Strategic Research Plan
Koolen-de Vries Syndrome
KdVS Foundation

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December 2022

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COMBINED Brain
Consortium for Outcome Measures and Biomarkers for Neurodevelopmental Disorders

Non-profit consortium led by **52+ patient advocacy foundations**, working with the clinicians, researchers and pharmaceutical firms that are developing treatments for the disorders they represent.

**Strategic Research Plan:** overview of current research / clinical landscape to identify gaps necessary to fill to develop effective treatments/ cures for kids with KdVS.

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Strategic Research Plan Contents

1. Koolen-de Vries Syndrome (KdVS)
2. Kansl1 Biology
3. Mechanism of KdVS
4. Key leaders
5. Conceptual Model of KdVS
6. Existing drug development resources
7. Current therapeutic strategies
8. Recommendations
Koolen de Vries Syndrome (KdVS) is caused by microdeletions in 17q21.31 and variants in the *KANSL1* gene.

Current prevalence estimates for both 17q21.31 microdeletion and *KANSL1* variants is 1:30,000-1:55,000 live births.

Among known KdVS patients, ~75% have microdeletion and ~25% have a *KANSL1* mutation.

Clinical presentation of KdVS is heterogeneous and may include: developmental delay, intellectual disability, impairments in motor skills, hypotonia, epilepsy, autonomic storms, characteristic facial features, ocular manifestations and congenital malformations in several organ systems (heart, lungs, kidney, orthopedic).

Currently there are no *clinical* therapies to restore cognitive or motor function. There is good seizure management with anti-epileptic drugs (AEDs).
**KANSL1 biology**

*KANSL1* is a nuclear protein, part of 2 complexes regulating histone acetylation: MLL1 and NSL1 complex.

*KANSL1* is widely expressed lifelong in both central and peripheral neurons (CNS and PNS) with peak expression during development between 9 pcw-24 pcw\(^5\) in the brain; *KANSL1* expression is highest in cerebellar hemispheres and cortex\(^6\).

*KANSL1* protein primary role is a chromatin modeler - regulating expression of target genes - currently unknown.

- Preliminary evidence that *KANSL1* may also acetylate non-histone targets (*unpublished, Willsey*)

*KANSL1* may create a unique episignature among patients distinct from other genetic neurological disorders.

- Episignature is a potential biomarker for KdVS.
Mechanisms of KdVS

A. Genetic Variants

B. NSL Complex

C. KdVS Pathology

Pathological effects of KANSL1 haploinsufficiency. In healthy controls, KANSL1 complexes with the non-specific lethal (NSL) complex which acetylates histones, loosens chromatin and allows for transcription of target genes. Under conditions of KANSL1 haploinsufficiency (KdVS), histones remain in place and transcription of KANSL1 target genes is suppressed."
Mechanisms of KdVS

ClinVar reports 71 pathogenic/likely pathogenic variants in KANSL1 with an additional 279 with unknown significance (Dec 2022)

~60% of known variants are frameshifts
- majority of frameshifts considered to be truncating variants

~20% of KANSL1 variants are nonsense
- pathogenic mutations considered to cause haploinsufficiency
- microdeletion (definitive haploinsufficiency) cause phenotype which resembles KANSL1 phenotype caused by pathogenic mutations (rodent and clinical)\(^7\)
Key Leaders in KdVS

**Radboud University Medical Centre**
David Koolen, MD, PhD
Bert de Vries, MD, PhD
Nael Kasri, PhD
Katrin Linda, PhD
Brooke Latour, PhD

**St Jude's Children's Hospital**
Heather Mefford, MD, PhD
Esmat Fathi, PhD

**McGill University**
Ken Myers, MD, PhD
Ahmed Sahly, MD, PhD

**Istituto di Medicina Genomica**
Marcella Zollino, PhD

**UC - San Francisco**
Helen Willsey, PhD

**Murdoch University**
Angela Morgan, PhD
Olivia Van Reyk, PhD
Miya St John, PhD

**Emory University**
Victor Faundez, PhD

**Tel Aviv University**
Daphna Landau Prat, MD

**Université de Strasbourg**
Yann Herault, PhD

**University of Newcastle**
Tracy Dudding, MD, PhD

**University of Amsterdam**
Frank Jacobs, PhD
Colette Moses, PhD
Conceptual Model of KdVS

*Draft* model based on literature review, interview with 1-2 caregivers, and discussions with KOLs

Goal of a conceptual model is to create an outline of symptoms and their impacts on patients and caregivers.

A formal conceptual model study requires in-depth open-ended interviews of wide sample of caregivers.

A conceptual model forms the basis for Natural History studies, standards of care, clinical outcome measures, and clinical trial endpoints.
Conceptual Model of KdVS

Complex seizure/epilepsy profile\textsuperscript{8-10}
- \(\sim 65\%\) status epilepticus\textsuperscript{8}

Seizure types reported:
- Focal impaired awareness seizure (60%)
- Generalized tonic-clonic seizures (25%)
- Continuous spike-waves in sleep (CSWS) (20%)
- Drop attacks (10%)
- Absence with eyelid myoclonia (10%)
- Tonic seizures (7%)
- Febrile seizures (7%)
Natural History Studies for KdVS

Gen-IDA (Dr. Mendel)
- International study of patients with genetic causes of ID and ASD
- cohort ~44 KdVS patients
- unique surveys
- data: accessible to approved researchers

Rare-X (Charlene Son Rigby)
- cohort of ~12 KdVS patients
- ClinGen surveys (validated in select neurodevelopmental populations)
- biorepository (biofluids, iPSCs)
- data: accessible to approved researchers

Simons Searchlight (Wendy Chung)
- cohort of ~3 KdVS patients
- validated surveys in neurodevelopmental populations
- biorepository (generation of iPSC models)
- data accessible to approved researchers
### Disease Concepts for KdVS

<table>
<thead>
<tr>
<th>Domain</th>
<th>Symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological</td>
<td>Epilepsy</td>
</tr>
<tr>
<td></td>
<td>Abnormal EEG/MRI</td>
</tr>
<tr>
<td></td>
<td>Status epilepticus</td>
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<tr>
<td></td>
<td>Febrile seizures</td>
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<tr>
<td></td>
<td>ESES</td>
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<tr>
<td></td>
<td>Hypotonia/Hypertonia</td>
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<tr>
<td></td>
<td>Tremors</td>
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<tr>
<td></td>
<td>Autonomic storms</td>
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<tr>
<td></td>
<td>Ataxia</td>
</tr>
<tr>
<td>Communication</td>
<td>Apraxia</td>
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<tr>
<td></td>
<td>Reduced phonetic inventories/ delayed speech</td>
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<tr>
<td></td>
<td>Limited babbling</td>
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<tr>
<td></td>
<td>Dysarthria</td>
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<tr>
<td></td>
<td>Reduced expressive communication skills</td>
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<tr>
<td></td>
<td>receptive language impaired</td>
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<tr>
<td></td>
<td>Echolalia</td>
</tr>
<tr>
<td>Sleep</td>
<td>Sleep deprivation (triggers seizures)</td>
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<tr>
<td></td>
<td>continuous spike-waves</td>
</tr>
</tbody>
</table>
# Disease Concepts for KdVS

<table>
<thead>
<tr>
<th>Domain</th>
<th>Symptom</th>
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<tbody>
<tr>
<td>Behavior</td>
<td>Hyperactivity</td>
</tr>
<tr>
<td></td>
<td>Impulsivity</td>
</tr>
<tr>
<td></td>
<td>Sociable</td>
</tr>
<tr>
<td></td>
<td>Anxiety / ASD or Autistic traits</td>
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<tr>
<td></td>
<td>Depression</td>
</tr>
<tr>
<td></td>
<td>Sensory difficulties</td>
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<tr>
<td>Motor</td>
<td>Impaired fine/gross motor skills</td>
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<tr>
<td></td>
<td>Delayed growth</td>
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<tr>
<td>Gastrointestinal</td>
<td>G tube / tube fed</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Dysmorphic craniofacial features, long face and bulbous nose that appear with age</td>
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<tr>
<td></td>
<td>Hypermobility</td>
</tr>
<tr>
<td></td>
<td>Septal deviation</td>
</tr>
<tr>
<td></td>
<td>Congenital heart defects</td>
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<tr>
<td></td>
<td>tracheomalacia/laryngomalacia</td>
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</tbody>
</table>
### Disease Concepts for KdVS

<table>
<thead>
<tr>
<th>Domain</th>
<th>Symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Ectodermal abnormalities</td>
</tr>
<tr>
<td></td>
<td>Abnormal hair pigmentation and texture</td>
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<td></td>
<td>Brittle nails</td>
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<td></td>
<td>Dental defects (enamel issues/spacing or shape)</td>
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<tr>
<td>Visual</td>
<td>Strabismus</td>
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<tr>
<td></td>
<td>Cataracts</td>
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<tr>
<td></td>
<td>Conductive or sensorineural hearing impairment</td>
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<tr>
<td></td>
<td>Conductive hearing loss</td>
</tr>
<tr>
<td></td>
<td>Partial blindness</td>
</tr>
<tr>
<td></td>
<td>Farsightedness</td>
</tr>
<tr>
<td>Other</td>
<td>Cryptorchidism</td>
</tr>
<tr>
<td></td>
<td>Kidney and urologic anomalies</td>
</tr>
<tr>
<td></td>
<td>Ectodermal abnormality</td>
</tr>
<tr>
<td></td>
<td>Musical interest/fascination</td>
</tr>
<tr>
<td></td>
<td>Pulmonary issues (shallow breathing)</td>
</tr>
</tbody>
</table>
Existing drug development resources

1. *In vitro models* (patient-derived)

1. *In vivo models* (zebrafish, frog, rodent)

1. Natural History/Patient Registry data
### In vitro models of KdVS

<table>
<thead>
<tr>
<th>Model</th>
<th>Where</th>
<th>Type</th>
<th>Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPSCs</td>
<td>Simons Searchlight</td>
<td><em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; Microdeletion</td>
<td>? 17q21.31</td>
</tr>
<tr>
<td></td>
<td>Simons Searchlight</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Radboud University</td>
<td><em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; <em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; <em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; Microdeletion</td>
<td>Gly178fs Gly179fs Lys180fs 17q21.31</td>
</tr>
<tr>
<td></td>
<td>COMBINEDBrain</td>
<td><em>KANSL1</em>&lt;sup&gt;-/+&lt;/sup&gt; Microdeletion</td>
<td>17q21.31</td>
</tr>
<tr>
<td>Neurons</td>
<td>Radboud University</td>
<td><em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; <em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; <em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; Microdeletion</td>
<td>Gly178fs Gly179fs Lys180fs 17q21.31</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Radboud University</td>
<td><em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; <em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; <em>KANSL1</em>&lt;sup&gt;-/-&lt;/sup&gt; Microdeletion</td>
<td>Gly178fs Gly179fs Lys180fs 17q21.31</td>
</tr>
<tr>
<td></td>
<td>COMBINEDBrain</td>
<td>Microdeletion</td>
<td>17q21.31</td>
</tr>
</tbody>
</table>
### In vivo models of KdVS

<table>
<thead>
<tr>
<th>Model</th>
<th>Where</th>
<th>Type</th>
<th>Genetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Herault (IGBMC, France)</td>
<td>17q.21.31 del (del) 17q.21.31 dup (dup) Kansl1-/+</td>
<td>Chr11:104,081,561-104,534,288 Exon 2 deletion</td>
</tr>
<tr>
<td>Mouse</td>
<td>Pan (Beijing, China)</td>
<td>Kansl1 lox-cre</td>
<td>genetic loci unknown</td>
</tr>
<tr>
<td>Frog</td>
<td>Willsey (UCSF, United States)</td>
<td>Kansl1-/+</td>
<td>sgRNA against exon 3</td>
</tr>
<tr>
<td>Drosophila</td>
<td>Schenck &amp; Kramer (Nijmegen, Netherlands)</td>
<td>Dmel/nsl1-/+ (Kansl1 orthologue)</td>
<td>Several RNAi constructs Kansl1/3 3FLAG/HA tags</td>
</tr>
</tbody>
</table>
Types of treatment strategies

Re-purposed drugs
Focus on FDA-approved compounds that can restore 1) cellular phenotypes and 2) \textit{in vivo} phenotypes

Cellular phenotypes: autophagy, increased oxidative stress, impaired synapse formation

\textit{Linda et al} 2022
Types of treatment strategies

Re-purposed drugs
Focus on FDA-approved compounds that can restore 1) cellular phenotypes and 2) in vivo phenotypes

Cellular phenotypes: impaired mitosis (impaired mitotic spindle formation)

Meunier et al 2015
Types of treatment strategies

Re-purposed drugs
Focus on FDA-approved compounds that can restore 1) cellular phenotypes and 2) *in vivo* phenotypes

*In vivo* phenotypes: abnormal brain development (frog, mouse), behavioral deficits

Koolen-de Vries Syndrome Foundation

Rosenthal et al 2021

Arbogast et al 2017

Arbogast et al 2017

Arbogast et al 2017

Arbogast et al 2017
Types of treatment strategies

Re-purposed drugs
Focus on FDA-approved compounds that can restore 1) cellular phenotypes and 2) *in vivo* phenotypes

*In vivo* phenotypes: abnormal brain development (frog, mouse), behavioral deficits
- Rescue of synaptic and behavioral phenotype with retinoic acid

Pan et al 2022
Types of treatment strategies

Genetic therapies attractive because KdVS is primarily a haploinsufficiency disorder

Gene replacement is feasible

Size of coding sequence: 3315 bases

AAVs cargo capacity ~4000-5000 bases

Another strategy: leverage endogenous regulators of KANSL1 expression

- KANSL1-AS strand
- KANSL2 / KANSL3 compensatory mechanism

| Kansl1 size by mRNA, coding sequence and protein |
|---------------------------------|----------|
| Template                        | Size     |
| mRNA (bases)                    | 195,474  |
| Exons (total/coding)            | 15       |
| Coding sequence (bases)         | 3315     |
| Protein (amino acids)           | 1105     |
| Transcripts (protein coding)    | 14       |
| Variants (SNP*)                 | 71       |
| Cell-specificity                | low      |
Resources and Gaps in KdVS Drug Development

**Disorder Identification**
- Active KdVS Fdn
- KdVS Patient Registry
  - Find patients
  - Faster diagnoses
- Mouse/frog/drosophila
  - Inducible mouse model
  - High throughput drug screens
  - Downstream targeting
- IPSCs Simons
  - Plasma/CSF biomarker
  - EEG Signature
  - ERP Biomarker

**Drug Discovery**
- High-Throughput screens
- Translational research:
  - In vitro -> In vivo
  - Gene Therapy/
  - Gene Replacement
  - Gene manipulation

**Drug Development**
- Conceptual Model
  - ICD-10 Code
  - Standard of Care
- Registry/Natural History
  - Expand to Clinical Site
  - Coordinate data collection/pool
  - International Natural History Study
  - Simons Searchlight Collaboration (iPSCs)
  - Facilitate publication of Gen-IDA data
  - Quarterly Research Roundtables

**Drug Approval**
- Future FDA Listening Session
- ORCA Validation
  - Seizure outcomes
  - Community priorities
- Future Endpoint working group

**Disorders - Modeling - Phenotyping - Treatment Strategy - Collaboration - Natural History - BOMs - Agency - Trials**
Types of recommendations for KdVS Foundation

**Patient Engagement Projects** are those which bring more attention to the disorder, increase the number of families involved in activities, educate families about the disorder, and empower families to advocate for their child and connect with others in the field. They may not require funding.

**Collaboration Projects** are those in which the KdVS Foundation can work with others who are planning or implementing projects which fill gaps in the drug development process. They may or may not require additional funding.

**Funding Projects** are those in which the KdVS Foundation can use its own funds to encourage projects which fill gaps in the drug development process. They require funding by definition.

**Strategic Decisions** are guidelines for choosing projects which build capacity for the entire field.
Recommendations for KdVSF: Patient Engagement

Patient Engagement Projects: Find patients and get them involved

- KdVS Foundation Contact Registry
  - Encourage all families to obtain a Clinical Research ID (CRID)
  - Patient /caregiver priority assessment(s)
    - Priority symptoms -> outcome measures to assess

- Participate in current Natural History Studies:
  - Rare-X, Simons, Gen-IDA

- Establish an international Natural History Study (i.e. CureGRIN Foundation)
  - Select sites in Europe, Australia, Canada, United States and South America
  - Standardized set of surveys

- Diagnose patients faster
  - Add KdVS to all relevant gene panels
  - Add KdVS to BegiNGS screen
  - Leverage Probably Genetic and FaceMatch to identify patients

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Recommendations KdVSF: Patient Engagement/Funding Projects

Biomarker development:

- Expand samples in publicly available Biorepositories (Coriell, Simons, COMBINEDBrain)
  - Biosamples focused on biomarker development
    - Collect biofluids (urine, blood, cerebrospinal fluid)
    - Cells (PBMCs, fibroblasts, iPSCs)
    - Tissue collections
  - Encourage biosample donations from international community
  - Coordinate biofluid collection at next family meeting in 2023
  - KdVS Foundation fund the initial collection and storage of samples

- Collect EEGs and ERPs
  - Provide seed funding to evaluate EEGs and ERPs as biomarker
  - Make EEGs and ERPs available in central repository
  - Link EEG/ERP to natural history data and biosamples

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Recommendations KdVSF: Collaborations

- Share established patient-derived models in vitro in a central repository.
- Share established animal models to validate high-throughput screens and validate recent work in rodents (retinoic acid)
- Collaborate with other rare disease groups with overlapping pathogenic mechanisms (ie, chromatin modifier, epigenome modifiers, impaired autophagy) to propose basket basic research experiments
- Host annual international KdVS Scientific and Family Summit
- Coordinate quarterly KdVS research symposium with KOLs to:
  - facilitate translational collaboration
  - select natural history sites
  - expedite first pilot clinical trials

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Recommendations KdVSF: Funding clinical projects

Outcome Measure Development:

- Fund the development of a Final Conceptual Model of KdVS
  - Qualitative interviews of caregivers/clinicians/educators to generate an unbiased patient profile
  - Submit study manuscript for publication
  - Articulate relevant surveys for Natural History Studies

- Coordinate a study to assess patient community priorities
  - Based on findings of Conceptual Model
  - Identify priority treatment targets

- Validate Observer Reported Communication Ability (ORCA) in KdVS
  - build on Simons data with qualitative interviews

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Recommendations KdVSF: Fund basic science with seed grants

● In vivo experiments:
  ○ Complete a metabolomic/proteomic study in brain tissue/blood of microdeletion/Kansl1 variant rodents
  ○ Develop an inducible/conditional model to identify therapeutic window
  ○ Treat with “hits” from repurposed compound screen in in vitro model
  ○ Replicate rescue effects of Retinoic Acid exposure in Kansl1 (and del) model
  ○ Identify novel acetylation targets of Kansl1 (lessons from CHD2-RD)

● In vitro experiments:
  ○ Identify episignature of KdVS as biomarker
  ○ Potential to use read-through technology (Ataluren) to restore phenotypes in nonsense variants;
  ○ Potential to suppress KANSL1-AS expression to increase expression of KANSL1
    ■ Potential for KANSL1/KANSL2/KANSL3 to compensate for KANSL1 knock-down ability of gene replacement for KANSL1 (AAVs)

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Strategic Decisions are guidelines for choosing projects which build capacity for the field

- Establish research priorities and allocate funding
  - Clinical characterization (40%)
  - Curative therapies (40%)
  - Disease modifying therapies (20%)

- Establish a formal Request for Proposal (RFP) program
  - Call for applications to address strategic gaps and research priorities
    - Seed grants to targeted recipients
  - Formal outside pre-review (excluding applicants)
  - Anonymous scoring rubric (NIH format)

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Figure 1: Mutations causal for Koolen-de Vries Syndrome. Koolen-de Vries Syndrome (KdVS) is caused by either a microdeletion in region 17q21.31 encompassing at least five genes (top), or by a mutation in the KANSL1 gene (bottom). KANSL1 mutations identified in various KdVS individuals are shown. The exons of the KANSL1 gene are shown in white, the untranslated region (UTR) in grey, and the introns as black lines. The figure is adapted from Koolen et al. (2016)\textsuperscript{67}. 