to SKS patients (seizure treatment, aggression, sleep, etc.)

The studies listed below are both studies looking at the effect of mTOR inhibitor treatment on aging. Keep in mind, these studies are done on an older population than the majority of known SKS patients, however their results may give information on starting points for dosing if an mTOR inhibitor study is initiated for SKS. Additionally, it has been hypothesized that there is a subset of SKS patients that have accelerated aging. If further investigation can identify these patients specifically, there may be a reason to push for mTOR inhibitor testing in these patients first.

- Everolimus Aging Study (EVERLAST) (NCT05835999)
    - The objective of this project is to determine if mTORC1 inhibition by 24 weeks of daily (0.5 mg/day) or weekly (5 mg/week) everolimus can safely improve physiological and molecular hallmarks of aging in humans.
    - https://clinicaltrials.gov/study/NCT05835999?term=mtor&rank=6
- Rapalog Pharmacology (RAP PAC) Study (NCT05949658)
    - The objective of RAP PAC is to identify safe and effective weekly dose(s) for the mTOR inhibitors sirolimus and everolimus that intervene on the underlying fundamental biology of aging.
    - https://clinicaltrials.gov/study/NCT05949658?term=mtor&page=2&rank=13

Because mTOR is in the same pathway as many other genes that cause seizure disorders, it may be possible that treatments for those disorders could be repurposed for SKS. An antiseizure medication originally approved for CDKL5 deficiency disorder is currently in Phase 3 clinical testing for treatment of seizures in TSC patients. The Phase 3 trial and the results from the Phase 2 trial are below.

- Adjunctive Ganaxolone Treatment (Part A) in TSC Followed by Long-term Treatment (Part B) (TSC) (NCT04285346)
    - To objective of this study was to determine preliminary safety and efficacy of ganaxolone as adjunctive therapy for the treatment of primary seizure types in patients with genetically- or clinically-confirmed TSC-related epilepsy through the end of the 12 week treatment period.
    - https://clinicaltrials.gov/study/NCT04285346?term=tsc&page=3&rank=22&tab=results
- Adjunctive GNX Treatment Compared With Placebo in Children and Adults With TSC-related Epilepsy (NCT05323734)
    - This is a Phase 3, global, double-blind, randomized, placebo-controlled study of adjunctive GNX treatment in children and adults with TSC-related epilepsy.
    - https://clinicaltrials.gov/study/NCT05323734?term=tsc&page=5&rank=47

A clinical study of particular interest seeks to better understand Mos-FED (Mosaicism in Focal Epilepsy Cortical Dysplasia Tissue), a type of epilepsy often caused by mosaic mTOR mutations.

While this is not a clinical trial with a treatment arm, it might be beneficial to reach out to the researchers to determine if certain SKS patients might fit into their inclusion criteria.

- Mos-FED (Mosaicism in Focal Epilepsy Cortical Dysplasia Tissue) (MosFED) (NCT06053671)
    - Focal cortical dysplasia (FCD) is a malformation of brain development, the most common cause of drug-resistant epilepsy and often caused by mutations in mammalian target of rapamycin (mTOR) pathway genes. The main outcome will be finding new causes of epilepsy with FCD and the development of new diagnostic and screening tools.
    - https://clinicaltrials.gov/study/NCT06053671?term=mtor&page=4&rank=32

Repurposing Drugs

If clinicians in the SKS community identify FDA-approved drugs that may help manage SKS symptoms, it is essential to understand the pathways for making these treatments accessible and to assess when a clinical trial may be warranted. Off-label use and compassionate use represent two key mechanisms for repurposing drugs without the need for a formal clinical trial. **The SKS Foundation should consider exploring repurposing opportunities** for off-label use, or investing in broader drug repurposing studies (see 'Research and Therapeutic Development' for more detail).

Off label use refers the use of an approved drug for an unapproved indication (disease or symptom) or condition. Each drug is approved for use in specific indications and when a treating physician prescribes a drug for an indication that is not on the list of approved indications then that drug is being used 'off label.' This is a legal and common practice and is justified when scientific evidence suggests that the drug will be both effective and safe in the treatment of the un-approved indication and is in the best interest of the patient. Tracking the outcomes of off-label use is sometimes undertaken by the prescribing physician, this is often done in isolation and not shared across patients. **The Foundation should work with the Medical & Scientific Advisory group to develop clinical protocols for making off-label/repurposed drugs available to SKS patients.** One example of this is sirolimus (an mTOR inhibitor), which has been prescribed off-label to some patients but there is currently no standardized approach to prescribing, observing and reporting for SKS patients. The Foundation/MSA should also consider if the current registry could be used to track outcomes or if a different mechanism is needed, to quickly identify a positive or negative affect.

Compassionate use and treatment under the **Right to Try Act** refers to use of an investigational drug being tested for another indication. In this case, patients and their physicians can petition the drug manufacturer to access the drug for use when the patient has exhausted all other possible treatments and has a life-threatening or serious condition. Compassionate use

applications must be reviewed by the FDA and an Institutional Review Board (IRB), while the FDA and IRB do not have to review applications under the Right to Try Act, only the treating physician and the drug manufacturer are required to approve of use under a Right to Try application. Given that SKS is not seen as immediately life threatening in most cases, this may be a more difficult case to make. If there are specific situations in SKS that are recognized as immediately life threatening and where an investigational treatment might help then the treating physician should consider documenting this case and making a request.

Importantly, in the context of rare diseases like SKS, off-label use of drugs or specialized use cases such as compassionate use can lead to the effective identification of new treatments, but only when information is shared, and more than one patient is treated. For this reason, registries and natural history studies can be very effective in the tracking of treatment trends in a rare disease community and dissemination of that information to researchers, clinicians, and patient foundations.

Research and Therapeutic Development

Resources and Tools

Key Recommendations:

- Consider if there are ways to **aid or speed up the ongoing work in flies** to both identify mTOR mutation-associated sleep and seizure phenotypes and use fly models for drug repurposing screens
- Continue **encouraging characterization of the various mice models** and timely communication of the findings so the Foundation knows if it is worth investing further in the current mouse models, new mouse models, or that mice models may not be an effective translational model for SKS.
- **Invest in the generation of SKS patient-associated mTOR mutations cell lines**

Animal Models

Animal models provide a means to study the effect of genetic mutations on behavior and biology, as well as test therapeutics for efficacy, safety, and toxicity. The existence of an effective translational animal model enables disease communities to test therapeutics on relevant models prior to moving into human testing. *While this lowers risks that arise if a treatment is moved directly into human trials without animal testing, models can also have the added benefit of accelerating discovery of new therapeutics, decreasing wasted funding, and increasing pharma interest in a specific disease.* Animal models are validated based on certain criteria and can have predictive, face, and/or construct validity.

- Predictive validity: a measure of how well a model can be used to predict currently unknown aspects of human disease (e.g. correlation between the animal and human as it relates to therapeutic outcomes)

- Face validity: a measure of how well a model replicates the disease phenotype in humans (e.g. does the animal model exhibit all the symptoms and behaviors found in the human condition?)
- Construct validity: how well the mechanism of disease in the humans is matched in the animal model (e.g. does the underlying biology match between the human and the animal)

While it would be ideal to have an animal model that had predictive, face, and construct validity, this is rarely possible. Important findings can still be made using an animal model which does not perfectly recapitulate the human condition. Additionally, while an animal model can be very helpful, they are not necessary for drug development. Many diseases do not have a testable animal model and are still able to move into clinical trials. Therefore, while important, they are not the only important consideration for drug development.

Animal Models for Smith-Kingsmore

Main issues with current animal models for Smith-Kingsmore syndrome:

- We are still in the early stages of development for the mouse and fly models of disease
- No clear phenotype
- Possibility that mice are resistant to effect of hyperactive mTOR, or they do not manifest like humans
- Homozygous mTOR mutant mice are not viable

Main consideration – what is the end goal?

- Is it to learn about mTOR biology? Determine effects of a certain mutations on animal physiology? Evaluate the effect of treatment on a particular behavior or biomarker? The answers to these questions can impact animal selection.

Fly model (Drosophila melanogaster)

Important work is currently ongoing in fly models of SKS. The fruit fly, or *Drosophila melanogaster*, is a model that has been used by geneticists for decades. Because of this, many techniques have been developed which make it a useful model for studying a genetic disorder such as SKS. Flies have a rapid reproductive rate and develop to adulthood quickly so scale up of fly colonies if very cost-effective and quick (compared to mammalian disease models).

As of October 2024, there is work ongoing in the lab of Dr. Joanna Chiu at UC Davis to study the impact of *MTOR* mutations on Drosophila behavior. Dr. Chiu's lab is studying sleep, seizures, and temperature sensation in the SKS flies. Additionally, she has interest in understanding the impact of mTOR mutations on nutrient sensing in these flies, and the time of feeding can

impact sleep or sleep quality. Because flies are known to have consolidated sleep, as humans do, she is interested to know if these mutations influence the duration and frequency of this type of sleep.

Another potentially important area of investigation that the flies could begin to answer is the impact of mTOR dysfunction on the blood brain barrier (BBB). The BBB creates a barrier between the brain and the rest of the body which protects it from toxins while allowing in important nutrients. mTOR activity has been known to disrupt the permeability of the BBB (*12*) which would result in less effective gatekeeping in the central nervous system. Patients with SKS are known to have unexpected reactions to certain drug treatments which may be the result of altered BBB permeability.

The results of work looking at the impact of mTOR mutation on the permeability of the BBB will be important for understanding the impact of mTOR mutations on seizures and sleep as well as determining if nutrients/the timing of nutrition impacts the behavior of the flies. This may be an area worth looking into in more detail clinically with SKS patients, by potentially using various contrast agents and MRI based techniques. If there are interesting findings in the flies this is worth considering in more detail.

There is anecdotal evidence from the SKS community that modifying diets can have beneficial effects on patients' seizure, behavior, and sleep patterns (from interviews within the community). Specifically, non-pharmacological changes to diet, such as the ketogenic diet, have been known to decrease seizures in some seizure patients and this has even been found in an animal model of TSC (*6*), this is also commonly seen in seizure disorders generally. This would be interesting to test if a model of SKS is found that has seizures.

Because mTOR and the mTOR pathway is highly conserved between flies and humans, it is possible to study effects of SKS-associated mutations to mTOR pathway and downstream proteins/pathways. Additionally, flies are highly suited to drug repurposing studies. Given the cost-effectiveness of fly models, the Foundation should **consider if there are ways to aid or speed up the ongoing work in flies** to both identify mTOR mutation-associated sleep and seizure phenotypes and undertake more comprehensive drug repurposing research.

Key questions flies can begin to answer:

- Impact of mTOR mutations on sleep (duration and frequency) and seizures
- How blood brain barrier (BBB) permeability changes with circadian rhythm
- Identification of quantifiable phenotypes in flies with SKS-associated mTOR mutations
- How restrictive eating impacts behaviors in the flies
- If SKS-associated mutations impact different tissues differently
- Because mTOR and the mTOR pathway is highly conserved between flies and humans, it is possible to study effects of SKS-associated mutations to mTOR pathway and downstream proteins/pathways
- Potential to set up a small molecule drug screen assay/drug repurposing screen

Mouse model

There are currently two known mouse strains with SKS-associated mTOR mutations see Table 2 below for details. The $mTOR^{\Delta R1480\text{-}C1483}$ mouse is the only mouse strain that has been characterized so far. This mouse has a four amino acid deletion that mirrors an SKS patient (ΔR1480-C1483) mutation. Mice that are homozygous for the mutation are not viable. This is unsurprising given the likely extent of the mTOR hyperactivation in these mice. A conditional model of a homozygous overactivation mTOR mutation in the brain may uncover a phenotype in the mouse that could be used to test therapeutics. However, this animal would not have face validity with SKS in humans and may take quite a bit of time to generate and identify a phenotype.

The second mouse, which is almost available, has an E1799K mutation in mTOR, which is a mutation found in a significant number of SKS patients (up to half of all reported cases in the literature) (*6*). Phenotyping of this mouse has not begun (as of October 2024). This work is ongoing in Dr. Andrew Liu's lab.

Table 2. Currently available mouse models of SKS

Animal Strain	Location and Access	Details
Jacks mutation Δ(R1480-C1483) 4amino acid deletion)	Created in Cincinnati At Andrew Liu's lab (general access unknown)	Homozygous mutant mice are not viable Heterozygous mouse, characterization ongoing but as of Fall 2024: • Do not see gross phenotype • Do not see a circadian rhythm phenotype • Heterozygotes have subtle sleep phenotype and activity level changes
$mTOR^{E1799K}$	Andrew Liu's lab	Phenotyping in progress

It has been hypothesized that the mutations found in mosaic SKS patients may have higher mTOR activation than non-mosaic mutations. Therefore, generation of a mouse model with one of the mutations found in a mosaic SKS patient may be more likely to have a significant, measurable phenotype. If none of the ongoing mouse models have a testable phenotype, Odylia recommends looking into the possibility of developing a mouse with a mutation found in patients with mosaic SKS, but also balancing this with cell line and fly model investment since the mouse may be problematic for modeling SKS ultimately.

Mosaic Mouse Models

Some patients with SKS have mosaic mutations in mTOR. Because their mutation in mTOR is not found in all cells of that individual, traditional mouse models will not have construct validity for these patients. Animals expressing a mutation in all cells will not be a perfect model for these patients; it is important to remember that they might not be fully indicative of the human condition, especially in terms of treatment side effects in healthy, non-mutated cells. However, there are a few options already available and genetic tools are advancing to a point where there is hope of creating a mosaic model in the future (Table 3).

Traditional mouse models give us insight into some, but not all patients with SKS. Mosaic mouse models might be a good opportunity to understand the subset of SKS patients with these types of mutations. Recently, great strides have been made towards creating more complex transgenic animal models. In the long-term, it would be worth exploring the creation of a mosaic mouse model, especially if none of the traditional models show phenotypes that are prevalent in the mosaic SKS population. Additionally, using a mosaic mouse model would allow researchers to understand the effect a treatment has, not only on mutated cells, but also on healthy cells.

The Foundation should proceed with caution before investing in this area as the utility of a mosaic mouse model for evaluating drugs which target specific symptoms may be unknown or complicated by the mosaic nature of the model. As of October 2024, there are mosaic mouse models used to model disease but so far none have been used for drug development. These mice can answer interesting biological questions specific to mosaic patients. Since they are not the majority of cases, the generation of mosaic mice are unlikely to be a top priority unless an interested researcher is identified.

Table 3. Different approaches to generation of mosaic mouse models.

Technique	Summary	Pros	Cons	References
ifgMosaic	This technology enables the examination of multiple and combinatorial gene function with high	- Can be used for multiple genes - Can look at any gene of interest	- Might get low rate of cells expressing transgene	(Pontes-Quero et al., 2017)

	temporal and cellular resolution	- Fluorescent markers allow for tracing of which cells contain the mutation		
MADM (mosaic analysis with double markers)	Mosaic analysis with double markers (MADM) offers one approach to visualize and concomitantly manipulate genetically defined cells in mice with single-cell resolution	- Fluorescent markers allow for tracing of which cells contain the mutation	- Doesn't work for all genes - Generates homozygous mutant cells for a candidate gene of interest and wild-type cells in an otherwise heterozygous background	(Contreras et al., 2021)
CRiSPR-Cas9 mediated	CRiSPR-Cas9-mediated, site-directed mutagenesis in mice generates mosaic founder mice	- Can look at any gene of interest	- Would need to choose a representative mutation - Varied efficiency of desired point mutation, and other non-homologous end-joined variants - No fluorescent marker	(Vasu and Fox, 2021)

Cell Line Generation

Cell Line Selection and Generation

Odylia recommends investing in the generation of several cell lines with mTOR mutations found in SKS patients. There are two key considerations before funding this work: (1) the selection of the cell line type and (2) the selection of the SKS mutations to generate in these cell lines. As a starting point, we recommend editing in *MTOR* mutations into an easy to grow cell line rather than generating patient derived samples. Established cells lines are easy to grow and by mutating the *MTOR* mutation into the cell line directly, variation due to differences in genetic background are not an issue. We recommend discussing with the Advisory group and selecting a cell line that is often used to study the function of mTOR, and relevant to disease biology, or an established cell line that can be derived into other tissue/organ-relevant cell types.

For the mutations to be studied, we recommend selecting several mutations that mirror the diversity seen in SKS patient population, such as mutations from each proposed clinical subgroup (activating non-mosaic, mosaic, and hypoactive *MTOR* mutations). The E1799K mutation would be important since it accounts for such a significant percentage of the population. Other mutations that represent a range of human phenotypes and are representative of where the majority of mutations exist in the protein would enable better

understanding of disease pathobiology and allow therapeutics to be more appropriately paired as needed with various SKS subgroups. This would be ideal to discuss with the MSAB.

Screening

The first hurdle in small molecule screening is identifying a robust cellular phenotype that is relevant to disease and is quantifiable and amenable to high-throughput screening. The 'screen-able phenotype' can be a number of things, and it may take some time to discover what the best phenotype(s) is to focus on. Once robust phenotypes are identified, these phenotypes can be used to measure if a potential therapeutic can ameliorate the phenotype associated with the mTOR mutation. Cell lines can be a robust tool for screening prioritized drug panels (i.e., current treatments on the market for closely related symptoms) or large, unbiased panels. Medications used to treat sleep irregularities, seizures, bone health, or behavioral disorders related to those seen in SKS should be prioritized for initial screening (*13*). Even though cells do not have some of these more complicated behaviors, these drugs can be tested against cellular phenotypes (i.e. mTOR activation, mTOR localization, insulin signaling, mTOR phosphorylation, etc.).

Key Questions for Cell-based Screens:

1. Can the underlying biology of the SKS-associated mTOR mutations be determined?
    - Are all mutations hyperactivating?
    - Are the many functions of mTOR effected equally?
    - Are there point mutations that decrease mTOR activity?
2. Is there a reliable phenotype of these cells that can be used to screen drug compounds? How does that relate to human symptoms?
3. What drug screens make the most sense to start testing?

Research and Therapeutic Considerations for Smith-Kingsmore Syndrome

Summary of Research Recommendations

Basic biology: Prioritize research into the underying biology of SKS, how SKS-associated mTOR mutations alter specific functions of mTOR, with a particular focus on seizure mechanisms and circadian rhythm disruption.

Therapeutic development:

- Drug repurposing and screening - focus on small molecule repurposing using cell and fly models with seizure/sleep phenotypes
- Targeted genetic approaches - develop mutation-specific ASOs (e.g., E1799K) as a flexible, precise therapeutic strategy; deprioritize broad gene therapy, genome editing, and cell therapy until feasibility improves
- Explore broader neurobiological avenues
    - Investigate seizure therapeutics from related mTORopathies (e.g., TSC, CDKL5) for potential crossover use in SKS
    - Collect more brain imaging and neurophysiology data (MRI, EEG, CT) to understand SKS-specific neurobiology

Key Biological and Clinical Questions

After reviewing the published research and interviewing families and researchers, we have assembled a prioritized list of key questions pertaining to SKS and mTOR. This list is prioritized based on potential impact on maintaining life and increasing quality of life for patients and families as well setting the SKS Foundation up for future therapeutic research. A large portion of these questions rely on strong iPSC development, and as such that should be a priority.

Biological questions

1. Is there an identifiable and screenable phenotype associated with SKS-mTOR mutations in a cell or animal model that relates to disease?
2. Which biological functions of mTOR or pathways are affected by mutations found in SKS patients? Are mTORC1 and mTORC2 affected equally?
3. How do activating mutations in mTOR impact seizures, circadian rhythms, etc.?
4. Are there epigenetic differences between patients with SKS?

Clinical questions

1. Are there more effective seizure treatments for patients?

2. Does timing of current drug treatments or nutrition impact behavioral phenotypes or sleep quality/disruption?
3. How do patients stratify genetically/phenotypically – are there any biological implications?
    a. Are there differences in the aging process in different patient groups?
    b. Are there any conclusions to be drawn about patients with the same mutations?
4. Are there currently understood clinical endpoints that can be used in a clinical trial?

Circadian Rhythms and mTOR

Because the circadian clock is known to be disrupted in SKS patients, drugs that are known to impact this feedback loop may be helpful in treating some of the sleep or behavior symptoms seen in SKS patients without needing to target mTOR itself. There is ongoing work in this field (*14*) which may have interesting and important implications for SKS patients. *Once an animal or cell model of SKS is developed that has a testable circadian rhythm phenotype, these types of therapeutics may be a good place to start testing.*

Therapeutic Modalities Overview

Developing a therapeutic that genetically treats SKS is difficult since mTOR is a ubiquitous protein with multiple critical functions in maintaining cellular health and proper function. Current genetic therapeutic technologies aim to express or silence a gene or protein. Finding a treatment for SKS will likely require fine tuning of the mTOR protein activity, targeting a specific function of mTOR, or targeting proteins downstream of mTOR through specific pathways.

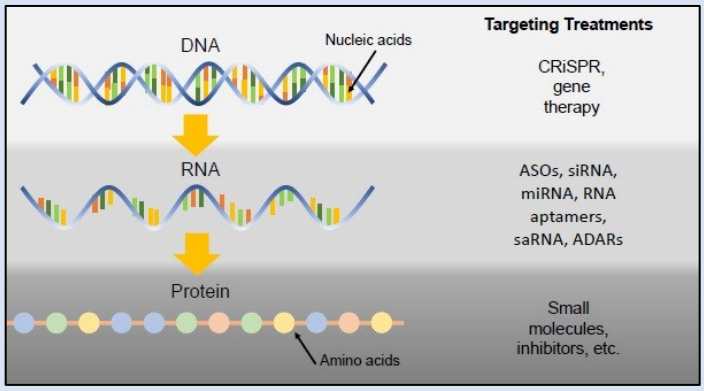
Additionally, developing a diverse portfolio of therapeutic approaches for SKS can increase the likelihood of finding an effective treatment, and better address different needs across the various genetic subgroupings of the SKS community. Below is a general list of different therapeutic modalities or approaches to drug development. Not all of these modalities are applicable to SKS treatment, we advise making prioritization decisions based on feasibility and biological considerations.

1. Small molecule screening: when screening compounds already approved by the FDA this is called drug repurposing. Examples include Tylenol and Zyrtec. While cost effective and sometimes the most efficient path to find a drug, this approach is what we call a "fishing expedition." By the nature of the term screening usually large libraries of chemical compounds are tested to see if they have an effect on a disease-relevant outcome in cells lines. For example, a screen might test a library of a thousand or tens of thousands of different compounds to see if the compounds affect a DNA repair mechanism when exposed to a DNA damaging toxin. Those compounds that protect the cell against damage would be studied further. While this can be a cost-effective approach when an effective compound is identified, there isn't always an effective compound identified or the outcome is not truly disease specific. Also, if there is a potential drug identified, it isn't always clear what the chemical or molecular mechanism of action is. It is also critically important to have a phenotype (whether in a cell or animal model) to test against.

2. Biologics (antibodies, soluble proteins, hormones): These are large protein-based molecules that are usually injected into the patient and target circulating proteins. Because of their size they do not usually penetrate cells easily or cross certain barriers in the body and therefore they must exert their effects outside of the cell. This limits their utility to certain tissues or localized use.

Inside of a cell, DNA is transcribed into RNA before being translated into protein (Figure 1). Therefore, a mutation in DNA can result in the mutation being carried into the RNA transcript. This mutation in turn may result in a change in amino acid in the protein which could affect function of the protein. Because only one copy of the RNA transcript for the mutated version of the protein is different from the wildtype (non-mutated) RNA transcript, it is possible to target this RNA specifically. This opens the door for RNA therapeutics. Additional methodologies can target DNA or protein.

Figure 1. Dogma of DNA



3. Cell therapies (bone marrow transplants, or iPSC delivery): The injection into or replacement of cells in the body is called cell therapy. Sometimes a functional cell is put into the body when the previous cells die off or are dysfunctional, and sometimes cells are taken from the body and treated with a gene therapy and then put back into the body.

4. Gene therapies and genome editing (AAV, lentivirus, CRiSPR): These approaches deliver genetic material (such as a functional gene) or directly edit the mutation in a genome.

5. RNA therapeutics (siRNAs, ASOs, aptamers, etc): Targeting RNA is sometimes easier than delivering DNA, especially when the aim is to decrease the expression or function of a protein. RNA targeting therapeutics utilize a diverse and growing number of approaches that can exert different effects on the RNA itself including upregulation, downregulation, and allele-specific targeting to name a few.

SKS Therapeutic considerations

Primary Obstacles and Considerations for Therapeutic Development

The challenges of SKS mouse models (discussed more in the Animal Models for Smith Kingsmore Syndrome section) and lack of a cell model is an obstacle to therapeutic development. It is important to keep in mind that animal models are not humans and are rarely perfect models of human disease, they can be extremely beneficial in both helping understand disease biology and identifying useful drugs. However, it is possible to develop therapeutics without an animal model that phenotypically mimics patients. A cell or animal (such as a fly) that can be used for high throughput screening is the most efficacious way to test many potential therapeutics at once in an unbiased way. At the same time, additional research should be put into understanding the biological mechanisms of disease.

Given the current understanding of SKS as of October 2024, we recommend investing in small molecule repurposing screening. Because mTOR is so ubiquitous and such an important kinase, we do not recommend manipulating its expression via gene therapies, genome editing, or silencing efforts. At this time there is little evidence to indicate that cell therapy or large biomolecule treatments would have the desired effect on mTOR. However, new therapeutics and new therapeutic modalities are constantly being developed so it is always important to keep an eye on and consider novel treatment options. Further understanding of the downstream effects of SKS MTOR mutations on certain pathways may also highlight alternative proteins that can be targeted therapeutically.

Small molecule screening of previously identified drugs is a highly cost-effective way to identify therapeutics. For this to be most effective, there first needs to be a cellular or behavioral phenotype identified. A cellular phenotype, for example in cell lines with SKS-associated mTOR mutations, would allow researchers to screen for drugs that alter that phenotype. Doing so in cell lines is an ideal place to start because you can look at multiple lines at once with different mutations without having additional confounding variables such as genetic background. Another way to perform this type of screen is in an animal model with an observable, testable behavioral phenotype. This is especially useful for complex behaviors such as seizures or sleep. Having appropriate genetic models of disease can help in better understanding the disease biology as well as therapeutic evaluation, but the throughput of testing therapeutics is

dramatically different between models. Cell lines and smaller animal models with rapid reproduction rates (such as flies and c. elegans) can be used for screening libraries of compounds or genetic interventions, while mice are most useful for evaluating the efficacy of one or a few therapeutics.

Additionally, because a significant number of patients have the same activating mutation, consideration should be given to the potential of developing a mutation targeting therapeutic. Of the published SKS patients, almost half have the E1799K allele(*6*). An ASO, antisense oligonucleotide, is a short, synthetic strand of RNA that is designed to bind to a specific RNA sequence in a cell. The primary purpose of an ASO is to modulate the expression of a specific gene by interfering with the production of proteins. ASOs can be designed to prevent the translation of specific messenger RNA (mRNA) into protein or can modify splicing patterns, which can ultimately reduce, enhance, or alter the protein produced by the gene. They are highly specific and can target specific missense mutations to modulate the amount of mutated mTOR present in cells without reducing the amount of nonmutated mTOR. Because ASOs are designed for specific mutations, an ASO designed against the E1799K mutation would not be applicable to patients with a different mutation. An additional advantage of an ASO is the ability to modulate the dose of the ASO since it is not a one-and-done type treatment. If safety margins are well established, it might be possible to eventually modulate mTOR activity by different levels by dosing the ASO up or down and seeing how the patient responds to the treatment.

Targeting mTORC1 vs mTORC2

One area of research not yet investigated is the impact of SKS-associated mTOR mutations on mTORC1 and mTORC2 function specifically. Understanding if there is a difference in their regulation of these complexes could improve biological understanding and therefore therapy development. There can be risk in targeting only one complex without understanding if there is a difference in impact. The risk in targeting only mTORC1 function is that there is a potential to cause feed-forward overactivation of mTORC2 that would not be preferred (interview with Dr. Aaron Besterman). mTORC1 and mTORC2 are both upstream and downstream of each other so parsing out the exact implications for the two is likely to be quite complicated (*15*). While this is a scientifically interesting question, it is unlikely to yield directly to therapeutic development in the short-term. *Odylia recommends keeping an eye on this type of research but to not expect it to yield actionable results in the short-term.*

SKS Neurobiology

Many symptoms of SKS can be linked back to aberrant neurodevelopment. Macrocephaly, seizures, aggressive behaviors, autism spectrum disorder and dysregulated sleep to name a few (*16*). As TSC and many other neurodevelopmental disorders caused by mutations in genes in

the mTOR pathway (See Related Patient Groups) are also associated with seizures, there is the potential for crossover of therapeutics among these disorders for those therapeutics that target a common pathway. Recent research points to mTORC2 to be a driver for epilepsy (*17*) pointing to the potential for mTORC2 targeted therapies to treat epilepsy in a more targeted manner. There is a new treatment for seizures in TSC patients currently in Phase 3 Clinical Trials that could also have an impact on seizures in SKS patients (See Clinical Trials).

On an anatomical level, there are gross neuroimaging anomalies in patients with SKS. The most common anomaly is a change to corpus callosum structure; however, this is not always consistent. There are also instances of polymicrogyria in SKS patients (from an interview with Dr. Aaron Besterman) which are often the loci for seizures (*18*). Further details on brain anatomy of SKS patients should be collected where available from self-reported data or sources such as MRIs, EEGs, or CT scans. *Additionally, discussion with the Advisory group would be useful to help the Foundation understand when and why some patients are diagnosed with SKS versus focal cortical dysplasia and where there is overlap in the diagnoses. While symptomatically there is not always overlap, there may be useful research or treatment options that could be shared across diagnoses.*

Broader opportunities for SKS Therapeutics

In addition to mTOR itself, there is potential for other treatments to directly impact SKS patients. There is significant research into how human circadian rhythms are impacted by diet, timing of exercise/nutrition, fine tuning of hormones or other circulating signals which may have beneficial effects on SKS patients. The additional hypothesis into differences in aging in patients with SKS also opens the potential for aging research to impact SKS treatment. Because so many mTORopathies in addition to SKS are impacted by seizures indicates that there may be a shared mechanism between these diseases. One new seizure medication is currently approved for treatment of seizures in CDKL5 deficiency disorder and is in Phase 3 clinical trials to treat seizures in TSC patients (See Clinical Trials Section). There has also been recent research into mTOR inhibitors that are targeted just to the brain (*19*). This research uses a brain permeable mTOR inhibitor and a non-brain permeable blocker of the inhibitor to keep the effects of the inhibitor localized to the brain. Additional research is needed both on the effectiveness and the safety of this treatment, but it might be an ideal candidate to treat some symptoms of SKS specific to the brain without having deleterious impact on other organs.

mTOR Biology and Background information

To understand the biological underpinnings of SKS, and to design targeted therapeutics, it is important to understand what mTOR is and what it does in our cells. The mTOR protein is a serine/threonine protein kinase in the PI3K-related kinase family (*20–23*). This means that

mTOR is an enzyme that adds a phosphate group to specific proteins with exposed serine or threonine amino acids. Biologically, this 'activates' these down-stream proteins and serves to regulate many different cellular functions. mTOR has several important functional domains including HEAT repeats, a FAT domain, FRB domain, kinase domain and a FATC domain (*24*) (Figure 2).

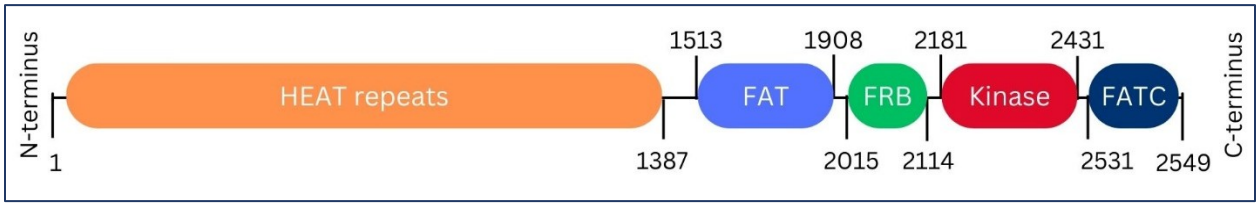


Figure 2. Functional domains of mTOR

Figure 2 displays the primary structure of mTOR. A protein's primary structure is the linear chain of amino acids that make up that protein. This image denotes several 'domains' which are distinct functional or structural units of a protein. They form distinct 3-dimensional structures, and many are found across different proteins. Within mTOR, the kinase domain is the catalytically active subunit of the protein, meaning that is the part of the protein responsible for its enzymatic function. The HEAT repeat domain, Huntington, Elongation Factor 3, PR65/A, TOR, exists on the exterior, solvent-exposed surface of mTOR (*5*). The FAT-domain regulates the kinase domain by restricting access to the catalytic core. The FAT domain is also responsible for the binding of mTOR to RAPTOR and RICTOR (*24*), these are important interacting partners of mTOR which will be discuss later in this section. The FRB (FKBP-rapamycin-binding) domain is thought to act as a 'gatekeeper' to the kinase domain with its rapamycin binding site interacting with substrates to grant access or not to the kinase domain (*24*).

Researchers have found that mTOR is intrinsically active, meaning that without regulation via regulatory domains or regulatory proteins it is always active, and that it has a highly restrictive catalytic core. They believe that mutations around this core create an opportunity for hyperactivity (*24*). Proteins exist in 3-dimensional space. That means that the linear primary structure depicted in Figure 1 only gives a part of the picture of what mTOR actually looks like in a cell. When the protein is folded properly, certain parts of the protein which are not close in the linear structure can actually be very close in the 3-dimenstional protein (Figure 3). In patients with SKS, researchers have found that predicted pathogenic variants cluster, not only in specific domains, but in 3-dimensional hotspots around the core of the protein where they are expected to disrupt proper folding of the protein (*5*).

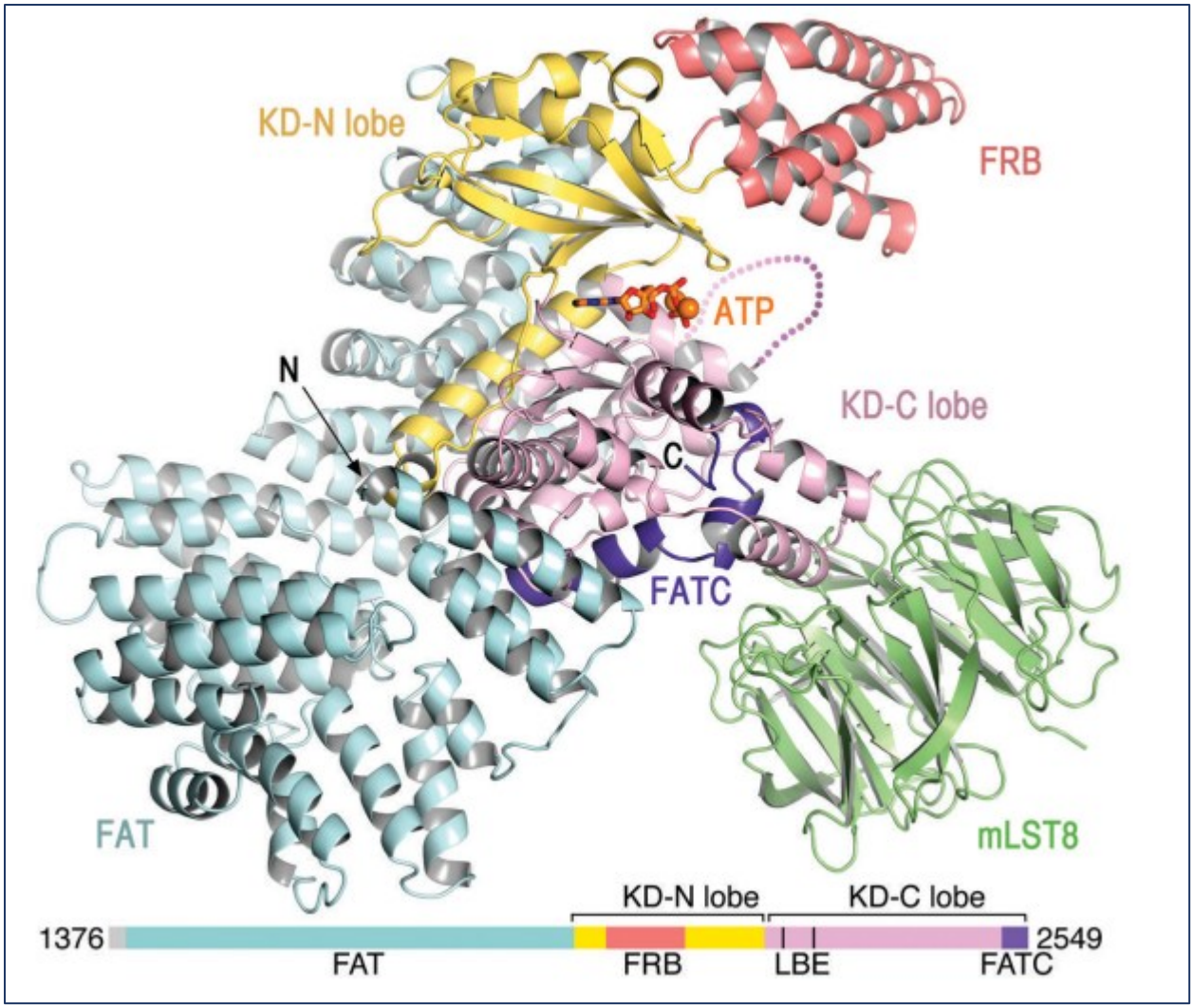


Figure 3. Three dimensional structure of mTOR showing the FAT (cyan) domain and the Kinase (pink) domain in close proximity. Image from Yang et al. (2013) (24).

The functional domains of proteins are important to keep in mind because mutations in different domains may have radically different outcomes to the function of the protein. The majority of SKS-associated variants of mTOR are localized to the FAT and Kinase domains (interview with Dr. Aaron Besterman) with a recent paper finding 86% of mutations in the patients studied localized to the FAT or Kinase domain (*6*). This may have impact on the activity of mTOR as well as its ability to bind appropriately to interacting proteins. Keeping records on what mutations have been found in SKS patients and at least some information on the phenotype of the patients is important to identify any patterns that may exist.

General Function of mTOR

The mTOR signaling pathway is a central regulator of normal cell growth and neurodevelopment (*5*, *22*) while dysregulated mTOR signaling is known to be implicated in cancer, diabetes, aging, and neurodevelopmental disorders(*22*, *25–27*). mTOR is the core subunit of two protein complexes, mTORC1 and mTORC2 (Figure 4). mTORC1 is responsible for regulating cell growth and metabolism through the production of proteins, lipids and nucleotides as well as suppressing autophagy (*22*). mTORC2 however, controls cell survival and proliferation and effects the insulin signaling pathway (*22*).

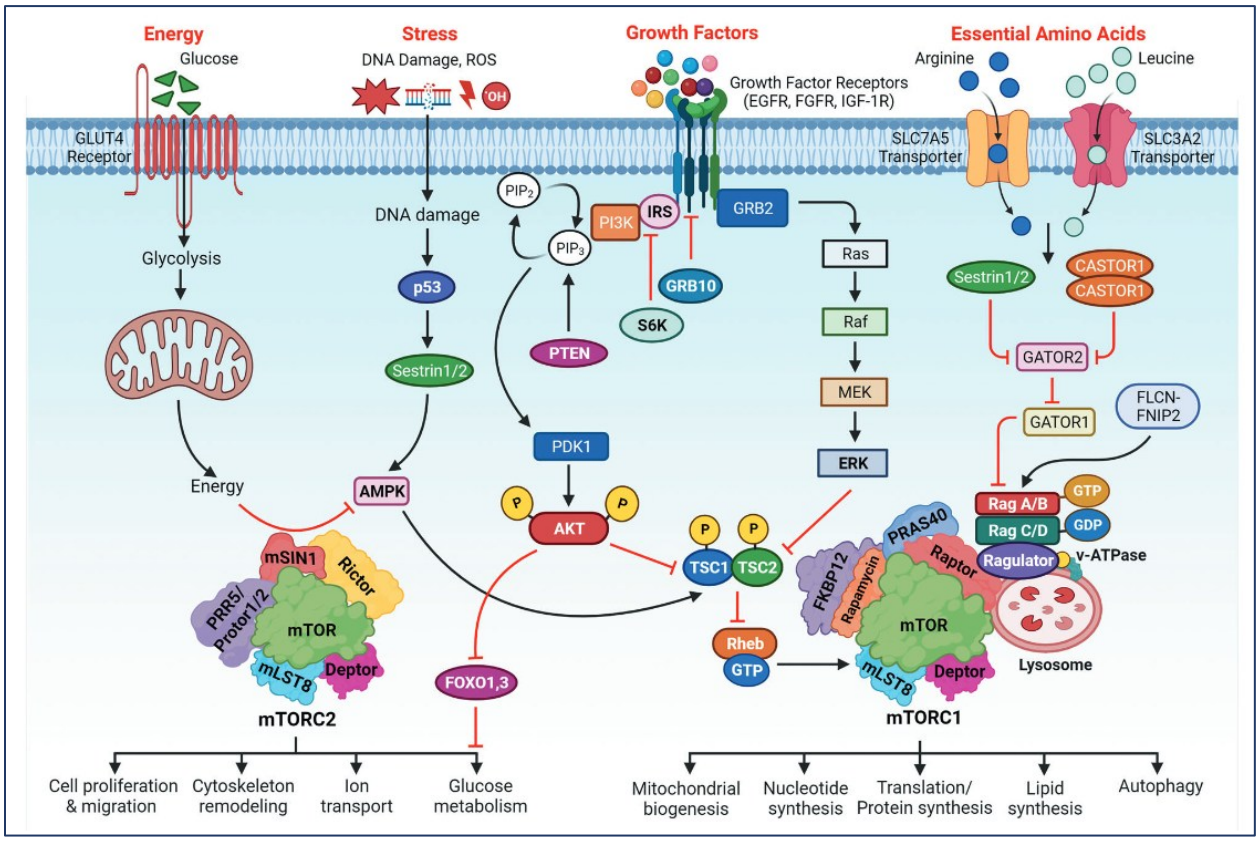


Figure 4. Major regulators of the mTOR pathway and function of mTORC1 and mTORC2 (*28*)

Neurobiology of mTOR

The many functions of mTOR are also critical within the brain. Extensive research has begun to reveal its pivotal role in cognition as well as how mTOR dysfunction is associated with neurological disorders. mTOR has been implicated in many brain functions including the proliferation of neural stem cells, the assembly and maintenance of circuits, experience-dependent plasticity, and regulation of complex behaviors like feeding, sleep and circadian rhythms (*15*, *21*, *29*). Studies have shown that mTOR activation in neurons can enhance synaptic strength and dendritic spine density, crucial for memory formation and cognitive flexibility (*22*). Overactivation of mTOR could therefore cause abnormal increases to synaptic

strength, dendritic spine density and an imbalance in excitatory and inhibitory neurotransmission which is linked with seizure pathway formation (*30*).

Mutations in mTOR and other genes in the pathway (*PIK3CA, Akt, PTEN, TSC1/2,* etc.) have been implicated in overgrowth and neurodevelopmental disorders, and seizure disorders (*5*, *15*). A significant amount of what we know about the impact of overactive mTOR on brain development and function is from research into Tuberous Sclerosis (TSC) and the genes that cause it, *TSC1* and *TSC2*. Loss of TSC1/2 regulation of mTOR leads to profound changes in neuronal architecture and differentiation including aberrant axon formation and defects in cellular maturation (*15*). In patients with TSC, mTORC1 hyperactivation correlates with a high occurrence of epileptic seizures and autism spectrum disorder (ASD) which indicates that mTORC1 signaling may be specifically involved in these symptoms (*22*).

mTOR and Circadian Rhythms

Circadian rhythms are the cycles in biological, physical, and behavioral changes that organisms go through in a 24 hour period. Many biological processes are governed by circadian rhythms including sleep, eating, hormones, body temperature, and the immune system. Dysregulation of this natural cycling can have grave impacts on quality of life. The protein mTOR is a known regulator of circadian rhythms (*31*). mTOR integrates signals from nutrients, growth factors, and energy status, linking metabolic function with the body's internal timing system. Studies show that typical mTOR activity follows a circadian pattern and influences key components of the molecular clock, such as the expression of clock genes like *PER1* and *BMAL1* (*32*, *33*). This connection further establishes that mTOR serves as a bridge between environmental cues, like food intake and light, and the internal processes that regulate the sleep-wake cycle. mTOR is also known to sense amino acids via mTORC1 (*34*) which is another way mTOR senses nutritional intake which can impact the function of the mTOR feedback loop.

Overactive mTOR signaling has been associated with altered sleep patterns and changes in metabolism, highlighting its role in synchronizing energy usage and circadian control. Mouse studies have found that mTOR over-activation speeds up the normal circadian clock oscillations while mTOR inhibition lengthens these periods (*35*). This can have an influence on neuroplasticity and cognitive functions, which are closely tied to the sleep-wake cycle. mTOR is essential for memory formation, synaptic plasticity, and learning, processes that follow circadian patterns. Abnormal mTOR activity can therefore lead not only to metabolic and sleep disorders but also to cognitive impairments (*15*). Understanding the complex relationship between mTOR and circadian rhythms offers potential therapeutic strategies for treating disorders like SKS or neurodegenerative diseases, where circadian disruptions are common.

Of note is that natural circadian rhythms are not controlled by mTOR itself but by the mTOR pathway which regulates the expression and activity levels of other genes and proteins, such as *BMAL1*. This gene is known to be regulated by mTOR and is part of the feedback loop that

regulates circadian rhythms as well as synaptic plasticity (*36*, *37*). Because the circadian clock is known to be disrupted in SKS patients, drugs that are known to impact this feedback loop may be helpful in treating some of the sleep or behavior symptoms seen in SKS patients without needing to target mTOR itself. There is ongoing work in this field (*14*) which may have interesting and important implications for SKS patients. *Once an animal or cell model of SKS is developed that has a testable circadian rhythm phenotype, these types of therapeutics may be a good place to start testing.*

Organizational Recommendations

As rare disease patient advocacy organizations are largely run by volunteers, it is ideal to take advantage of the expertise that already exists within the SKS community. Odylia recommends reaching out to the already existing SKS community to determine if there are additional people who are interested in volunteering time. This can be done with the parents and caregivers as well as with extended family and friends who are eager to help by tapping into relevant skill sets that can be helpful to an organization such as graphic designers, lawyers, coders, marketers, etc. Identifying critical needs and making that ask is an easy way to engage the community without directly needing to ask for monetary donations.

In addition to tapping the SKS community already established, there is a lot that can be gained by combing forces with other rare disease patient advocacy groups. In the Related Patient Groups section below we compiled several related diseases and their affiliated patient advocacy group. Because they are all caused by mutations in the same pathway, it is highly possible that what works for one disease may be useful for others.

Additionally, we hope to increase the researchers interested in SKS and SKS biology. In the Academic Groups section we aimed to identify novel researchers to the SKS Foundation who are doing work highly relevant to SKS and SKS drug development. As new research is funded by the Foundation it may be worthwhile to have discussions with these and other researchers to broaden the field of SKS research. It might also be useful to think about combining research efforts with related disorders. In this way, the SKS Foundation can combine funding efforts with related disorders whose effected genes overlap with SKS. This allows for research to be done on both diseases at once which oftentimes can be done with minimal added costs.

The SKS Foundation already has some great information available for families and clinicians. After interviewing families and caregivers of those with SKS, additional resources would also be greatly appreciated. Odylia recommends working towards generating additional informational fliers to help families navigate some of the difficult symptoms of SKS. This effort could benefit from data from the registry or questions directed at the community. It is not necessary to have all the answers; many families find it comforting to not be alone in the struggle. Information on what treatments have been tried as well as what has worked (or not) for some would arm families with valuable information as they navigate treatment for their own child. Additionally,

certain periods of life (especially puberty) seem to impact SKS patients and cause drastic changes in behavior, response to drugs, etc. Generating a document or a place for families to learn from each other would be greatly utilized by the community. There was extremely positive feedback on the conferences that have been held by the SKS Foundation. Due to the expense of these conferences, it is unlikely they will be able to become more frequent. However, fostering that community feeling does not have to stop. Because there are so few known patients with SKS and because they are scattered across the globe, having a resource online for community engagement would help families navigating life and treatments alone.

Recommendations for resource building: generating additional informational forms on the following topics or resources would help caregivers as noted in interviews with caregivers and community research

- Navigating difficult symptoms in SKS (seizures, behavior, sleep, etc.)
- Treatments overview (what is recommended for which symptom, what has been tried, what doesn't work, a place where families can report on what has worked for them)
- SKS changes in puberty
- Fostering communication and engagement with the community

Related Patient Groups

There are a large number of diseases that are caused by mutations in genes that impact mTOR function (Figure 5). If we consider just the rare genetic diseases (excluding rare cancers) it is possible that treatments that work for one may benefit others. Below is a table with many of these rare diseases listed along with the gene effected, the impact on mTOR activity, the symptoms the disease shares with SKS and a link to the organization or foundation for each disease. They are loosely organized by those with the most overlapping symptoms with SKS at the top and those with the least overlapping symptoms at the bottom.

While many of these diseases do not have significant overlap in symptoms with SKS, many of them, including Peutz-Jeghers syndrome, Von Hippel-Lindau disease, APDS, and Proteus syndrome, are known to have a higher rate of malignant cancers. So far, there has not been an increase in cancer seen in SKS patients, but it is something that the Foundation should continue to monitor with the community especially because there is some overlap between mutations found in cancer and those found in SKS patients.

Table 4. Diseases in the mTOR pathway

Disease	Gene Effected	Impact on mTOR activity	Overlapping symptoms with SKS	Organization
Tuberous sclerosis	TSC1 or TSC2	Increased mTOR activity	Seizures, autism spectrum disorder, hyperactivity, aggression, intellectual disability	https://www.tscalliance.org/
PIK3CA-related overgrowth spectrum (PROS)	PIK3CA	Increased mTOR activity	Seizures, intellectual disability, autism spectrum disorder	https://birthmark.org/pros-pik3ca-related-overgrowth-spectrum/
PTEN hamartoma syndrome	PTEN	Increased mTOR activity	Developmental delays, autism spectrum disorder	https://allaboutapds.com/
Proteus syndrome	AKT1	Increased mTORC1 activity	Intellectual disability, seizures	https://www.proteus-syndrome.org/
Peutz-Jeghers syndrome	LKB1/ STK11	Increased mTORC1 activity	Seizures	https://www.smartpatients.com/communities/peutz-jeghers-syndrome
Von Hippel-Lindau disease	VHL	Increased mTORC1 activity	Seizures	https://vhl.org/
APDS (activated PI3K delta syndrome)	PIK3CD or PIK3R1	Increased mTOR activity	Developmental delays	https://allaboutapds.com/

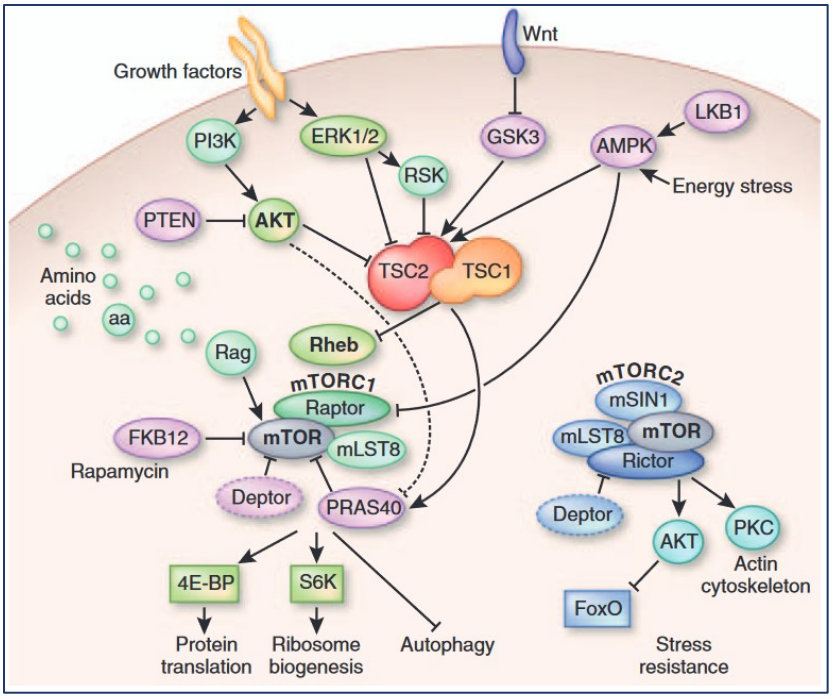


Figure 5. mTOR pathway including many proteins also known to be mutated in other neurodevelopmental disorders.

Academic Groups

Investigating new therapeutics and advancing knowledge of the pathways disrupted in SKS patients requires dedicated clinicians, researchers, and patients/patient's families working together. While there are already many dedicated researchers studying SKS, it is always good practice to reach out to additional researchers who work in related fields or whose research interests are in alignment with the SKS community. We always recommend expanding your research network whenever possible to encourage fresh ideas and critical feedback on current approaches. These investigators have expertise that could be helpful in continuing progress towards solving some of the predominant concerns of SKS patients and their families.

Laura Mantoan Ritter, MD, PhD
Kings College London
Website: https://www.devneuro.org/cndd/about-detail.php?recordID=2329
Email: laura.mantoan@kcl.ac.uk

- Dr. Mantoan Ritter investigates novel somatic mTOR pathway mutations in human epilepsy. Her focus is on novel diagnostic and therapeutic tools and precision treatments for super-refractory and genetic forms of epilepsy.
- She is currently working on the mTOR Pathway diseases node which aims to study the mTOR pathway rare diseases.

Professor Joseph Bateman BSc, PhD
Kings College London

Website: https://www.kcl.ac.uk/research/bateman-research-group
Email: joseph_matthew.bateman@kcl.ac.uk

- Dr. Bateman is interested in the regulation of neurogenesis by mTOR signaling and its role in epilepsy. He is focused on basic processes in neuronal function and development.
- He is currently working on the mTOR Pathway diseases node which aims to study the mTOR pathway rare diseases.

Professor Carla Green, PhD
UT Southwestern
Website: https://labs.utsouthwestern.edu/green-lab
Email: Carla.Green@UTSouthwestern.edu

- Her lab studies the molecular mechanism of the mammalian circadian clock and how it controls rhythmic physiology.
- studying more broadly the general role of the circadian clock in metabolism and aging.

Professor Stephanie Baulac
Paris Brain Institute (ICM)
Website: http://www.baulacleguernepilepsy.com/home
Email: stephanie.baulac@icm-institute.org

- Her lab does cell reprogramming and genome editing of human cells to generate iPSCs-derived cortical spheroids to establish in vitro disease modeling of focal and mosaic cortical malformations.
- Use this approach to generate human neuronal models disease caused by mutations in genes belonging to the mTOR pathway.
- Her goal is to determine how pathogenic mutations in mTOR genes impact cortical development and neuronal activity. She also has data on suppressing how mTORC2 specifically can reduce seizures in animal models.

Professor Kevan Shokat
UCSF
Website: https://shokatlab.ucsf.edu/
Email: kevan.shokat@ucsf.edu

- His lab uses chemical genetics to study and target signaling proteins, with the aim of finding new ways to treat human diseases.
- They are working to develop new mTOR inhibitors including RapaLink and Sapanisertib.

Professor Jonathan Lipton
Boston Children's Hospital
Website: https://www.liptonlab.com/
Email: jonathan.lipton@childrens.harvard.edu

- His lab is investigating circadian clocks in synaptic function and dysfunction and the molecular mechanisms linking clocks to sleep homeostasis.

- His lab studies other rare diseases that effect circadian rhythms and he comes from a background in TSC research

Professor Ruifeng (Ray) Cao
Rutgers University
Website: https://sites.rutgers.edu/ruifengraycaolab/
Email: ruifeng.cao@rutgers.edu

- His lab is interested in mTOR, the mammalian circadian clock, and autism spectrum disorders. He is very interested in the role of mTOR in the brain.
- They are specifically interested in the interaction of Bmal1, autistic phenotypes, and mTORC1 hyperactivation

Professor Dudley Lamming
University of Wisconsin-Madison
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Email: dlamming@medicine.wisc.edu

- His lab is interested in understanding and manipulating the mTOR signaling pathway through dietary, pharmaceutical or genetic interventions in an effort to provide insight into the treatment of age-related diseases, including diabetes, Alzheimer's disease, cancer, and Hutchinson-Gilford Progeria Syndrome.
- He may have interest in Smith Kingsmore syndrome, the mTOR mutations themselves or this interesting hypothesis about differences in aging among the SKS patients

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Interview References

Odylia would like to that all the researchers, clinicians, SKS Foundation Board Members, and caregivers who took the time to speak with us during this process. Below is a list of all the researchers/clinicians spoken to and the date of the interview. The identities of the caregivers and families will remain confidential.

Dr. Aaron Besterman, UCSD; July 18th 2024

Dr. Andrew Liu, UF; July 19th 2024

Dr. Joanna Chiu, UC Davis; July 31st 2024

Dr. Julian Martinez-Agosto, UCLA; August 13th 2024

Dr. Carlos Prada, Lurie Children's Hospital; September 4th 2024