

2019-
2021

Schaaf-Yang Syndrome Research Plan

Schaaf- Yang Syndrome Research Plan 2019-2020

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INTRODUCTION

Schaaf-Yang syndrome [SYS, OMIM #615547], first identified in 2013, is a rare genetic disorder affecting multiple systems. SYS is caused by mutations in the maternally imprinted, paternally expressed *MAGEL2* gene, located in the Prader-Willi syndrome (PWS) region of chromosome 15q11-13 [NCBI Gene ID: 54551]. To date, all reported mutations are predicted to result in a truncated protein.

Many clinical features of SYS overlap with PWS, including profound neonatal hypotonia, feeding difficulties, developmental delay/intellectual disability, behavioral problems and endocrine abnormalities. However, additional features are more common in SYS than in PWS, such as contractures, autism and severe intellectual disability.

The full phenotypic spectrum of SYS remains to be defined, and the natural history of the disorder is incompletely characterized. Interestingly, *MAGEL2* mutations have been determined to underlie some cases previously reported as Opitz-C or Chitayat-Hall syndromes. Further, while cellular studies have identified a role for the *MAGEL2* protein in membrane protein recycling and endosomal sorting through facilitation of the retromer recycling pathway, questions remain about the normal function of this protein and how disruption of its cellular function contributes to disease. Optimal standards of care have yet to be established for individuals with SYS, and novel therapeutic interventions have yet to be advanced.

Goal: With the input of experts, the ‘state of the field’ of SYS research will be assessed and a strategy to efficiently advance the science of SYS will be developed. This SYS/*MAGEL2* Advisory Group (SMAG) will identify and prioritize key research questions, and highlight current opportunities and needs. Recommendations will be made with respect to resource development, collaborative opportunities, and targeted research initiatives to advance the understanding of *MAGEL2* biology and SYS, develop and test therapeutic interventions, and improve the lives of individuals living with this disorder. It is expected that the recommendations will guide FPWR’s SYS research program for the next 3 years.

Deliverable: A report summarizing current SYS knowledge and research priorities, as well as recommendations for research initiatives and specific research tools to advance the field.



CURRENT STATE OF SYS RESEARCH

Patient Perspective

- Current care includes ‘usual’ early intervention for individuals with developmental disabilities: physical therapy, occupational therapy, speech therapy
- Family concerns include developing standards of care to improve clinical outcomes and to help with reimbursement issues. There is a need for evidence-based clinical care guidelines.
- Growth hormone replacement therapy is being used by some families (may need a “PWS” diagnosis for reimbursement) but little is known about optimal dose, appropriate age for initiation, and how to manage GH therapy in the context of sleep apnea, which can be a significant finding.
- Developing/accessing effective interventions to improve speech, communication, intellectual development are also a high priority.
- Sleep disruption is also of concern to families.
- Behavioral challenges that have overlap with the PWS behavioral phenotype (eg, anxiety, OCD behaviors, cognitive rigidity) may be present in older children
- Novel therapeutics (eg, oxytocin) are of interest to families
- To date, the understanding of family concerns, unmet needs and disease impact are based primarily on anecdotal experience; a more formal assessment of the scope of needs and concerns of SYS families has not yet been performed.

Clinical Aspects of SYS

- Currently 150+ patients with truncating mutation identified – there are likely thousands of SYS patients, but identification is slow and generally coming from private sources
- The natural history of SYS, including morbidity & mortality consists of <100 published cases, primarily of truncating mutations. [see McCarthy et al, 2018 [a](#), [b](#)]. Thus, these studies are at an early stage, defining common features of SYS, as well as the spectrum of severity.
- Neonatal hypotonia with infantile feed problems is a near universal finding to date
- Endocrine changes include high levels of ghrelin, low IGF-1 and GH deficiency
- Developmental delay, intellectual disability and autism spectrum disorder are consistent features of SYS cases described to date
- Additional features present in many/most individuals include respiratory distress, sleep apnea, other sleep disturbances, gastroesophageal reflux, constipation, temperature instability, hypogonadism and distal joint contractures
- Bone phenotype - scoliosis and low bone density are common and need to be monitored carefully
- Optimization of care needed for GH, other endocrinopathies, osteoporosis, apnea, sleep

Molecular Genetics

- Truncating mutations lead to phenotype
- Phenotype of truncating (missense, in/del) is highly variable and clear genotype/phenotype correlations have not yet been defined.
- Missense mutations are more complicated to discern, require family/parent of origin studies (not typically determined)
- The variability in phenotype suggests the possibility of gain of function activity for some MAGEL2 mutations.

Cellular Biology

- MAGEL2 is a member of the MAGE family of ubiquitin ligase regulators
- MAGEL2 functions in a complex with the TRIM27 E3 ubiquitin ligase and the USP7 deubiquitinating enzyme to facilitate the retromer recycling pathway through ubiquitination and activation of the WASH actin nucleation promoting factor.
- A role for MAGEL2 in receptor internalization/trafficking has been described
- Additional interacting proteins are being defined/investigated

Animal Models

Two different mouse models of *Magel2* deficiency have been widely used to date.

- The C57BL/6-*Magel2*^{tm1Stw/J} ([Stock No: 009062](#)) ([Kozlov, 2007 Nature Genet](#)) has a *Magel2*-lacZ knock-in allele that interrupts the endogenous *Magel2* gene and leads to expression of β -galactosidase fusion protein in cells that normally expression *Mage2*. This model has been used to define disrupted pathways (dopamine/serotonin pathway, sleep/circadian rhythm, counterregulatory response to hypoglycemia, activity, body composition, muscle, oxytocin) and evaluate potential therapies (oxytocin, endocannabinoids, diazoxide, OEA, setmelanotide).
- Muscatelli and colleagues created a mouse model, *Magel2 tm1.1Mus* {Schaller, 2010 #4086}, with a 3.4 kb deletion encompassing the promoter and ~ 3kb of the transcribed sequence, resulting in the lack of *Magel2* transcription from the mutated allele. Phenotype includes impaired feeding with poor suckling, higher neonatal mortality, and social and cognitive deficits. Administration of exogenous oxytocin rescues these defects.
- Two new models have been developed by the Muscatelli group and are being characterized.
- A conditional knockout is being developed through the FPWR Preclinical Animal Network, and is expected to be phenotyped in 2019.
- FPWR has developed a rat model (8bp deletion at ~360 bp into coding sequence of *Magel2*) – currently being phenotyped at Baylor College of Medicine.

Therapeutics

- Application of drugs/devices/supplements that are FDA approved /available or are in development to SYS. Examples: Growth hormone, oxytocin/carbetocin [Preclinical and clinical development]
- A number of other compounds (some FDA approved, some not yet approved/available) have been tested in mouse models of *Magel2* deficiency

SYS/MAGEL2 Advisory Group Members – Current and Expected Research Focus

Sebastien Bouret, PhD – normal development of CNS pathways involved in feeding and glucose regulation; developmental effects of Magel2 deficiency on the hypothalamus, including oxytocin biology; gene-environment interactions

Carrie Mahoney, PhD- evaluation of oxytocin, orexin and MCH neurons in a Magel2 deficient mouse model, with a focus on sleep, social interaction, and social memory.

Jennifer Miller, MD- clinical trials

Francoise Muscatelli, PhD Preclinical studies of oxytocin rescue in Magel2 deficient mouse; characterization of novel Magel2 mutant mouse models

Ryan Potts, PhD - molecular and cellular function of MAGEL2, MAGEL2 interacting proteins, proteomics of MAGEL2 deficient models

Rodney Samaco, PhD - characterization of Magel2 mutant rat model

Christian Schaaf, MD, PhD - Characterization of the SYS phenotype, genotype/phenotype correlations, clinical study of GH therapy, gene activation approaches for SYS

Yossi Tam, DMD, PhD – molecular basis of bone phenotype in Magel2 deficient mice

Rachel Wevrick, PhD – preclinical studies of pharmacotherapies in a Magel2 deficient mouse model, circadian rhythm, MAGEL2-interacting proteins and normal function

OPPORTUNITIES & NEEDS

Research Tools Needed

- **Antibody to MAGEL2** – Existing Abs reviewed, none are found to be acceptable for most lab uses. Ideally, an Ab would be crossreactive with both mouse and human proteins, and would be able to detect endogenous levels of protein.
- **Well characterized iPS cells** with *MAGEL2* deletion, patient-specific select mutations – these will be important but there are challenges in differentiating into the ‘right’ neurons (eg, functional classes of hypothalamic neurons, cortical neurons).
Note: although hypothalamic dysfunction is clearly present in SYS, it may not be the most pressing concern and hormone replacement works well. Unlike PWS, hypothalamic hyperphagia/ behavior around food are not the #1 priority. It may be more important to focus on cortical neurons in the context of autism/behavior and ID.
- Other cellular models: models of muscle and bone phenotypes
- Functional assays – assays that allow assessment of missense mutations
- Additional animal models, tissue specific / temporal knockouts
 - Models of patient-specific *MAGEL2* mutations
 - Conditional model to allow tissue specific and temporal specific knockout
 - Stop-floxed model to validate gene replacement strategies
- Biorepository – dental pulp stem cells, other patient specific cells
- Proteomic analyses
- Development of additional, well characterized assays (cellular/animal model) for drug/biologic screening is needed

Needs - Natural History & Clinical Trial Development

- Evaluation of clinical “knowns and unknowns”, building from the Schaaf’s group natural history data
- Evaluation of additional ways to gather patient information– eg, development of SYS-specific question set for Global PWS Registry or VIP Connect
- Analysis of current barriers to evaluating novel interventions – eg, identification of patients, lack of natural history data, clinical expertise, lack of standards of care, reimbursement issues, etc.
- Developing an understanding of what interventions families are doing, with a plan to provide families with a way of documenting ‘n of 1’ trials as rigorously as possible.
- Consensus on ObsRO (observer reported outcome) assessments/scales for families to use for natural history and ‘n of 1’ trials
- Patient experience data is needed to support industry in development of novel therapeutics, as per the FDA patient focused drug development initiative. These studies should define the patient perspective on the impact of SYS on the individual and family, as well as document efficacy and limitations of current therapies available to individuals with SYS, and define unmet medical needs. Symptoms: endocrine (GH deficiency, other), sleep disturbances, behavior, ID, bone, GI issues. Sampling of the entire population will be important to gain an understanding of the scope/variability of needs.

- Endpoints for SYS therapeutic trials
- Development of a 'first clinical trial' for SYS patients – consensus that a trial of GH therapy in SYS is needed. Endpoints: outcomes of growth, body composition, quality of life and exploratory endpoints of motor and cognitive development. To be determined: if this first effort should be US only or US and EU; source of GH for the study; final endpoints.

Outstanding Research Questions

- **Top priority:** Genotype/Phenotype: Need to definitively determine if SYS is caused by loss of function or if a gain of function is associated with some mutations; is a truncated protein or protein with an alternative C-terminal end (frameshift) produced in some patients? Resources needed - cell and mouse models with patient specific truncating mutations
- How does mutation of *MAGEL2* impact other genes in the region (eg, are there changes in expression of other genes)?
- Understanding basis of intellectual disability in SYS: cellular and pathway changes as well as connectivity/structure/developmental changes in the brain
- How are other neurotransmitter systems altered during development in the absence of *MAGEL2*?
- Defining a measurable cellular phenotype that extends to patient samples and is clinically important
- Functional (cellular) assay to evaluate new mutations and for drug screens
- Is there convergence in the function of *MAGEL2* and other PWS-region genes?
- What is the role of *MAGEL2* in peripheral organs?
- Clinical evaluation of sleep, sleep maintenance and/or circadian health in humans with SYS
- Genotype / phenotype:
 - Delineating how loss of *MAGEL2* function or gain of function from a truncated protein results in the observed phenotypes
 - Understanding the basis of phenotypic variability in SYS (mutation/phenotype correlation)

Molecular Therapy for SYS

Preclinical development of novel therapeutics to address the underlying molecular disruption in SYS:

- Gene activation (activation of maternal *MAGEL2*; overlaps with ongoing efforts in PWS)
- Gene replacement – development and testing of gene therapy vectors encoding *MAGEL2* – need to determine if some mutations exhibit gain of function that might interfere
- CRISPR editing of point mutations in *MAGEL2*
- Mutation compensation – small molecules, genetic manipulation to compensate for loss of *MAGEL2* function
- Common questions re: molecular interventions – can phenotype be rescued at later points in development; what level of *MAGEL2* function needs to be recovered to impact phenotype; what are possible off target and on target consequences of recovering *MAGEL2* function

NEXT STEPS

1. Generate antibodies for MAGEL2, N and C term.

2. Generate cell models

- Isogenic iPSCs with common SYS mutations
 - c.1996dupC (accounts for 40-50% of all mutations)
 - c.1996delC most severe phenotype
 - truncating mutation before the alternative start (prior to AA 612)
 - truncating mutation at beginning of MAGE homology domain
- Dental pulp stem cells from SYS patients

3. Functional assays – pilot funding to develop

- Specific assays to characterize pathogenicity of missense mutations, nonsense mutations, in frame deletions, and duplications.

4. Animal model phenotyping - - add onto PCAN – phenotype

- Additional phenotypic assessments through FPWR's Preclinical Animal Network
- Generate tissue specific Magel2 knockouts to decipher which tissues are driving specific phenotypes in the mouse
- Generate Lox-STOP-Lox mouse to allow reintroduction of Magel2 to determine phenotypic rescue at different developmental time points

5. Patient Experience Data and SYS Natural history

- Develop survey for caregivers on most challenging symptoms, current therapies, unmet needs, impact
- Promote enrollment in patient registry

6. Develop of a GH study for SYS. Define:

target population, inclusion/exclusion criteria
study design
endpoints
safety parameters to be examined
source of GH
investigators and clinical trial sites

SYS/MAGEL2 Advisory Group (SMAG)

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| Michael Born, Parent Advocate | San Diego |
| Sebastien Bouret, PhD | Children's Hospital Los Angeles |
| Carrie Mahoney, PhD | Beth Israel Deaconess Medical Center |
| Jennifer Miller, MD | University of Florida |
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