Insights from the NIH Undiagnosed Diseases Network

World Rare Disease Day Symposium

University of Michigan
February 25, 2022

William A. Gahl, MD, PhD
Director, NIH Undiagnosed Diseases Program

Acknowledgments

Director of Pediatric UDP: Cynthia Tifft, MD, PhD

Director of Bioinformatics: David Adams, MD, PhD

UDP Chief Neurologist: Camilo Toro, MD

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50-100 dedicated support personnel and volunteer consultants at NIH.

Kind and collaborative patients and families!

UDP
(May 19, 2008)

- **Goals:**
  - **To assist patients with unknown disorders reach an accurate diagnosis**
  - **To discover new diseases that provide insight into human physiology and genetics**

Intramural UDP Operations

- Applicants submit medical records
- Referring physician sends summary letter
- UDP Director triages submitted records
- Intramural NIH consultants review records
- UDP Director makes final disposition
- Patients/physicians receive a standard letter; advice conferred in ~25% of cases
- If accepted, 1-week inpatient CC admission

UDP Investigations

1. Customized (Personalized) patient phenotyping to rule out known diseases.
2. Genetic studies
   a. Commercial testing
   b. SNP arrays
   c. Exome and genome sequencing
3. Functional studies (assays, model systems)

UDP Numbers

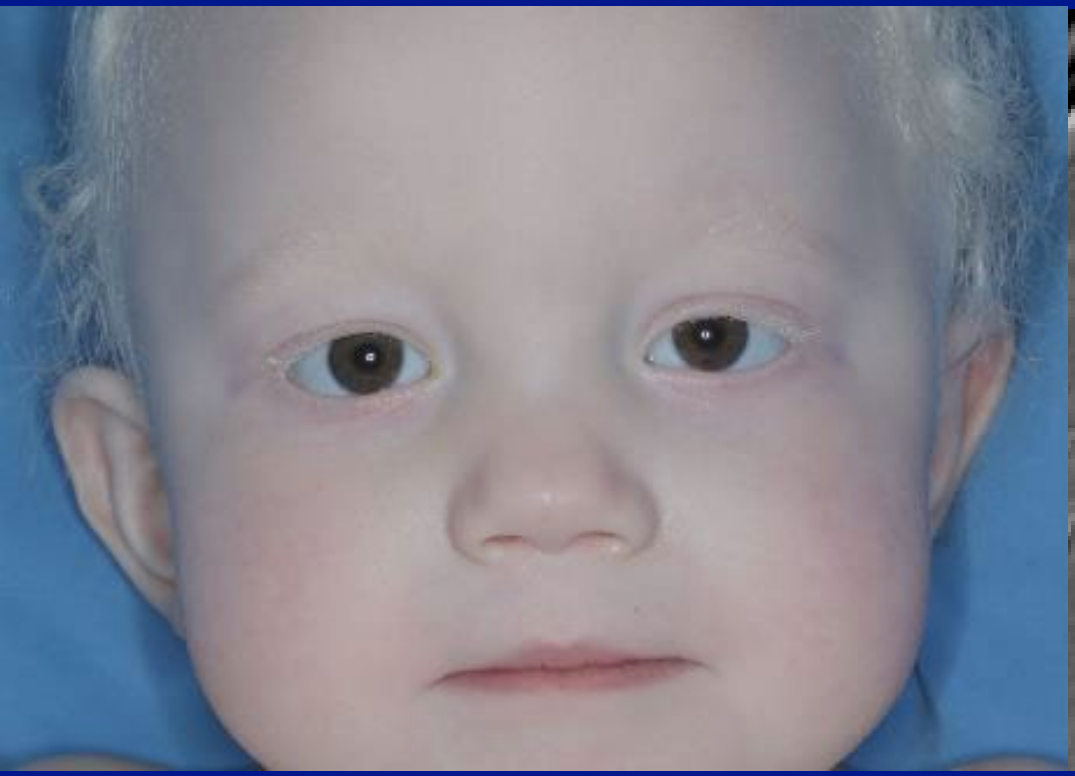
- Medical Records: >4500
- Admitted & Evaluated: ~1500
- Children: ~40%
- Neurological: ~50%
- Publications >190
- Some diagnosis: ~30%

Discovery

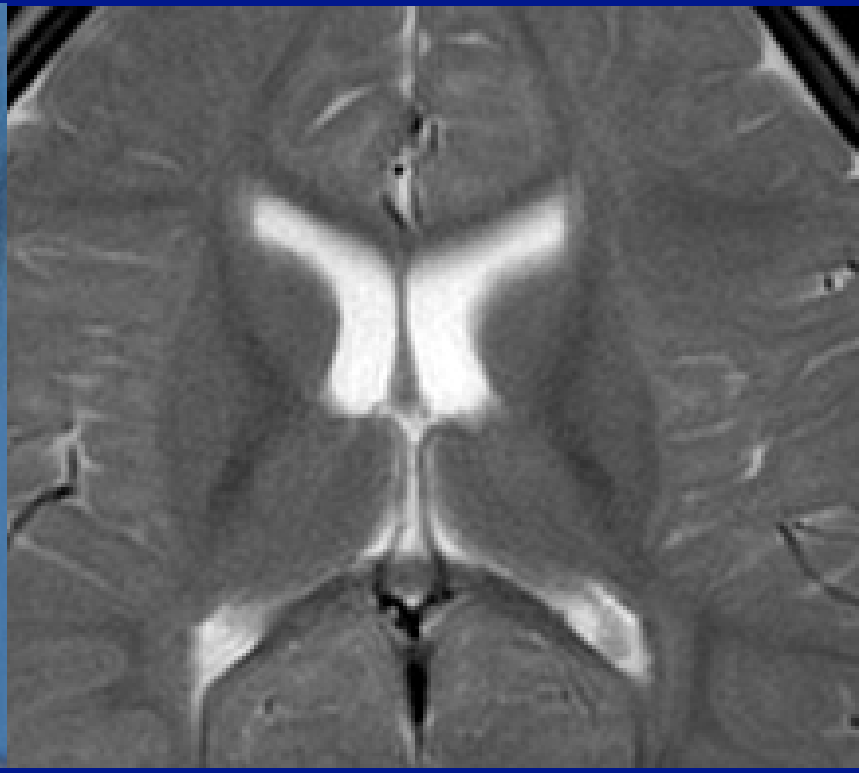
UDP 10898

- 18 month female; failure to thrive 2.5 mo
- Intestinal dysmotility; TPN dependent
- Cutaneous hypopigmentation; poor visual acuity
- Hepatosplenomegaly; nephromegaly
- Storage in liver, duodenum, colon
- Hypotonia; severe developmental delay
- Brain MRI: Delayed myelination
- Frequent respiratory infections and UTIs
- NO osteopetrosis

18 months

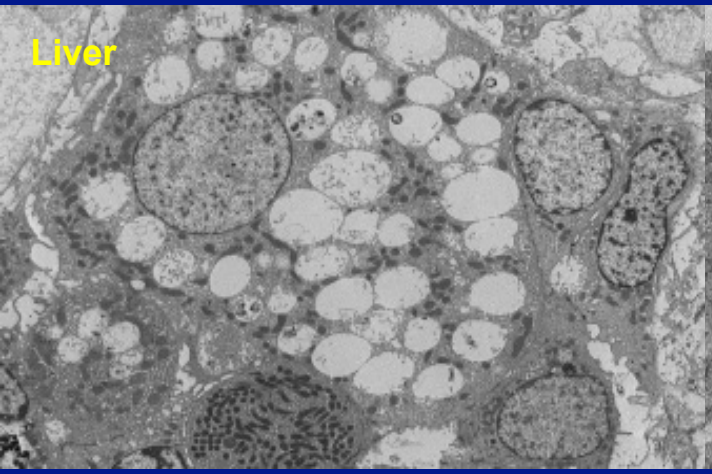


Delayed Myelination

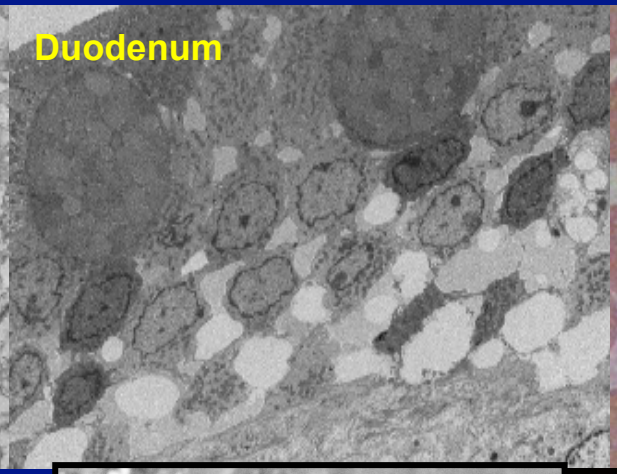


Dr. Austin Larson, Children's Hospital of Colorado

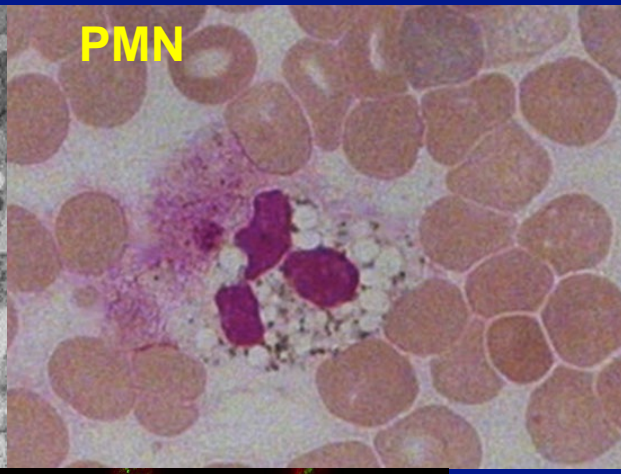
Foamy Histiocytes



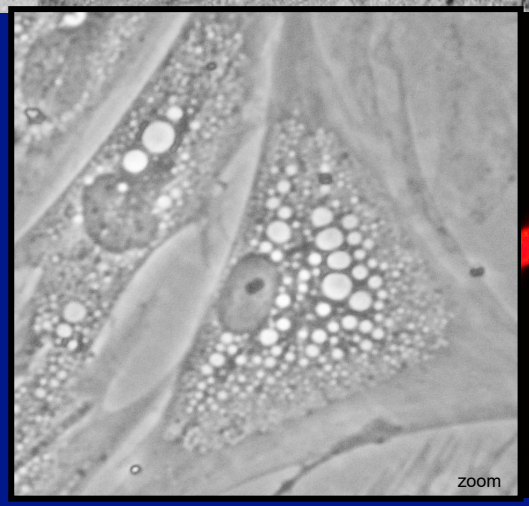
Cell Inclusions

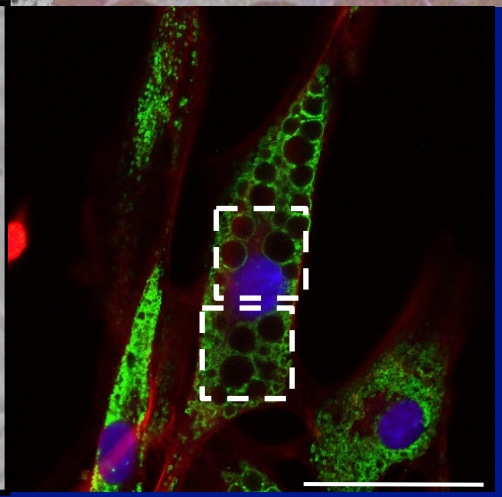


Vacuoles



Fibroblast Vacuoles

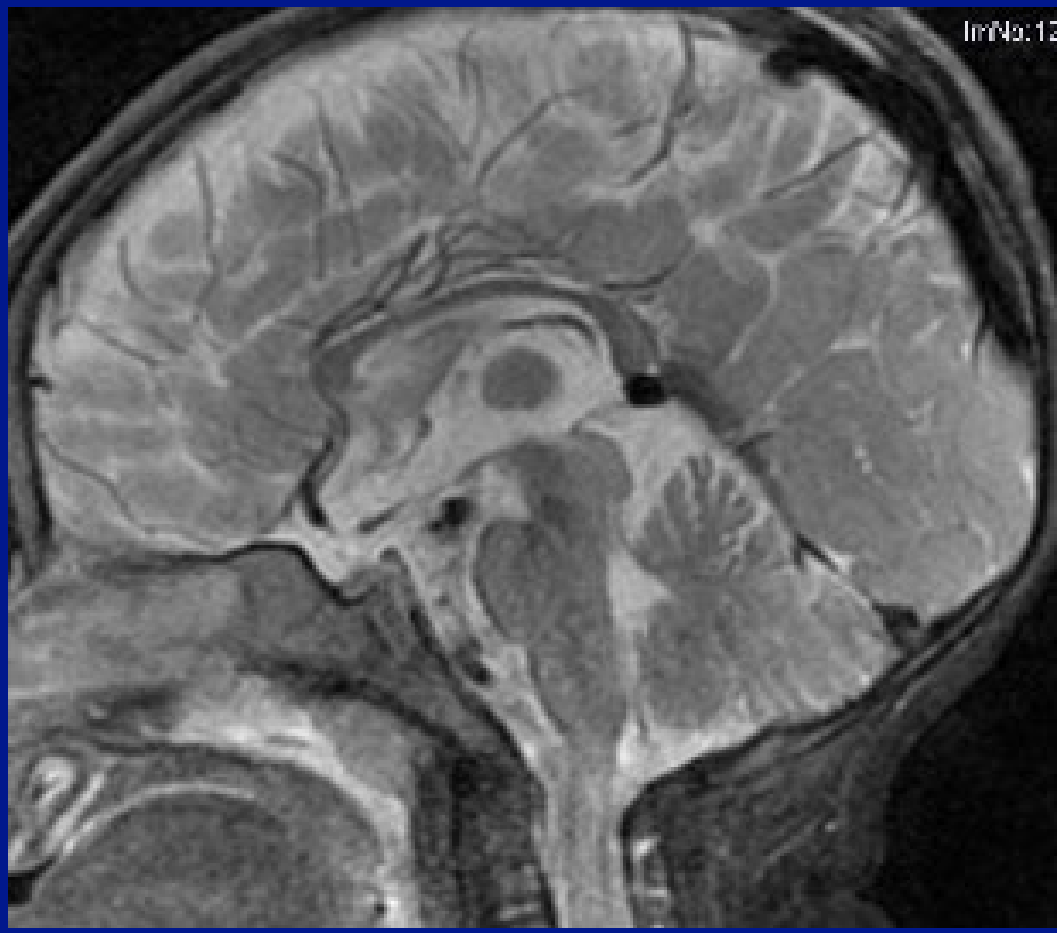




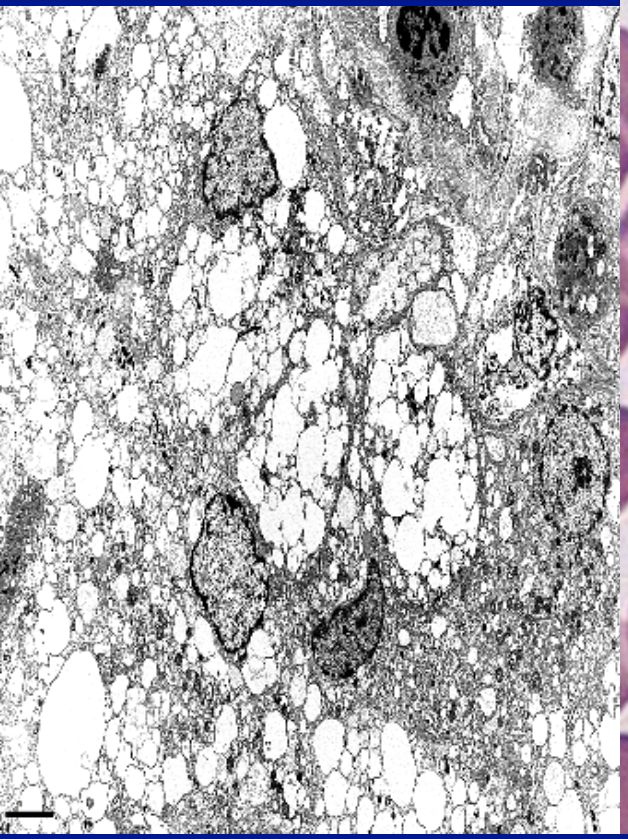
Patient 2

- 14 month Ghanaian male
- Cutaneous hypopigmentation
- Hepatosplenomegaly; nephromegaly
- Storage documented in kidney
- Developmental delay
- Brain MRI: Delayed myelination
- Hearing loss
- NO osteopetrosis

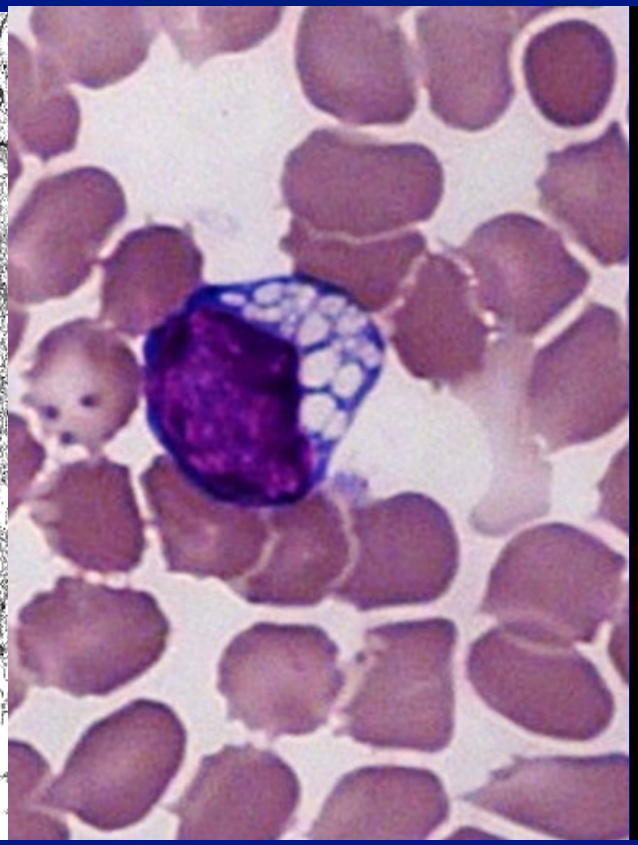
Delayed Myelination



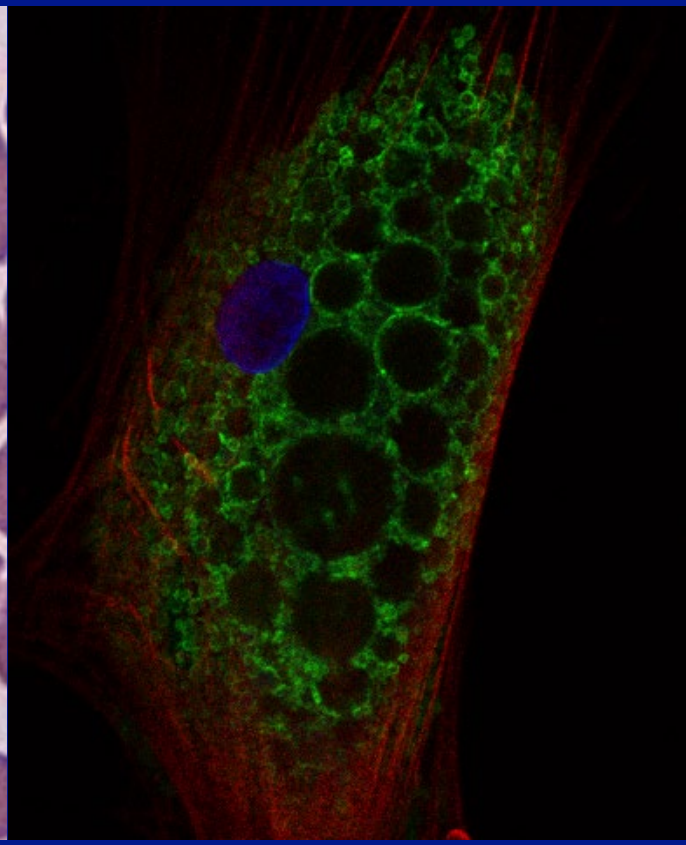
Kidney Macrophages Inclusions



Lymphocyte Inclusions

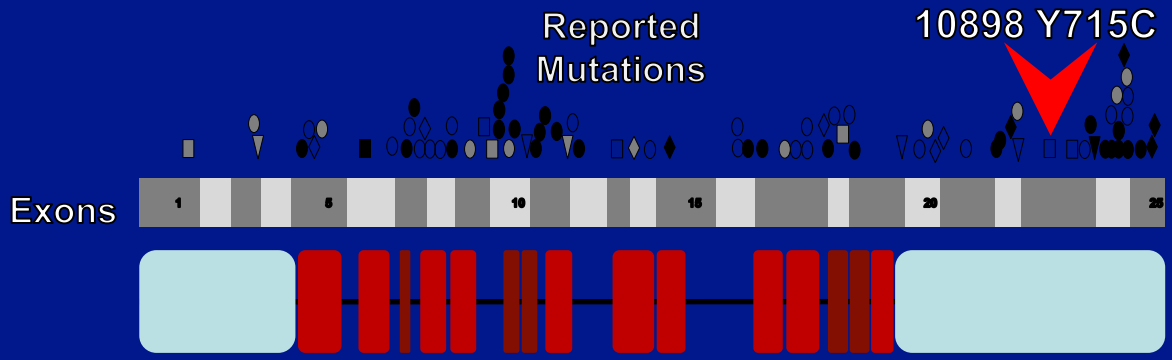


Fibroblast Vacuoles



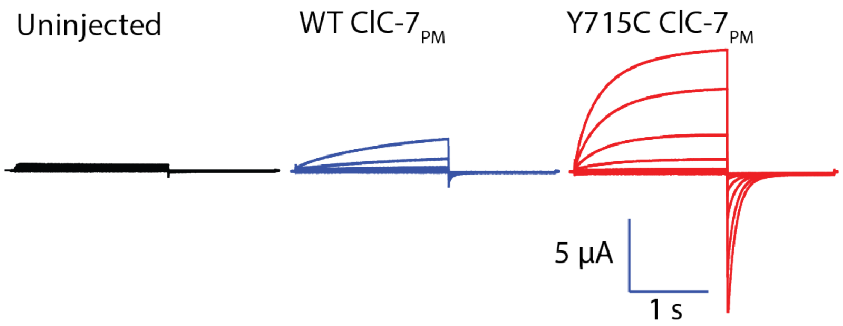
Exome Analysis

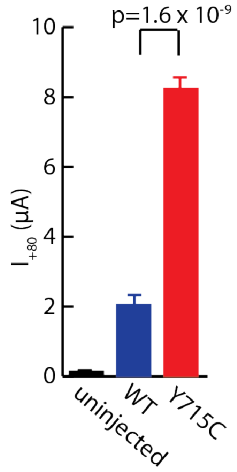
- BOTH children have a *de novo* mutation in *CLCN7; i.e.*, c.2144G>A; p.Y715C
- CLCN7: chloride transporter on late endosome/lysosome
- Chloride balances the protons that acidify the lysosome
- CLCN7 loss of function mutations cause osteopetrosis
- Our patients have one normal allele & NO osteopetrosis
- ?Gain of function mutation?



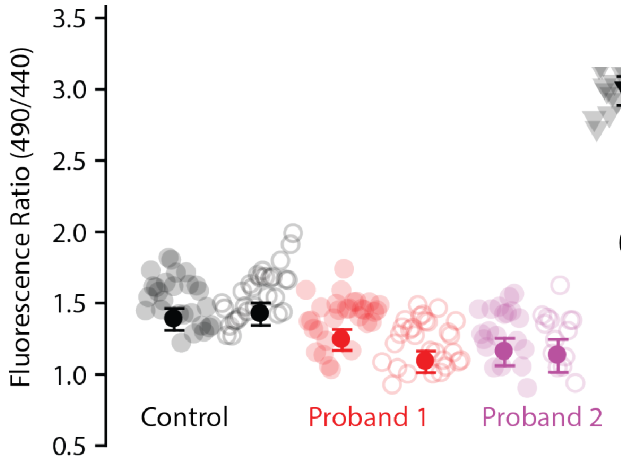
Alissa Becerril, Joseph Mindell, NINDS

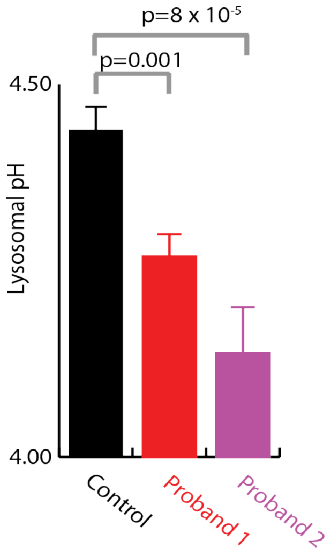
Chloride Currents in Xenopus Oocytes Expressing Wild-type and Y715C ClC-7





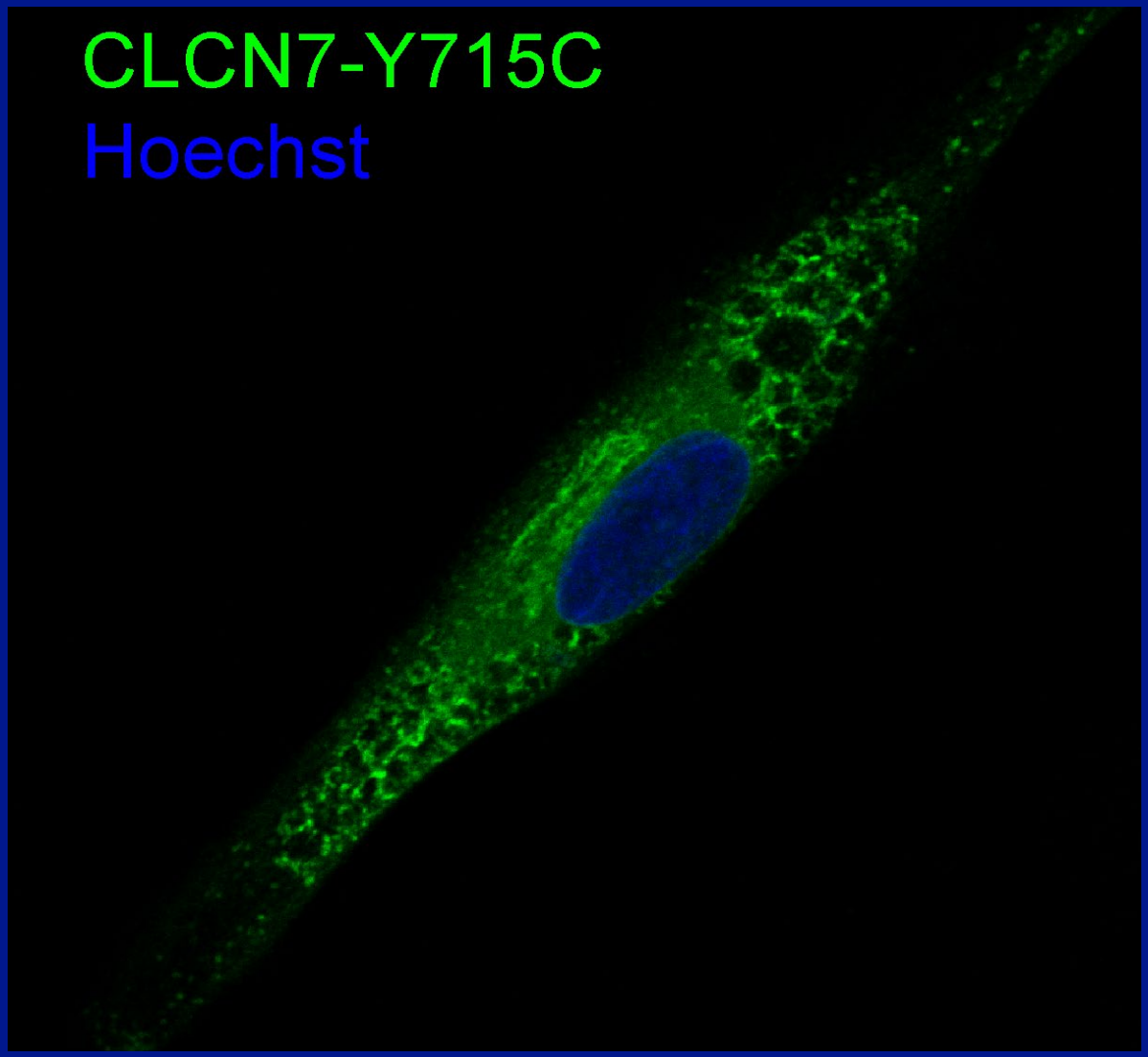
Oregon Green Fluorescence Measuring Fibroblast Lysosomal pH





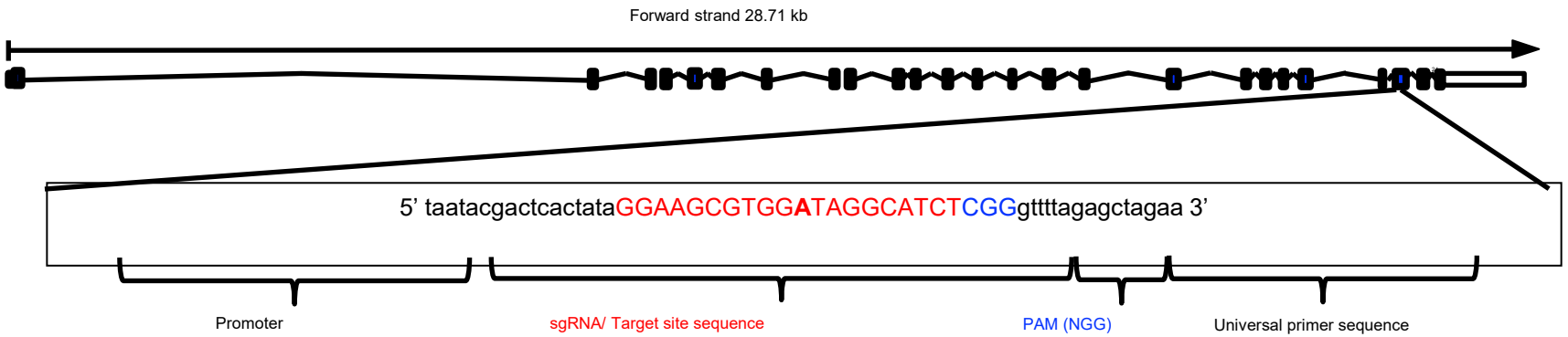
Mary Weston, Joseph Mindell, NINDS

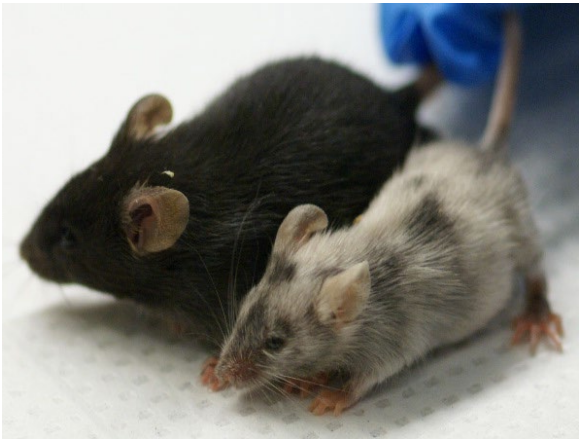
Wild-type Fibroblasts Transfected with CLCN7-Y715C Recapitulate the Patients' Cellular Phenotype: Large Cytoplasmic Vacuoles

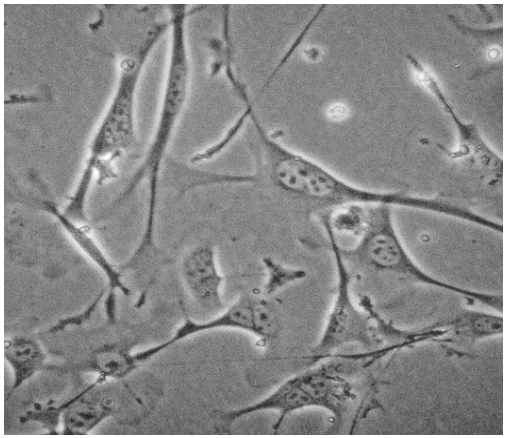


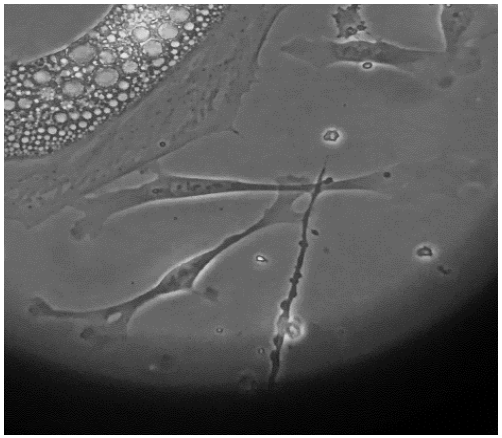
Mary Hackbarth

A Mouse with Knock-in of the Human CICN7 Y715C Paralogue (Y713C) Mimics the Human Phenotype

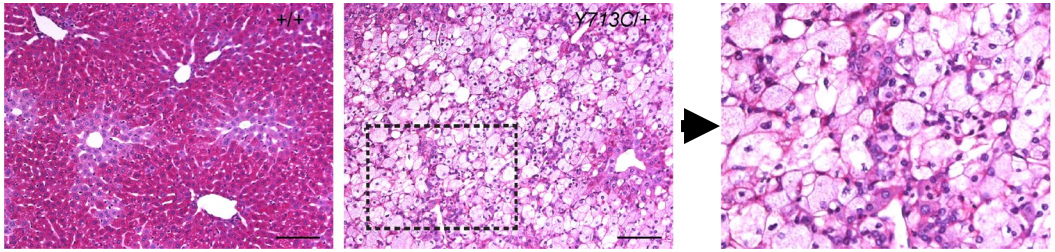








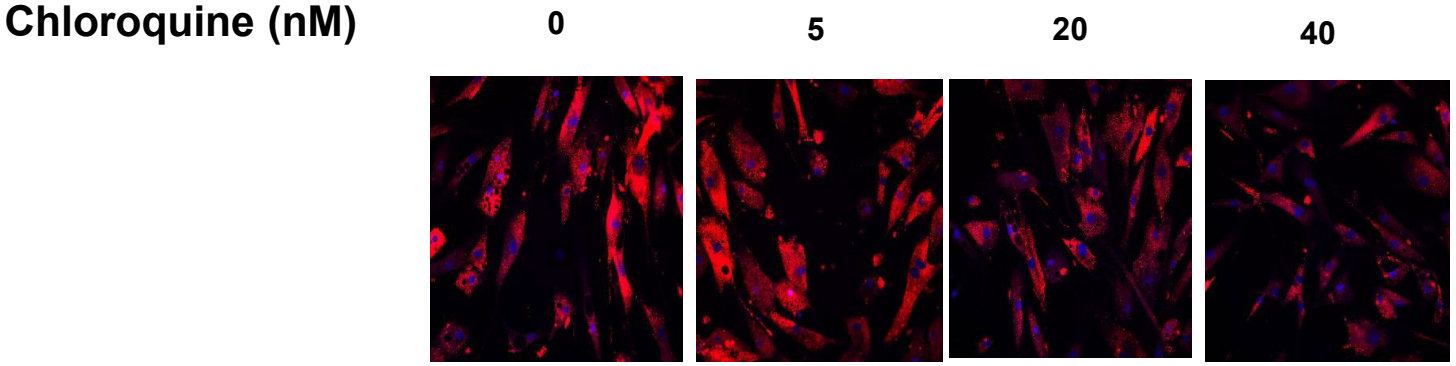
Liver Pathology



Normal **Y713C** **Zoom**

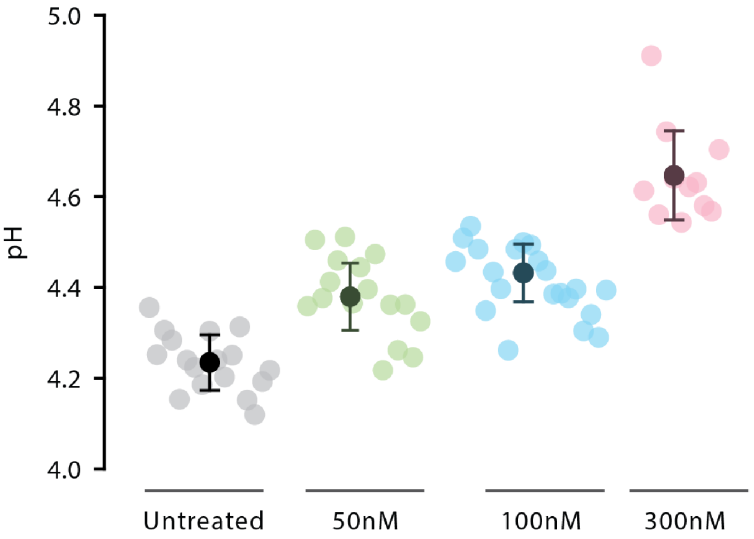
Ralu Nicoli, May Malicdan

Chloroquine Alkalinizes Endosomal and Lysosomal Compartments



LysoTracker Red Staining

pH Determined by Oregon Green Fluorescence Ratio



Alissa Becerril, Joseph Mindell

Chloroquine Treatment

- Dr. Debra Day-Salvatore, St. Peter's Hospital, New Brunswick, NJ
- IRB-approved protocol; written informed consent
- 1 or 2 mg/kg weekly chloroquine G-tube
- Chitotriosidase fell 26% (5690 to 4184 nmol/h/ml)
- Decreased kidney size on ultrasound
- Increased energy and activity
- Both children eventually succumbed.

New Disease-Gene Associations as of 2019

1. Arterial calcifications	NT5E
2. Spastic paraplegia, spinocerebellar ataxia	AFG3L2
3. Skin/skeletal lesions, FGF23 abnormal	NRAS
4. Upregulated interferon signaling	IFIH1
5. Stroke and vasculopathy	ADA2
6. Epileptic encephalopathy	AARS
7. Ablepharon macrostomia	TWIST2
8. York Platelet Syndrome	STIM1
9. Developmental delays	CAD
10. Cirrhosis, developmental delays	PP1R15B
11. Dystonia	KMT2B
12. Neurodevelopmental disorder	EBF3
13. Mitochondrial encephalopathy	TIMM50
14. Developmental and growth delays	GARS
15. Infantile parkinsonism	WARS
16. Developmental neuroregression	UBTF
17. Saul-Wilson syndrome	COG4
18. Microcephaly, seizures, cerebral atrophy	VARS
19. Developmental delays, dysmorphisms	TRAF7
20. Delays, cardiac defects, dysmorphisms	TMEM94
21. Delays, hair & liver defects, dysmorphisms	CCDC47
22. Neuropathy, ataxia, dystonia	COX20
23. Delays, microcephaly, brittle hair & nails	CARS

Rare Diagnoses

- Kearns-Sayre with cerebral folate deficiency
- Neuroaxonal dystrophy with spheroids
- Call-Fleming syndrome (vascular strokes)
- CSF tetrahydrobiopterin deficiency
- Spastic paraplegia due to *SPG7* mutations
- Hereditary Spastic Paraplegia with *SPG4* muts
- Stargardt's due to *ABCA4* mutations
- Noonan syndrome due to *PTEN* mutation
- Amyotrophic lateral sclerosis with *SOD1* mut
- GM1 gangliosidosis due to *GLB1* mutations
- Progressive supranuclear palsy
- Joubert syndrome

Very Rare Diagnoses

- Telomerase deficiency
- IgG4 sclerosing fibrosis
- Anti-synthetase syndrome
- *NOD2* mutations (father & child)
- *FOXG1* mutation in 2 year old
- Dejerine-Sottas syndrome/hypertrophic neuro
- *POLG1* in late-onset ataxia
- *DNAH1* ciliopathy
- SLE with cerebellar ataxia and anti-GWB Abs
- Smith-Magenis syndrome with *RAI1* mutation
- Pitt-Hopkins syndrome with *TCF4* mutation
- Amyloid myopathy
- Dystonia, dysarthria due to *ND3* mito mut

Very Very Rare Diagnoses

- Myoclonus epilepsy without renal failure – due to *SCARB2* mutations (5 in world)
- Ichthyosis Follicularis with Atrichia and Photophobia (IFAP) with *MBTPS2* mutations (6 families in world)
- Neurodegeneration with brain iron due to c19orf12 mutations (20 families)
- ALS-Frontotemporal Dementia due to c9orf72 expansion
- Cytosolic PEPCK deficiency due to *PCK1* muts
- *KDCT7* in two sibs with ataxia, Sz (2 families)
- Nephrolithiasis & 24-hydroxylase deficiency (few families)

Very Very Rare Diagnoses

- Congenital Disorder of Glycosylation type 2b (2nd and 3rd cases in world then)
- Adducted Thumb-Clubfoot Syndrome & *CHST14* mutations (1st case in U.S.)
- Spinocerebellar ataxia, myoclonic epilepsy & *AFG3L2* muts (1st AR case)
- Autosomal Dominant Leukodystrophy & *LMNB1* duplication (~10 in world then)
- Adenylosuccinate lyase def. (~60 cases)
- Hereditary Muscular Neuropathy type 6 due to *IGHMBP2* muts (oldest pt. known)
- Fatty acid 2-hydroxylase def. (~50 cases)

More Diagnoses - 1

- Leukodystrophy & spheroids with CSFR1 muts
- Leucoencephalopathy, Calcifications, and Cysts due to SNORD118 mutations
- Oculodentodigital Dysplasia due to GJA1 mutation
- Dysmorphisms & delays due to TRAF7 mutation
- Microcephaly, dysmorphisms, autism spectrum due to CTNNB1 de novo mutation
- Dysmorphisms & delays due to KMT5B de novo
- Vomiting, ITP, delays due to DDX3X mutation
- Neu-Laxova Syndrome 2 due to PSAT1 muts.

More Diagnoses - 2

- Tremor and spasticity due to *GAN* de novo mutation
- Connective tissue and GI disorder due to *TUBB2B* de novo
- Mitochondrial disorder due to *MTATP6* mutation
- Kleefstra Syndrome due to *EHMT1* de novo
- Fahr's due to *SLC20A2* mutations
- XMEN (X-linked immunodef, EBV infection, neoplasia due to *MAGT1* mutation
- Relapsing polychondritis
- Hereditary Spastic Paraplegia 76 & *CAPN1* muts
- AR Limb-Girdle MD 2Z due to *POGLUT1* muts

More Diagnoses - 3

- Leigh syndrome and mitochondrial complex I deficiency due to biallelic *NDUFAF6* mutations
- SMA type II-III with no *SMN1* and 3 copies of *SMN2*
- VEXAS (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic) in several patients with somatic mutations in *UBA1*
- Dysmorphisms, hepatitis, bruising, lipodystrophy due to *C1r* (complement subcomponent) mutation
- Late-onset metachromatic leukodystrophy
- 15 year old boy with ataxia, dysarthria, weakness due to a de novo *IRF2BPL* mutation
- Sanfilippo C with only neurodegeneration

More Diagnoses - 4

- Neurodegeneration due to bariatric surgery and methotrexate treatment causing folate deficiency
- Demyelinating peripheral neuropathy, CMT-like, due to a de novo *POLR3B* mutation
- Cardiac abnormalities and dysmorphisms due to iduronidase deficiency (mild Hurler syndrome)
- Sensorimotor neuropathy due to AAGGG expansions in the *RFC1* gene
- Developmental and intellectual delays, ataxia, dysarthria, seizures due to a *DNML1* mutation
- Autoimmune polyglandular syndrome type 2 due to a de novo *BACH2* mutation

Treatable Diagnoses

65 Year-old Man with Recurrent Meningitis

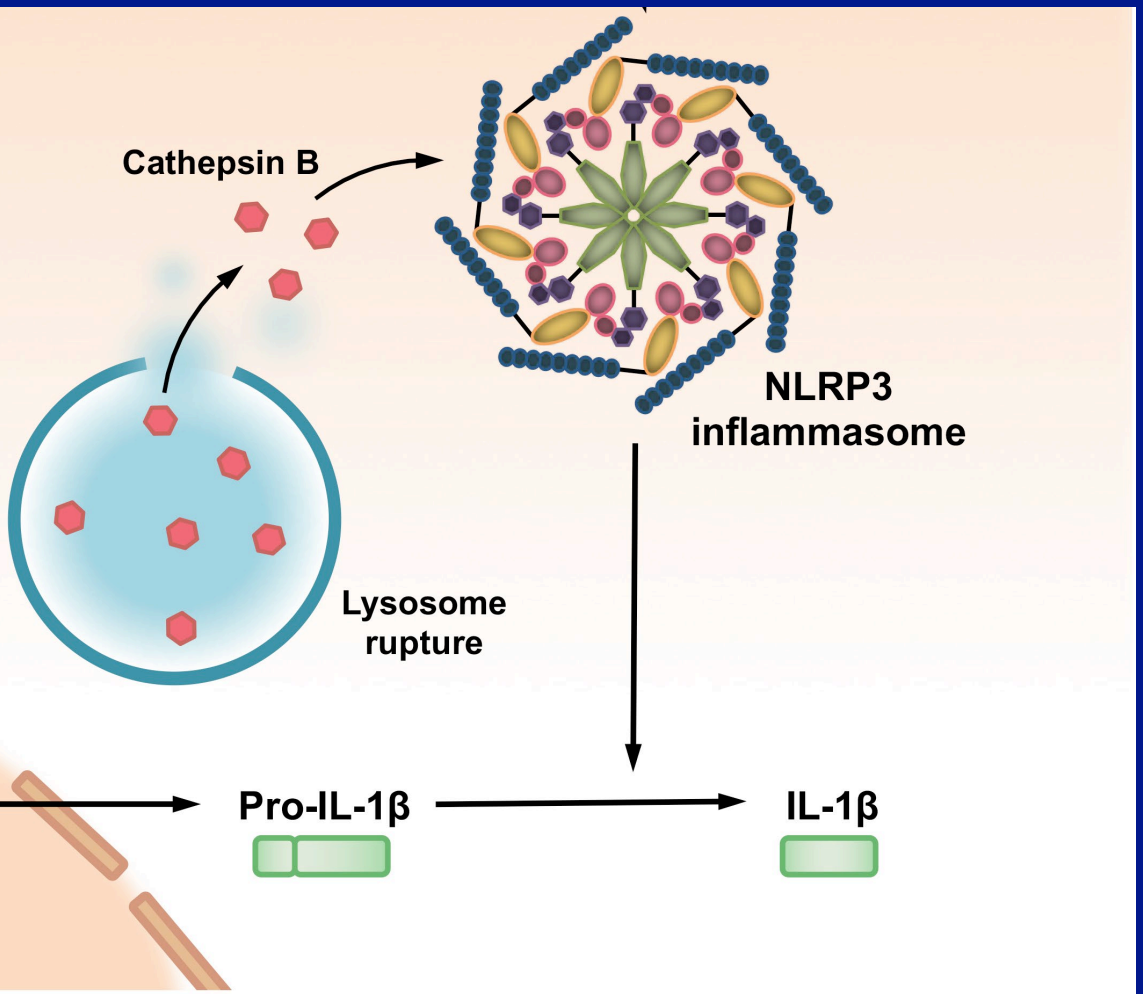
- Age 59 - First episode of meningitis; followed by autoimmune sensorineural hearing loss
- Acute: Headache, unsteady gait
- Chronic: Uses wheelchair, memory decline
- Age 59-65 – 27 more episodes
- LPs: Lymphocytic pleocytosis
- Aseptic; steroid responsive
- Negative imaging & rheumatology evaluation
- Normal labs, including CRP, ESR

65 Year-old Man with Recurrent Meningitis

- Exomes: Thr915Met in NLRP3
- NLRP3: Familial cold autoinflammatory syndrome or Muckle-Wells syndrome
- Heterozygous; gain of function

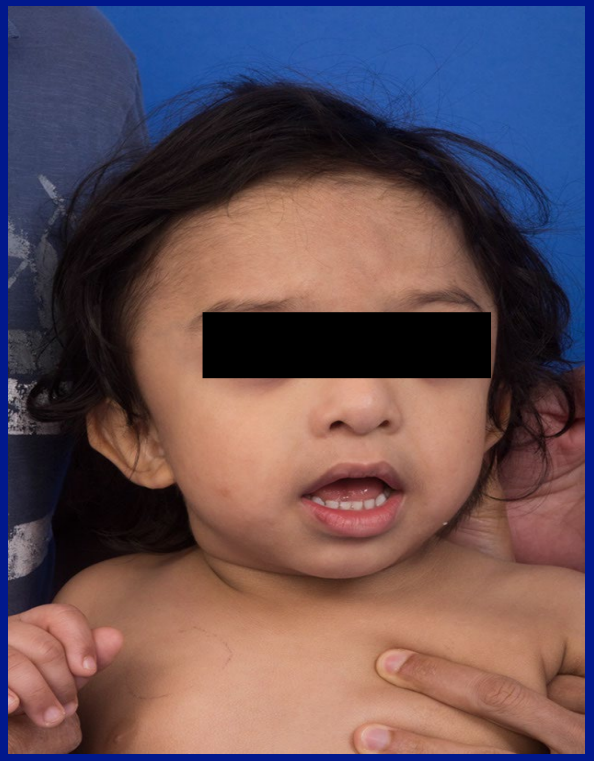
Donna Novacic, MD

- NLRP3 is part of the Inflammasome.
- A gain of function mutation will increase IL-1 activity.



- We treated with the IL-1 receptor inhibitor, anakinra
- In 4 hours, he walked and talked normally

UDP 11763: 3 Year-old Boy with Irritability



- Global developmental delays
- Macrocephaly (>95%)
- Short stature (<5%)
- Dysmorphic features
- Dysplastic pulmonic valve
- **Irritable, aggressive & injurious behavior**
- Optic atrophy
- Interstitial lung disease
- CSF Tetrahydrobiopterin 8 (18-50 nmol/L)

Precilla D'Souza, Cynthia Tifft

Genetics

Negative or Normal

- *PIK3CA, NRAS,* Noonan Spectrum Panel, Chromosome Analysis

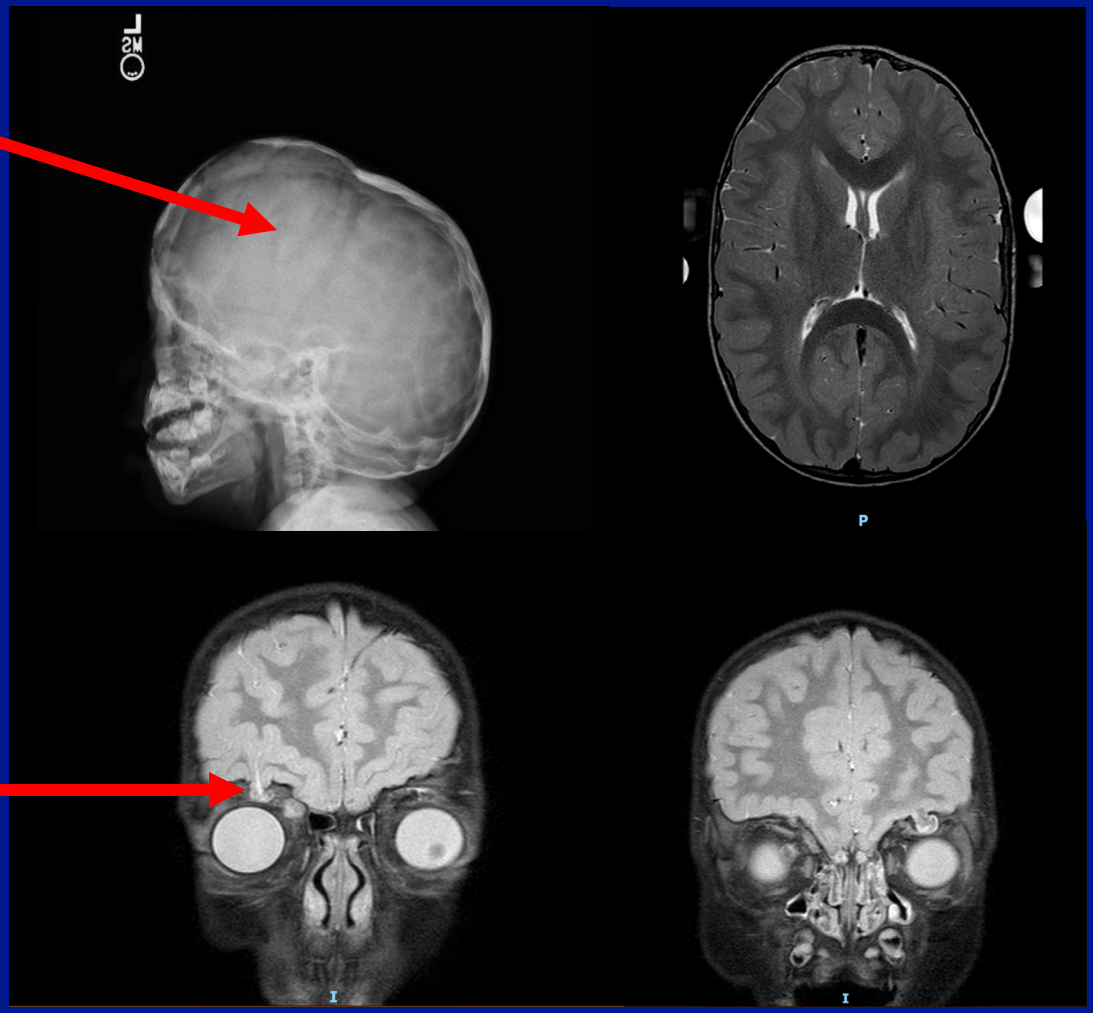
Positive or Candidates

- Chromosome Microarray - 277 KB interstitial deletion of 7q11.22-q11.22
- Baylor: *SETD2, IL1RAPL1, POLA1*
- NIH Pipeline: *GABRE, JMJC1C, KLHL8, PSMC3IP, SLC2A11*

Ellen Macnamara, GC

Cranial X-Rays and Brain MRI Under Anesthesia

- Copper-beaten skull, pancraniosynostosis
- Paucity of sub-arachnoid space and thinning of the ventricles
- Frontal lobes herniating through the orbits



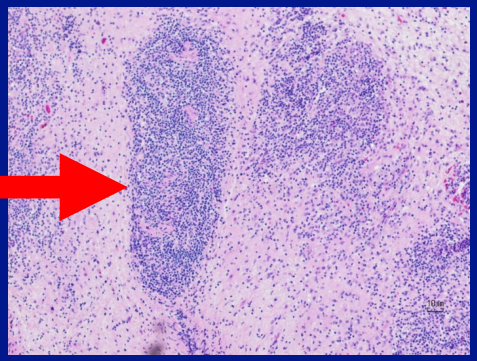
Gilbert Vezina, MD

Emergency Decompression Surgery by World Expert Saved this Boy's Life

Dr. James T. Goodrich MD, PhD
1946-2020
Director, Pediatric Neurosurgery Montefiore Medical Center
Professor at Albert Einstein College of Medicine
Died of Covid in 2020 caring for others.

UDP 10656 – A 10 Year-old Girl with Necrotizing CNS Vasculitis

- Normal early development
- Age 6 years: Headache, pharyngitis, ataxia
- MRI: Cerebellar FLAIR enhancing lesions
- CSF: 10 WBCs (88% lymphs), gluc 58, protein 31, 2 RBC
- Several clinical & MRI relapses; headaches, ataxia
- 2010: Cerebellar biopsy: T-cell perivascular infiltrate
- 2011 Thalamus biopsy: Similar
- Refractory to steroids, IVIG, cyclophosphamide, Cellcept, natalizumab



Ariane Soldatos, MD

NIH UDP Admission - 2015

- **Not infectious, oncologic, paraneoplastic, rheumatologic**
- **Flow cytometry (Dr. Bibi Bielekova NIAID)**

WBCs/ml CSF	**46 000** (ref range <5000 cells/ml)
Innate	**+++ granulocytes and monos**

- **Genetic testing: Compound heterozygous mutations in *PRF1***
  - Perforin is a T-cell protein that opens pores in target cells
  - Loss-of-function mutations can cause CNS-isolated Hemophagocytic Lymphohistiocytosis (HLH)
- **Functional studies in proband**
  - Absent perforin expression in cytotoxic cells
  - Decreased NK cell function

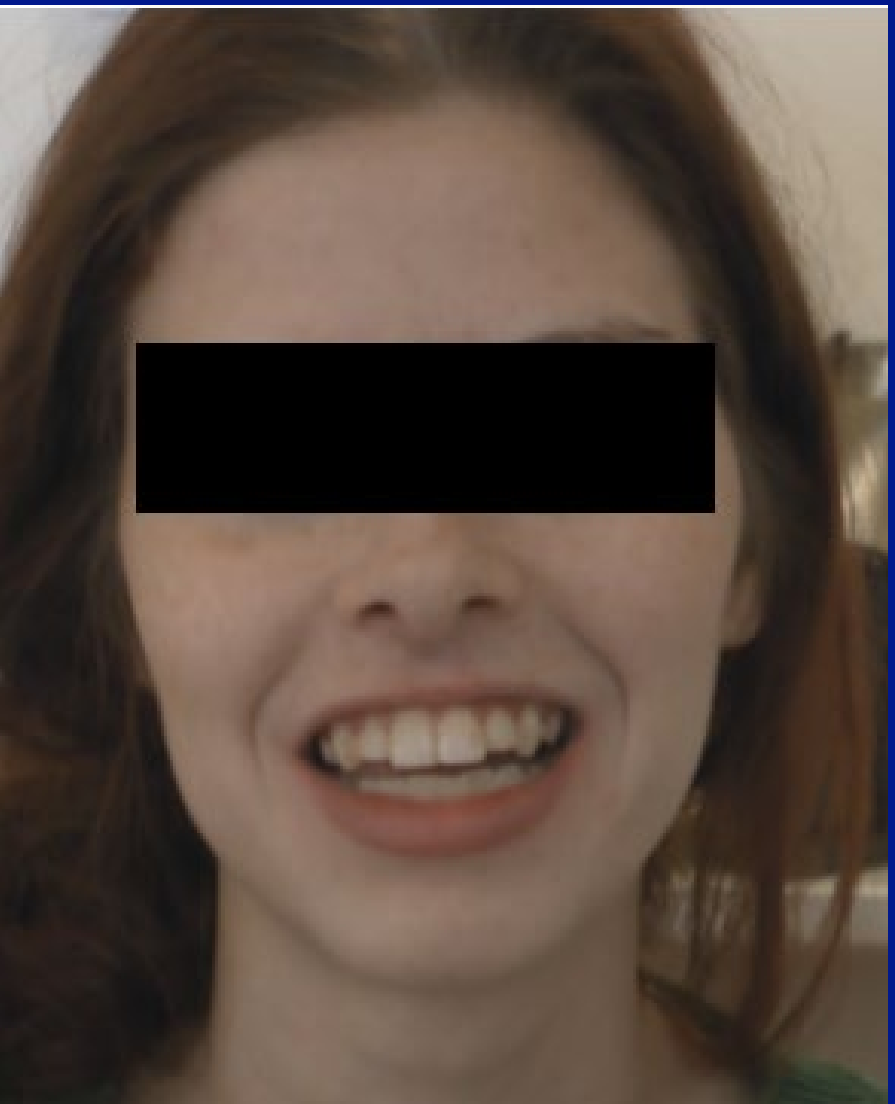
Ariane Soldatos, MD

Treatment: Bone Marrow Transplant

- **Result: CNS inflammation subsided; weaned off chronic steroids; improved neuromotor disability**
- **Implications:**
  - Brother tested for *PRF1* mutations
  - At referring medical center, 3 other children with refractor idiopathic brain inflammation were tested, found to have HLH, and transplanted.

Ariane Soldatos, MD

22 year old woman with dystonia

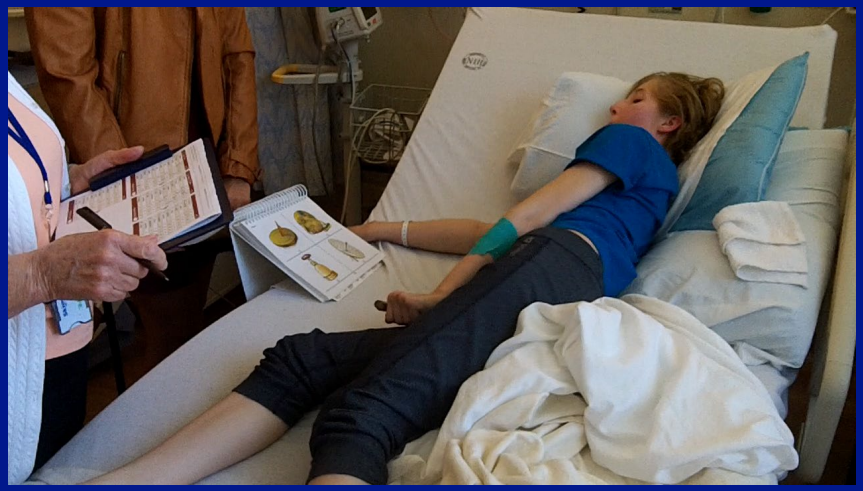


- ✓ Abnormal pen gripping
- ✓ Right foot deformity and twisting with gait
- ✓ Involuntary tongue movements
  - ✓ Speech
  - ✓ Swallowing
  - ✓ Nutrition
- ✓ Monoallelic *KMT2B* mutation
- ✓ Histone lysine methyltransferase deficiency

Dr. Manju Kurian, director of a Dystonia Clinic in London, sees *KMT2B* on the UDP's list of candidate disease-causing genes and calls Dr. Gahl. She has >20 dystonia patients with *KMT2B* mutations, is writing it up as a new disease gene, and says that several patients responded well to deep brain stimulation (DBS). She publishes a paper in Nature Genetics, including our patient, and another paper in Brain, showing the benefit of DBS.

Ariane Soldatos, MD, sees a 20 year old with progressive dystonia in the UDP

- 3y: Toe-walking
- 4y: Hypernasal and declining speech
- 5-6y: Left foot drag; “clumsy”
- 11y: Impaired gait, wheelchair for long distances, dystonia, choreoathetoid movements of upper extremities; started oral baclofen
- 14y: Intrathecal baclofen pump; non-ambulatory
- 15y: Lost ability to write; anarthria
- 20y: Spells letters with fingers to communicate; opisthotonic posturing; IT and oral baclofen, trihexyphenidyl, tizanidine, diazepam, clonazepam



Ariane Soldatos, MD

Diagnostic testing

Negative testing for:

- ***DYT1*** **GAG946 deletion**
- ***PKAN*** **gene**
- **Mitochondrial DNA (MELAS, NARP)**
- **Exome sequencing**
- **CSF neurotransmitters and pterins**
- **Muscle biopsy: Not nemaline rod myopathy**

Research genome positive for:

- **De novo *KMT2B* mutation: c.12_24dup13: p.Ser9GlyfsX111**
- **13bp duplication in exon 1 introducing a premature stop codon**

Ariane Soldatos, MD

Post-Deep Brain Stimulator

Ariane Soldatos, MD

Conclusions

- ***KMT2B*** **(DYT-28) is a relatively common cause of monogenic dystonia .**
- **Oromotor involvement is prominent.**
- **Some cases are reminiscent of NBIA.**
- **It is very responsive to DBS.**
- **Sharing to find similar cases is critical for new gene/disease discovery and treatment!**

Sharing by the NIH UDP to find second cases of new diseases

1. UDPICS Database
   - Phenotypes - Phenotips ontology
   - Exome sequences, variant analyses
2. Search UDPICS for variants in your gene.

Expansion

The Undiagnosed Diseases Network (UDN); Phase I (2014-18)

- UDP, 7 Clinical Sites, Coordinating Center, 2 Sequencing Cores, Metabolomics Core, Model Organisms Screening Center, Central Repository
- Formal data sharing agreements
- Consent: PII to be shared within UDN, de-identified data with others.
- First patients: August 2015.

Undiagnosed Diseases Network

Seven clinical sites, a coordinating center, two DNA sequencing cores, a metabolomics core, a model organisms screening center, and a central biorepository

The NIH site will continue to enroll about 150 patients per year, each of the clinical sites will ultimately enroll about 50 patients per year.

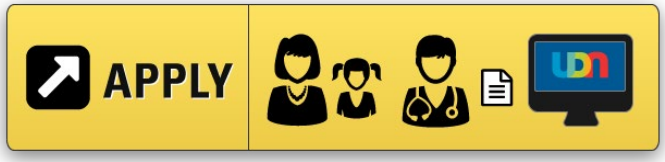
UDN: 8/15-1/22

- Applications 5310
- Accepted 2133
- Evaluated 1749
- Diagnosed 505
- Patient exomes ~500
- Patient genomes >1000

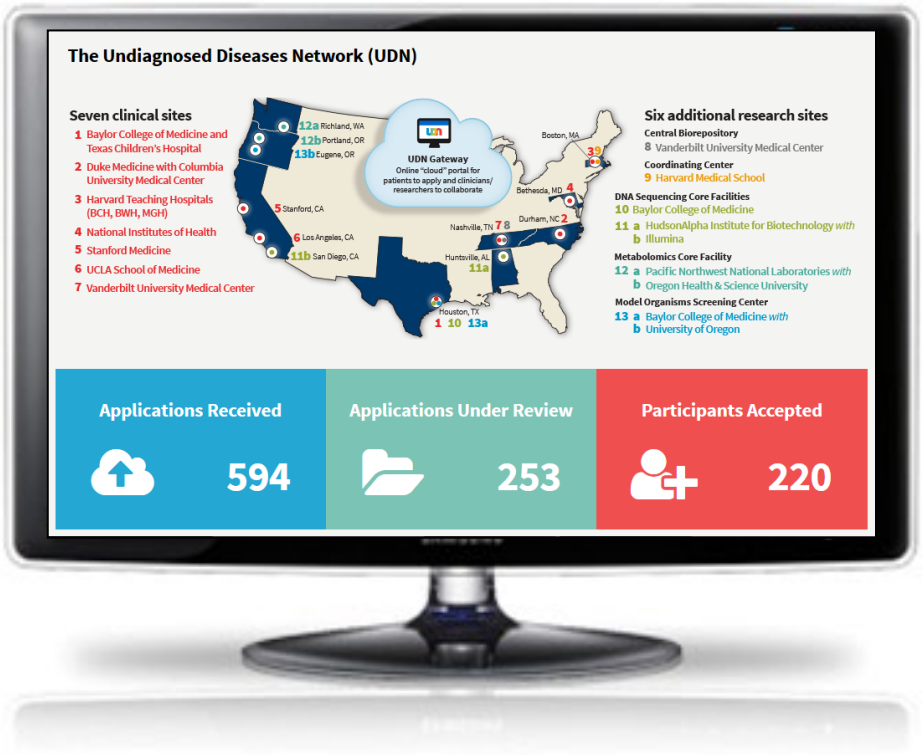
The UDN: Phase II-2018-2022

- 11 Extramural Clinical Sites (Harvard, Vanderbilt, Duke, Baylor, Stanford, UCLA, Wash U, U. Washington, CHOP-Penn, Miami, Utah)
- Coordinating Center, Sequencing Core, Metabolomics, Model Organisms, Repository

The UDN Gateway



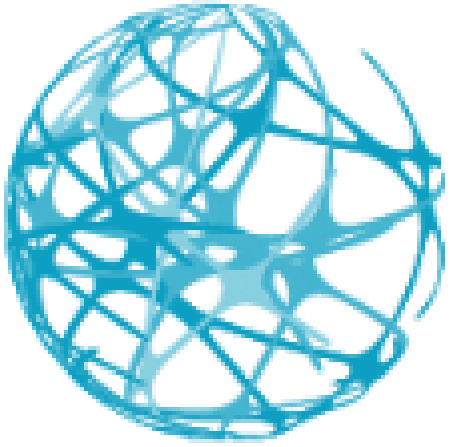
Click “Apply” button on any UDN website for more information



http://undiagnosed.hms.harvard.edu/apply/

Worldwide Access: UDNI

Undiagnosed Diseases Network International(UDNI): White Paper for Global Actions to Meet Patient Needs
Molecular Genetics and Metabolism 116:223-5, 2015.



Undiagnosed
Diseases Network
INTERNATIONAL

Website:

http://www.udninternational.org/

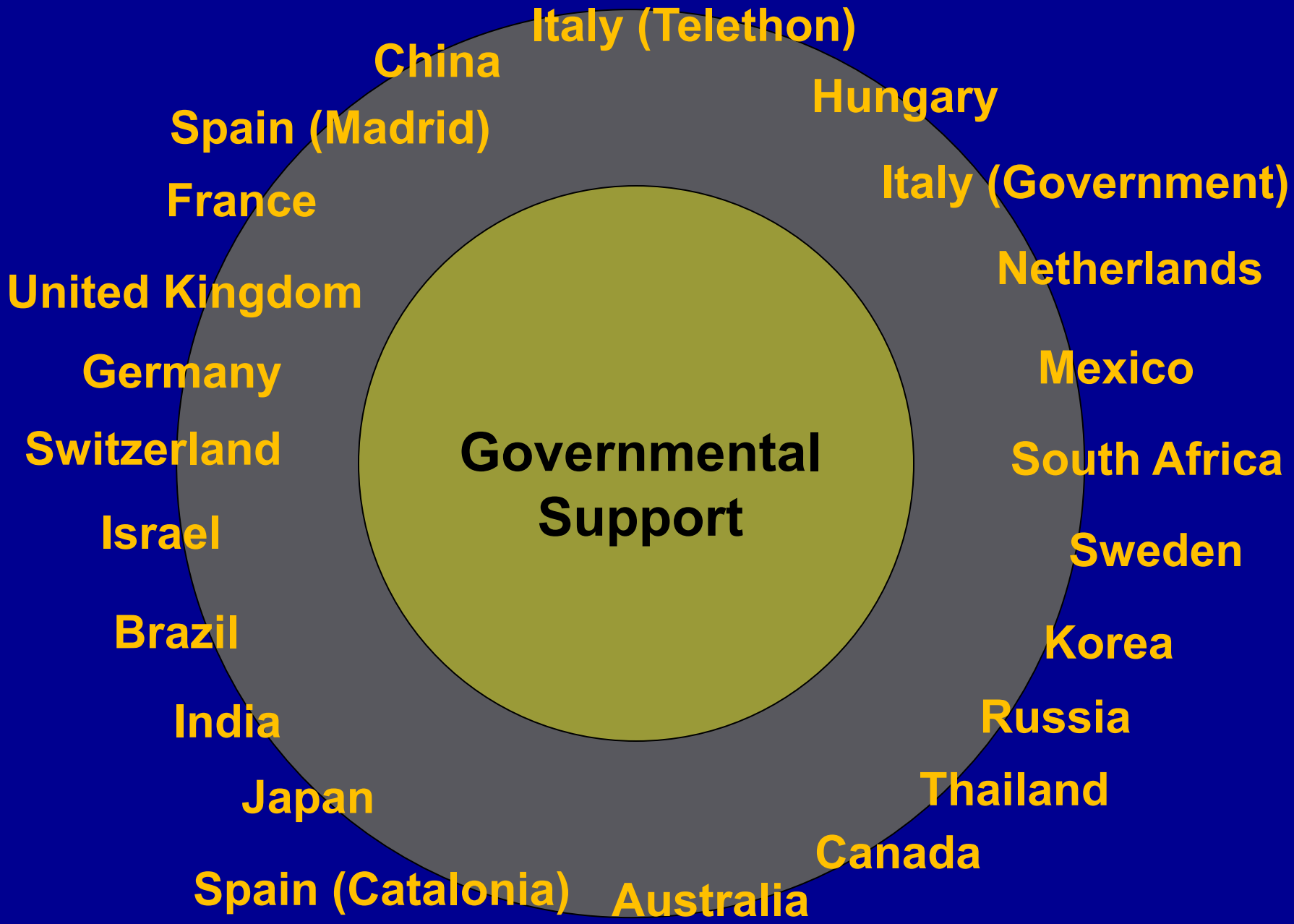
UDNI Meetings

(NIH Common Fund, Wilhelm Foundation, Local Sponsors)

- Rome September 2014
- Budapest – June 2015
- Vienna – February 2016
- Tokyo – November 2016
- Stockholm – August 2017
- Naples – June 2018
- New Delhi – April 2019
- Nijmegen – February 2020
- Mayo Clinic, Minnesota - April 2021
- Torino, Italy – January 2022

UDNI Charter, Committees, Data Sharing Policy, Best Practices

The UDNI is a Global Network



CONCLUSIONS: Rare and Undiagnosed Diseases Programs

- Require strong phenotyping of patients
- Foster new disease discovery
- Lead to insights into common diseases
- Help desperate patients
- Often require functional studies
- Sometimes do not need NGS
- Hugely benefit from data sharing
- Are needed throughout the world