The Doctor

Luke Fildes

The Undiagnosed Diseases Network: National and International

ITINERARE

Rare Disease Initiative Zurich Radiz Symposium
Kinderspital Zurich

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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 Numbers

- Medical Records: >4000
- Admitted & Evaluated: >1500
- Children: ~40%
- Neurological: ~50%
- Some diagnosis: ~30%
- Publications ~190

UDP Investigations

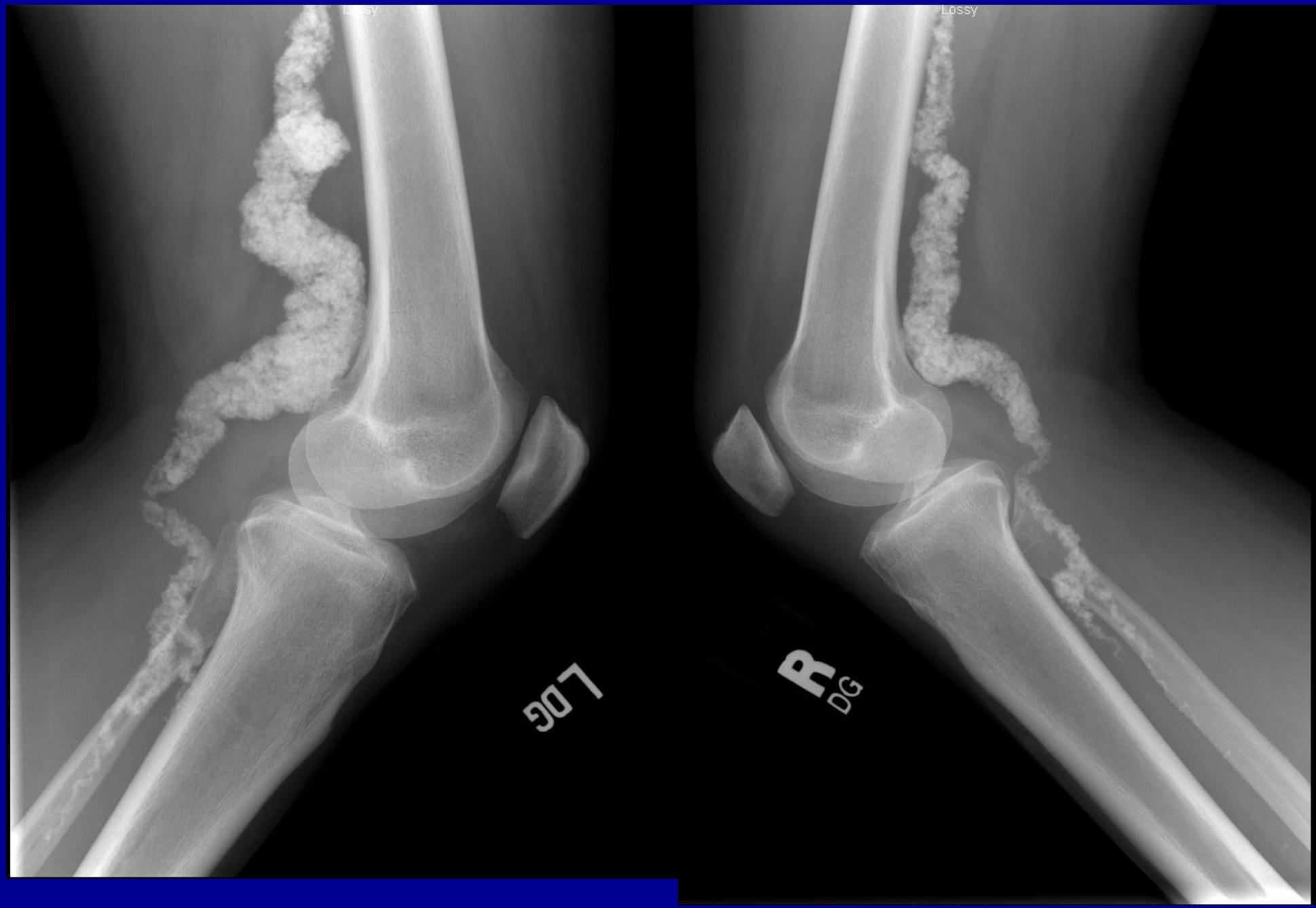
1. Customized (Personalized) patient phenotyping to rule out known diseases.
2. Genetic studies
   a. Commercial testing
   b. SNP arrays
   c. Exome and genome sequencing
3. Functional studies (assays, model systems)

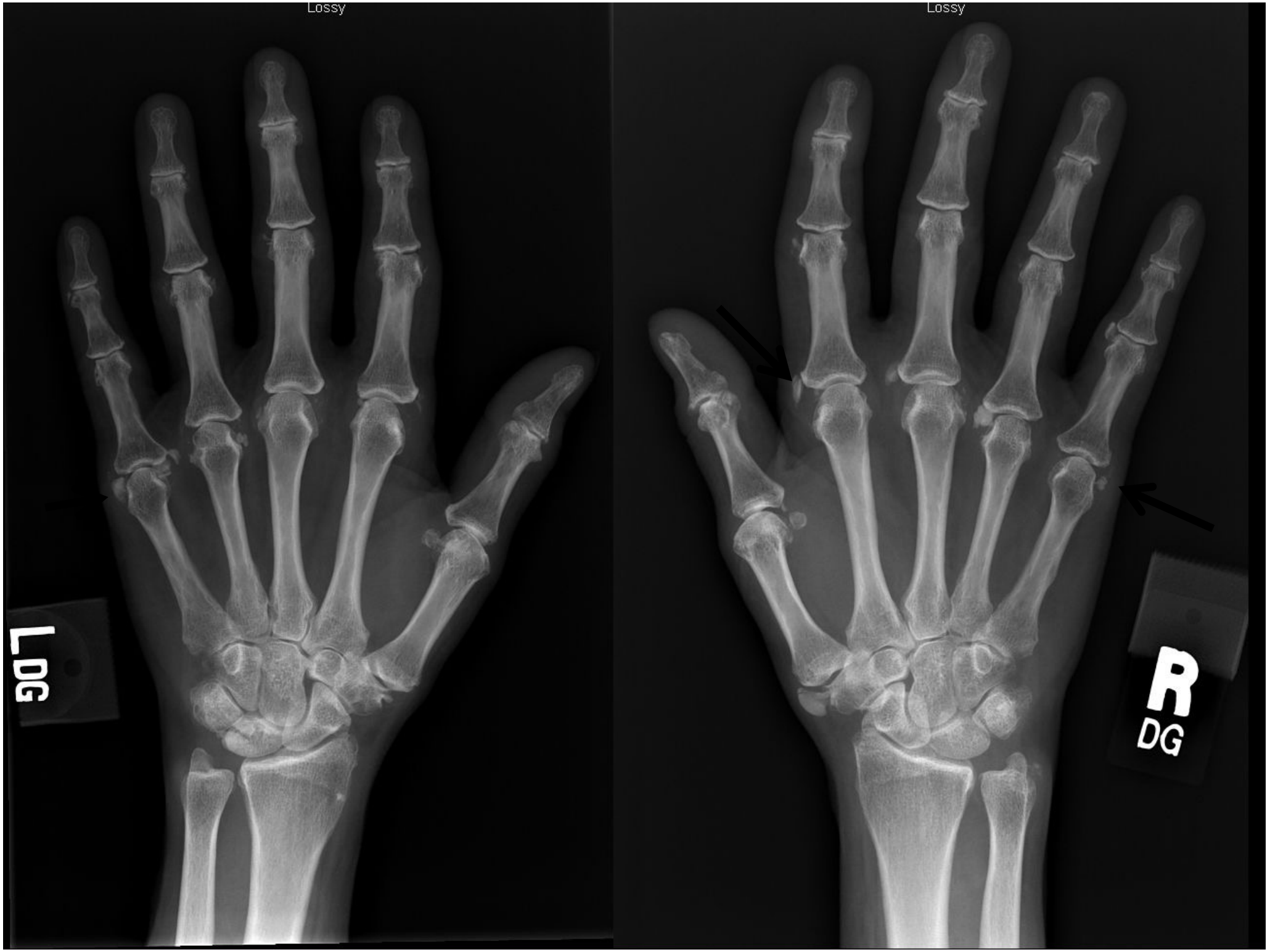
Discovery

5 Adult Siblings with these Clinical Symptoms and Signs:

- Intermittent claudication of calves, thighs, buttocks
- Chronic ischemic pain of the feet
- Joint pain in the hands
- Arterial calcification of lower extremities
- Spared coronary arteries

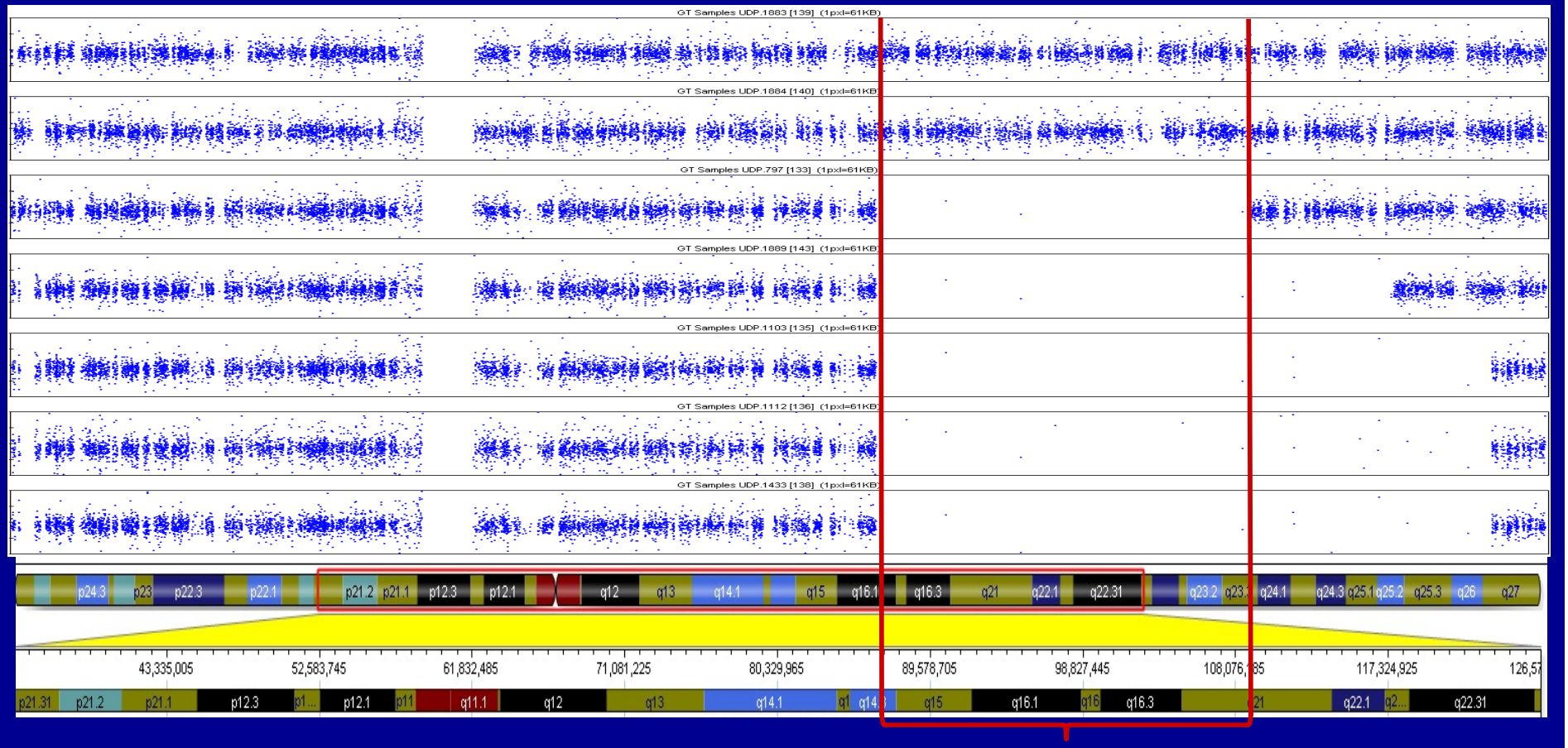
Femoral-Popliteal Artery Calcification




Lossy
Lossy
L
DG
R
DG

Parents were 3rd Cousins
SNP Array: Chromosome 6q14.3-6q21

Affected Parents



Region of Identical Homozygosity

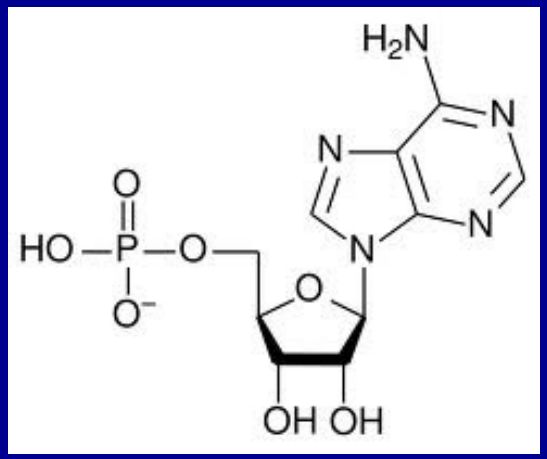
Dr. Tom Markello

Linkage Region

- Region of homozygosity: **22.4MB**
- 7977 total SNPs without a single A/B genotype in any locus
- 92 genes, about 902 exons
- No structural genes of the extracellular matrix
- One good candidate gene: ***NT5E***, encoding CD73, an ecto-5'-nucleotidase

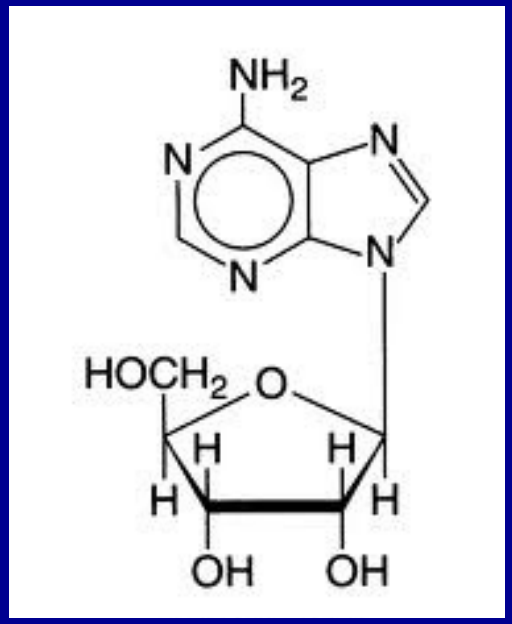
T. Markello, C. St. Hilaire

NT5E Encodes CD73



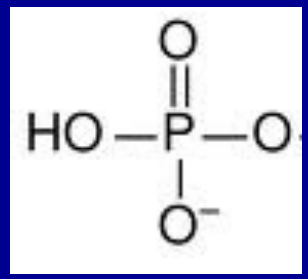
AMP

CD73



Adenosine

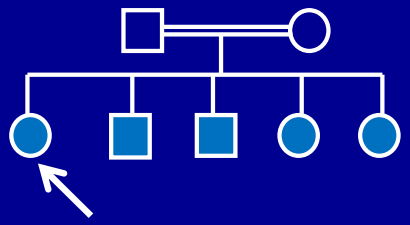
+



Pi

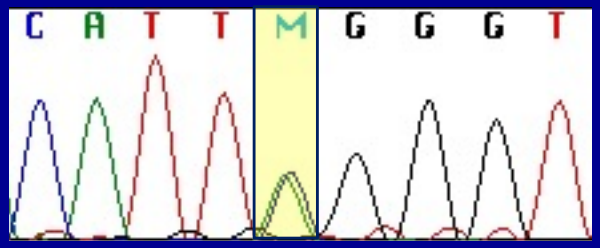
NT5E Sequencing Analysis

Family 1 (UDP)

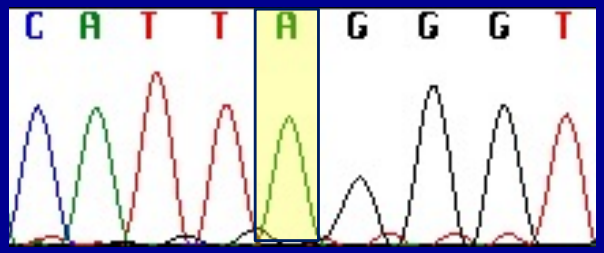


c.662C>A, S>X

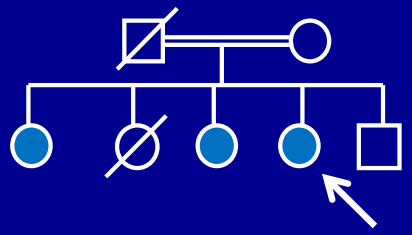
Parent



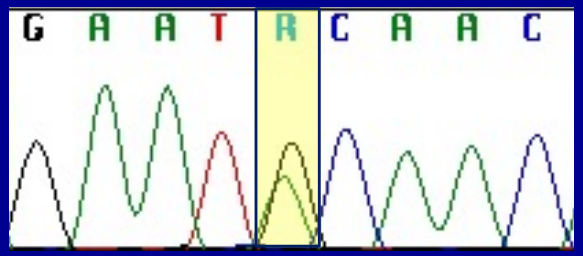
Affected

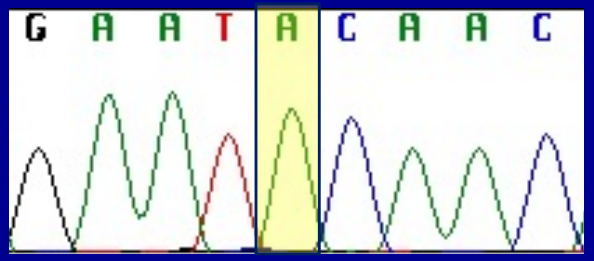


Family 2 (Kleta)

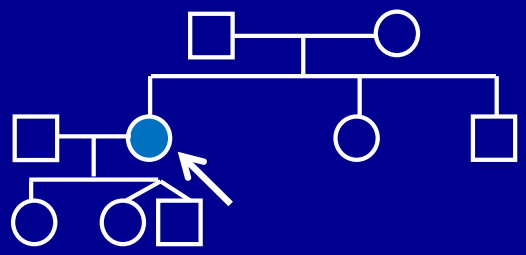


c.1073G>A, C>Y



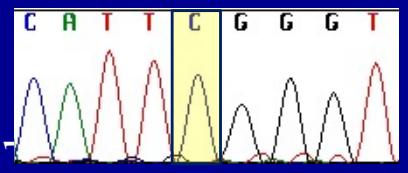


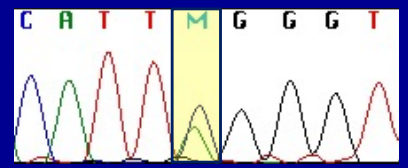
Family 3 (Nussbaum)



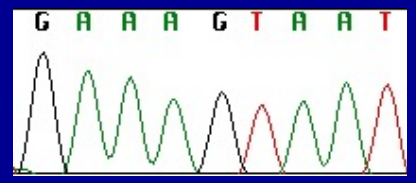
c.1069dupA/c.662C>A

Affected Contro

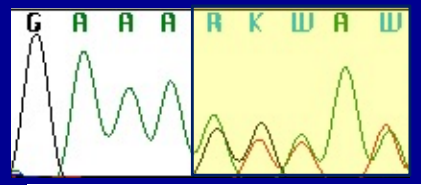




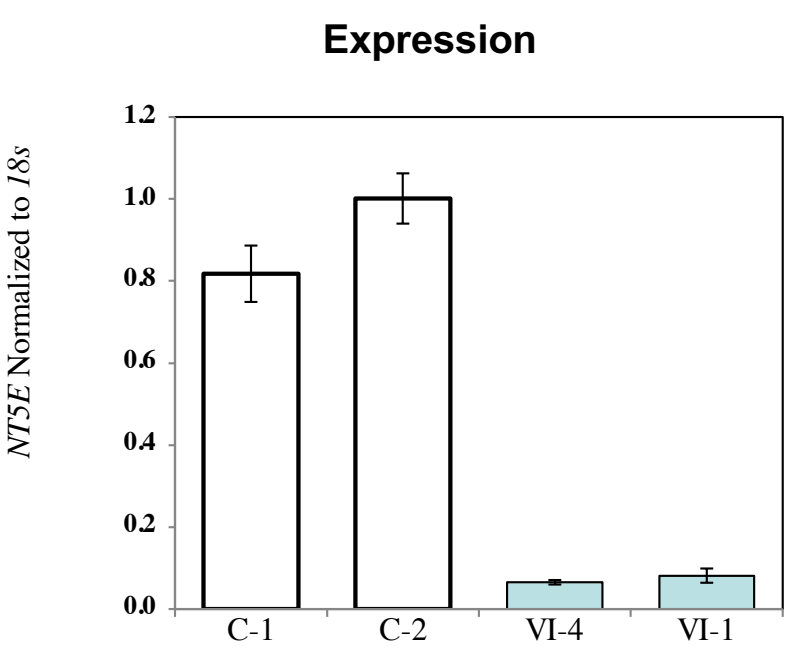
Control

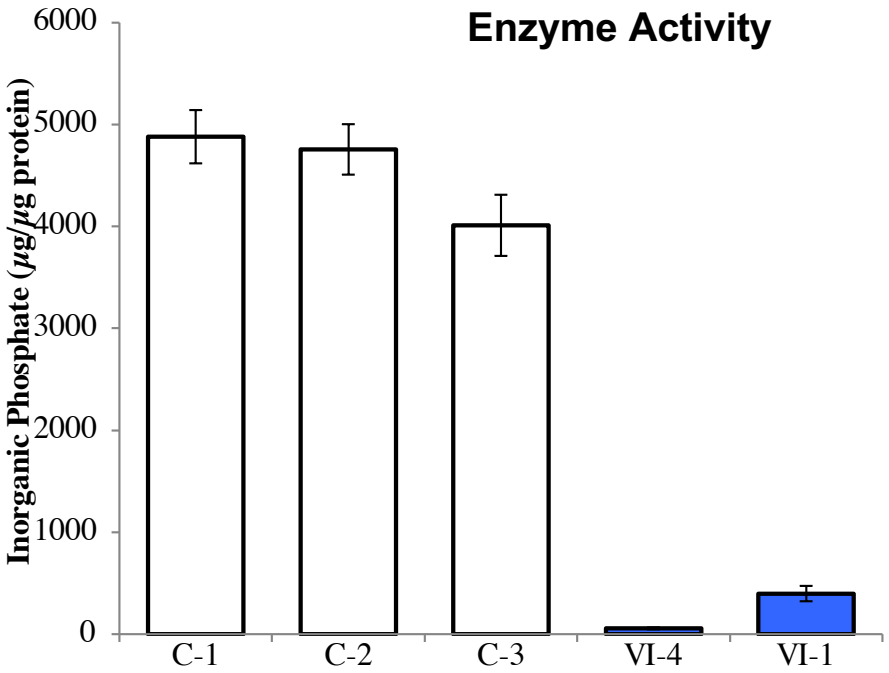


Affected



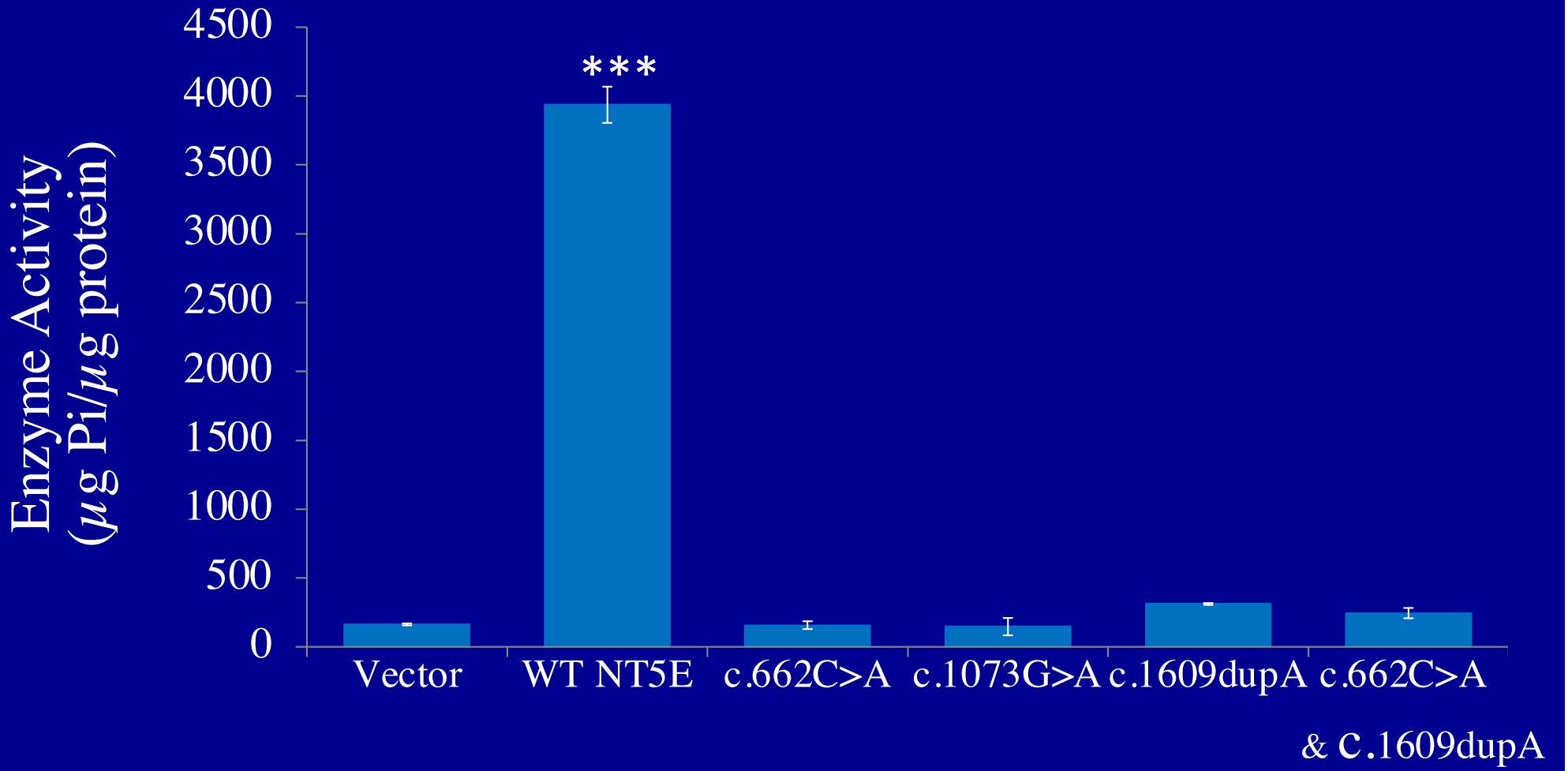
S. Ziegler





Drs. C. St. Hilaire, M. Boehm

Enzyme Activity in Mutagenized Constructs

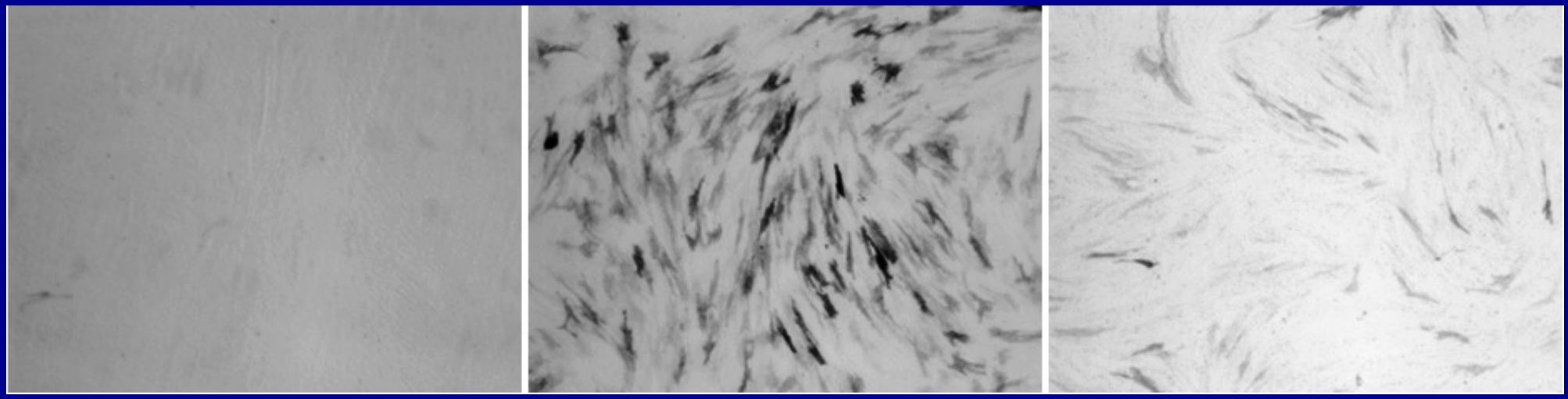


Vectors containing patient *NT5E* mutations transfected into HEK293 cells

St. Hilaire C, Ziegler SG, et al. NEJM 2011.

Increased Fibroblast Staining for Alkaline Phosphatase

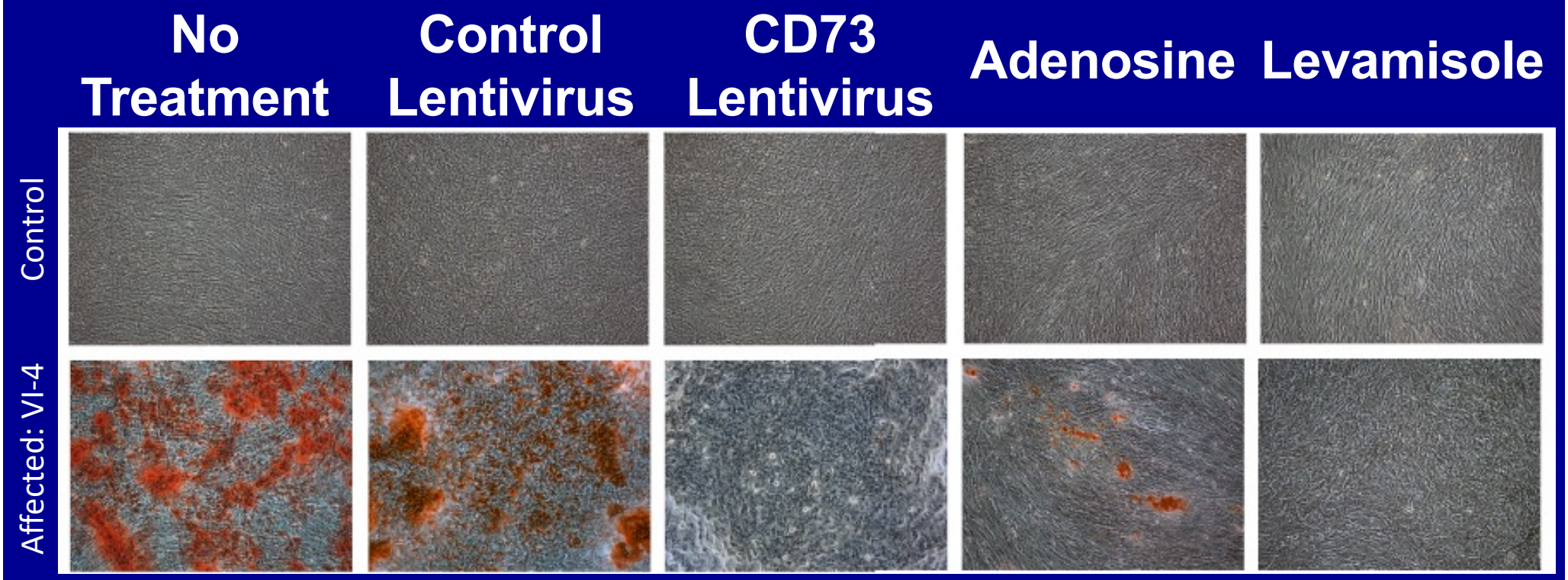
Control | Affected | Affected + Adenosine



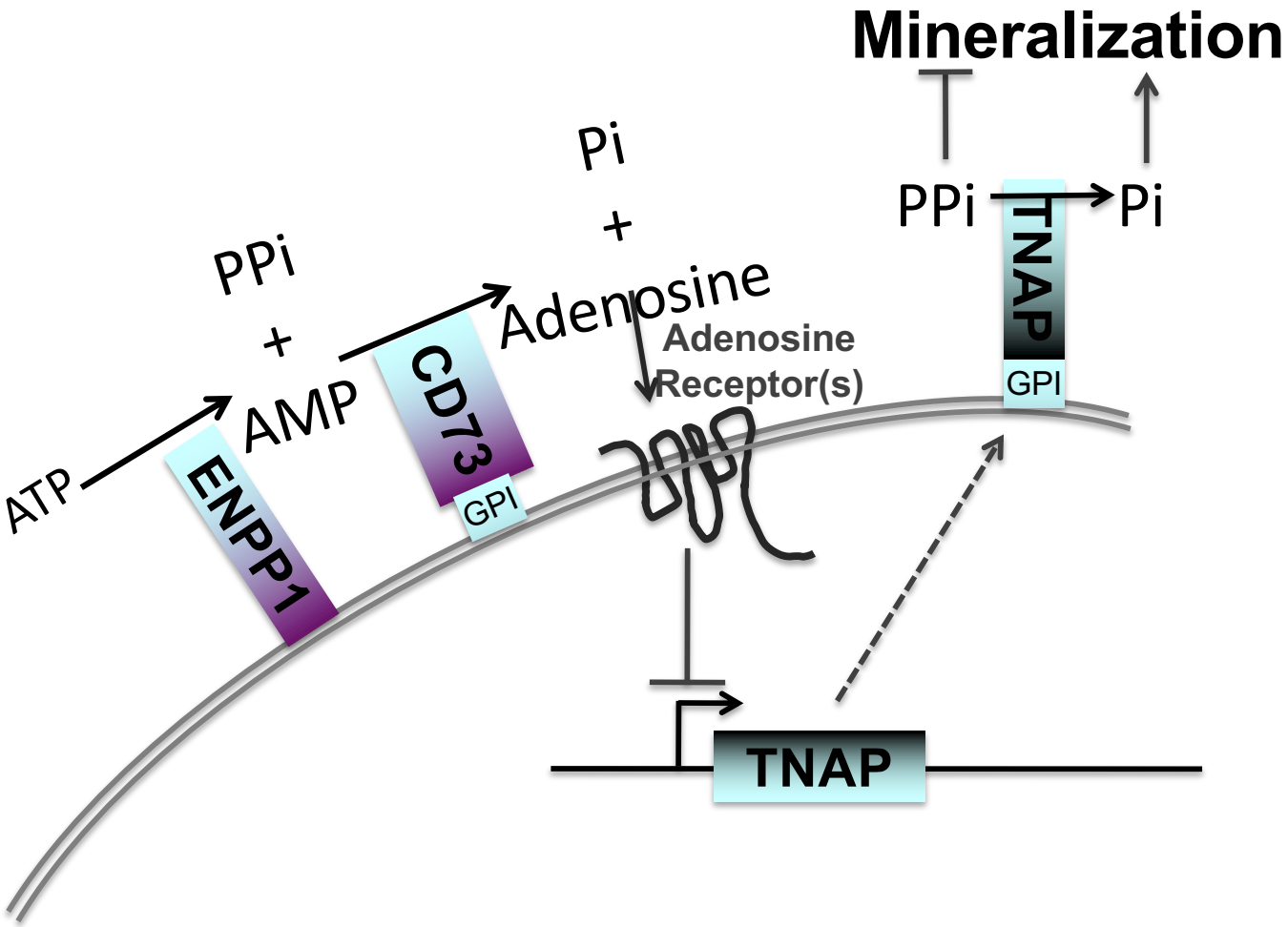
Adenosine treatment of cells reduces alkaline phosphatase staining.

St. Hilaire C, Ziegler SG, et al. NEJM 2011.

Rescue of Cell Calcification by a CD73 Lentivirus, Adenosine, or an Alkaline Phosphatase Inhibitor (Levamisole)



St. Hilaire C, Ziegler SG, et al. NEJM 2011.


Mineralization
PPi
Pi
TNAP
Pi
+
PPi
Adenosine
Adenosine
Receptor(s)
+
AMP
CD73
GPI
ATP
ENPP1
TNAP

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

Diagnoses

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

- Spermine synthetase mutations with developmental delays (Snyder-Robinson)
- XP with dementia due to ERCC1 mutation
- Delays and seizures due to PIGT mutations and GPI anchor deficiency
- Stargardt syndrome, Pelger-Huet anomaly, and others with chromosome 1 isodisomy
- Movement disorder due to *PLA2G6* mutations
- Osteopetrosis due to *LRP5* mutation
- Mowat-Wilson syndrome due to *ZEB2* mut
- Fahr's disease due to *PDGFRB* mutations
- Spasticity & leucodystrophy due to *DARS* mut
- Leucodystrophy due to *AARS2* mut

More Diagnoses - 2

- EMARRD (Early myopathy, AReflexia, Respiratory distress, Dysphagia) due to *MEGF10* mutations
- Neurodegeneration due to *BTK* mutation
- Cognitive & motor decline with *C19orf12* muts
- Waardenburg type 2 due to *SOX10* deletion
- SLE with cerebellar ataxia and anti-GWB Abs
- GM2 gangliosidosis and Sanfilippo disease
- TEMPI syndrome with erythrocytosis muts
- Choreo-acanthocytosis due to *VPS13A*
- Aicardi-Goutieres due to *RNASEH2B, A* muts
- SPG11, NPC1, STIM1, GARS, A-T, NGLY1, MNGIE, CAV3

More Diagnoses - 3

- Kohlschutter-Tonz syndrome (Sz, neurological regression) due to *ROGDI* mutations
- Delays, hypotonia, strabismus due to biallelic *UNC80* mutations
- CVID, aplastic anemia due to a *CTLA4* mut
- Myofibrillar myopathy with de novo *BAG3* mut
- X-linked intellectual disability, facial dysmorphisms due to *RLIM* mutation
- Desminopathy
- Fatal Creutzfeldt-Jacob; PrPSc/PrP27-30
- Oculodentodigital Dysplasia due to *GJA1* (connexin 43) mutations
- Chorea, hypomyelination-de novo TUBB4A mut

More Diagnoses - 4

- Spasticity, dementia, leukoencephalopathy due to homozygous POLR1C (RNA Pol III) muts
- Dementia, dystonia, brain atrophy due to chromosome 19 telomere fraying
- Microcephaly, delays, dysmorphisms with de novo *SPTAN1* mutations
- Brain atrophy, delays, visual defects, seizures with an X-linked *MED12* mutation
- Hemophagocytic lymphohistiocytosis due to perforin defect
- Chorea, hypomyelination with *TUBB4A* mut
- Hemiplegic migraine, cerebellar ataxia, myopathy with *CACNA1A* mutation

More Diagnoses - 5

- 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 - 6

- 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 - 7

- 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

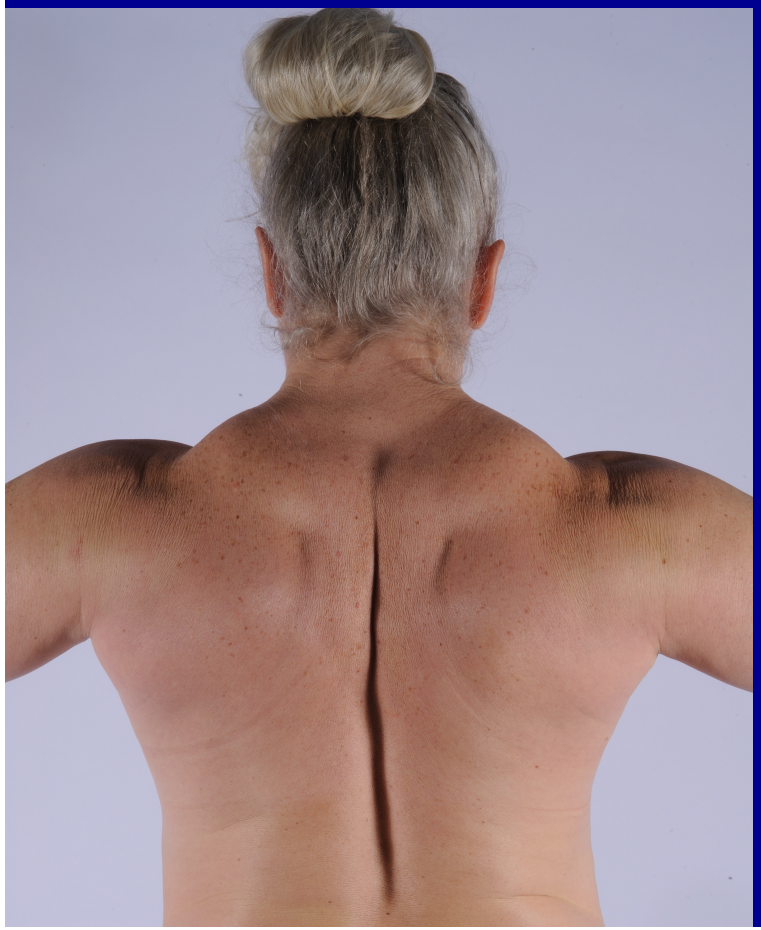
More Diagnoses - 8

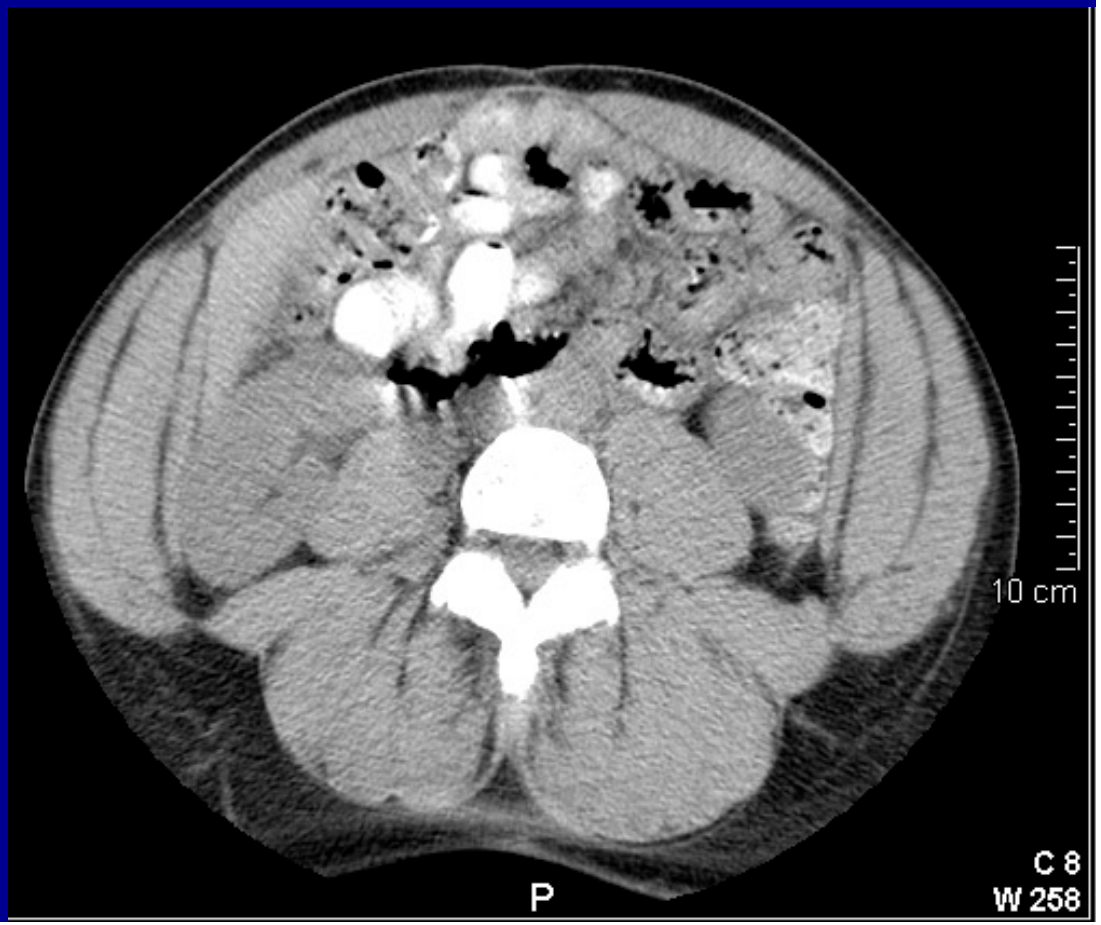
- Neurodegeneration due to bariatric surgery and methotrexate treatment causing folate deficiency
- Demyelinating peripheral neuropathy, CMT-like, due to a de novo *POLR3B* mutation
- Cardiac abnormalities and dysmorphisms due to iduronidase deficiency (mild Hurler syndrome)
- Sensorimotor neuropathy 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

Diagnoses/Treatments

52 Year old woman with increased muscle without increased strength

- No drugs; normal growth hormone
- EMG myopathic; normal initial muscle biopsy



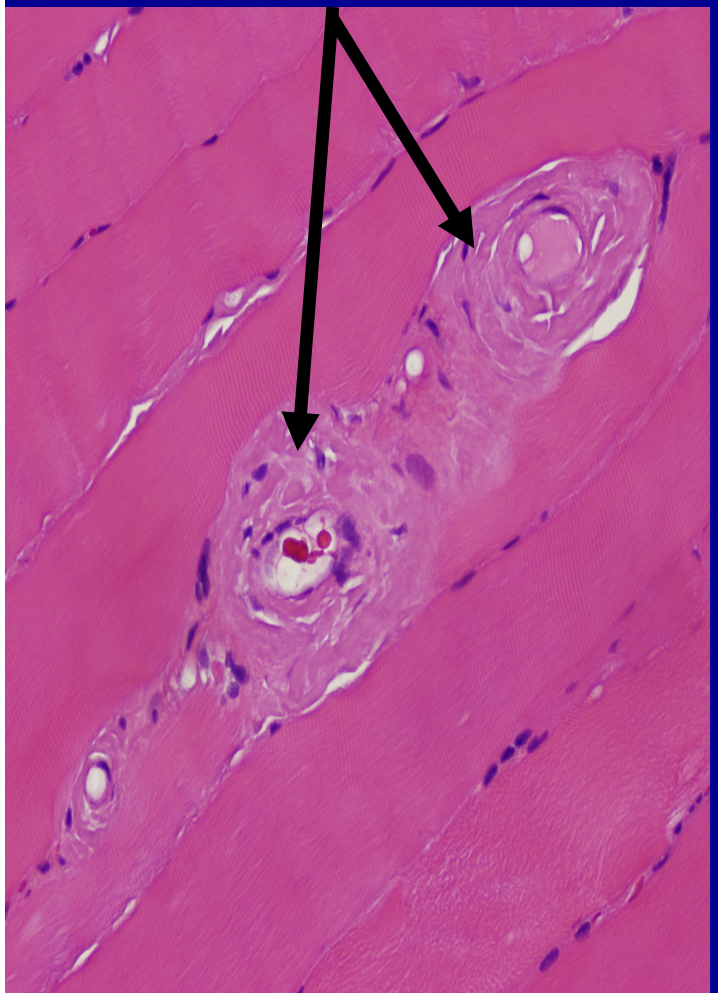


52 Year old woman with increased muscle:

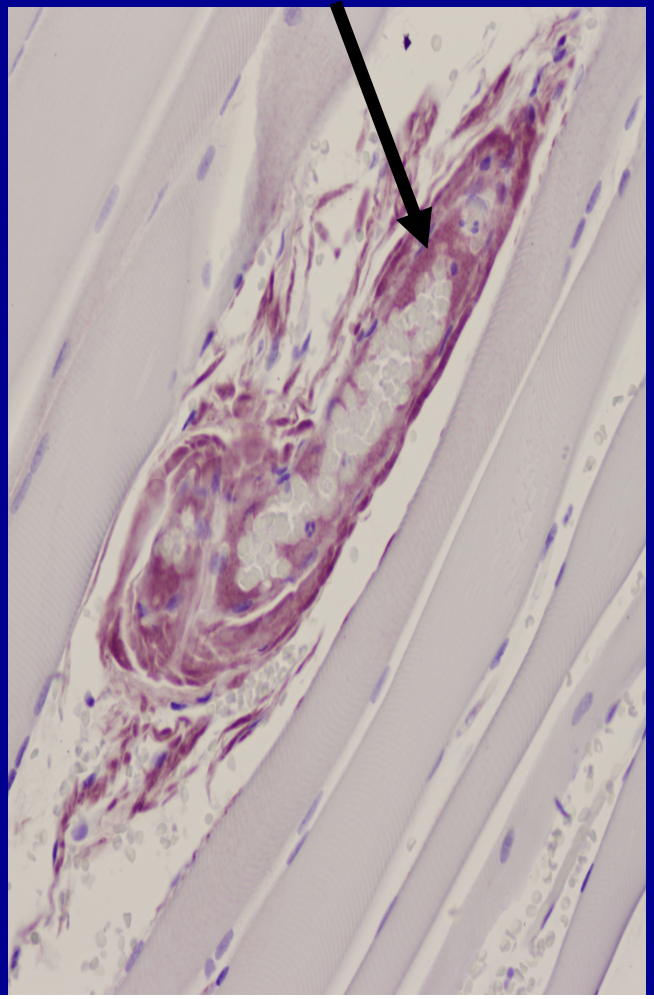
Amyloid Myopathy

Bone marrow: 10% plasma cells

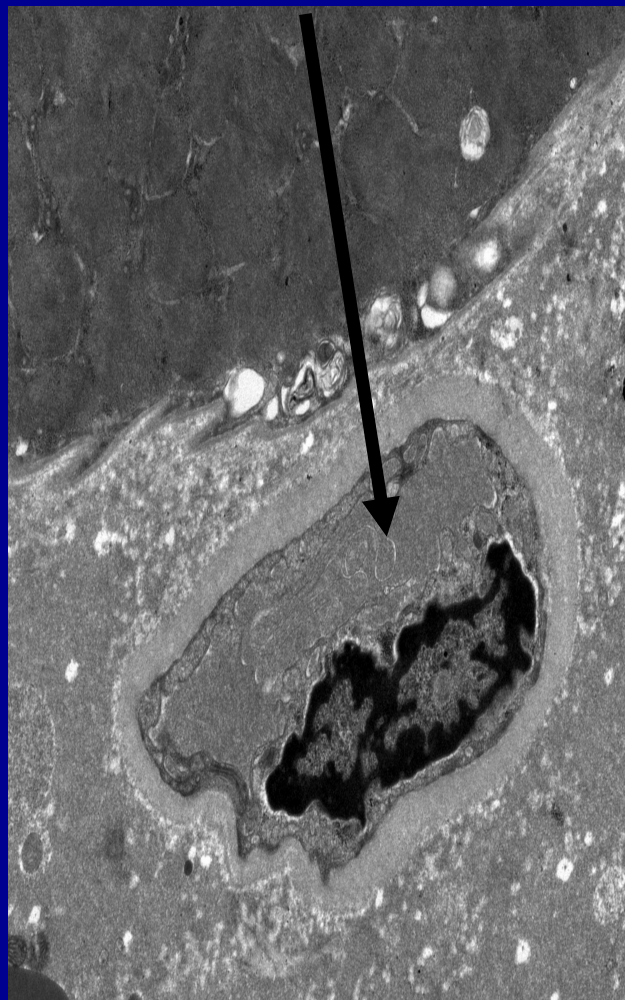
Thick vessel wall



Congo Red Stain



Protein aggregate



Outcome (Dr. Irini Manoli)

- Became short of breath, fatigued.
- Referred to country's amyloidosis experts at the Mayo Clinic.
- Underwent stem cell transplant in June, 2009; slightly rocky course.
- Began feeling better within weeks.
- Gradually recovering, with normalization of muscle mass.

65 Year-old Man with Recurrent Meningitis

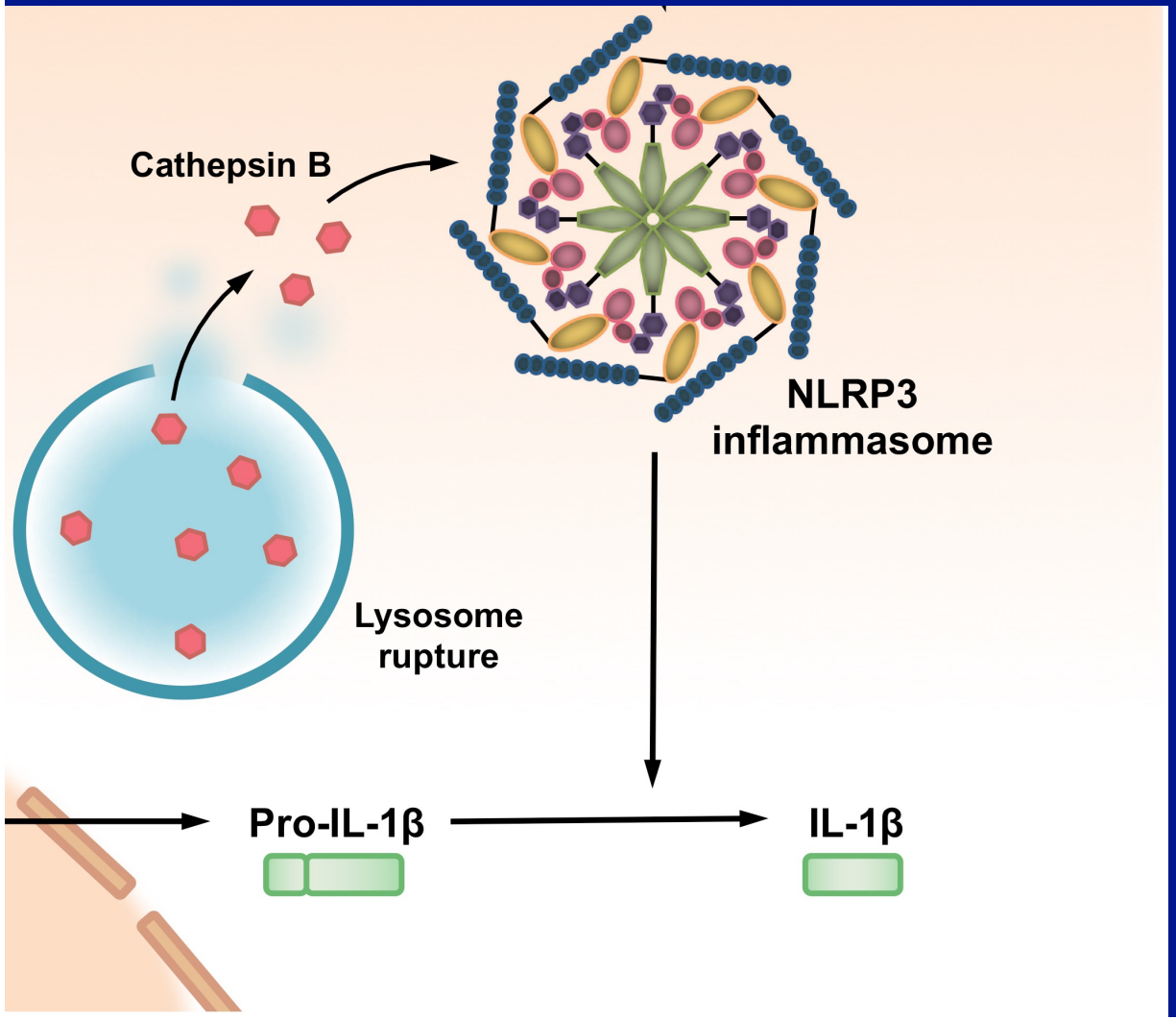
- Age 59 - First episode of meningitis; followed by autoimmune sensorineural hearing loss
- Acute: Headache, unsteady gait
- Chronic: Uses wheelchair, memory decline
- Age 59-65 – 27 more episodes
- LPs: Lymphocytic pleocytosis
- Aseptic; steroid responsive
- Negative imaging & rheumatology evaluation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

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.

Nature Genetics 49:223-37, 2016.

Meyer E ……., Kurian Manju A

Mutations in the histone methyltransferase gene KMT2B cause complex early-onset dystonia

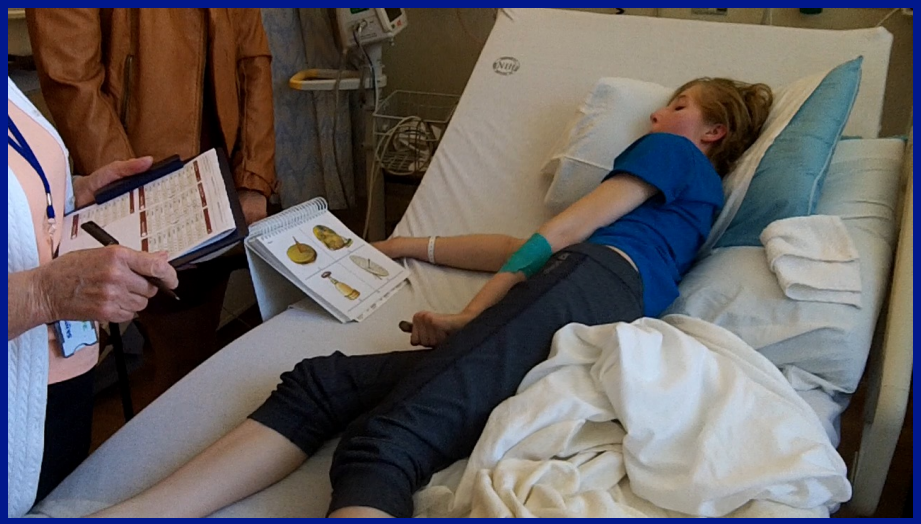
Brain 143:3242-61, 2020

Cif L ……., Kurian Manju A

KMT2B-related disorders; expansion of the phenotypic spectrum and long-term efficacy of deep brain stimulation

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.
3. List variants & phenotypes on web.
4. Provide limited access to UDPICS.

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.

UDN: 8/15-4/21

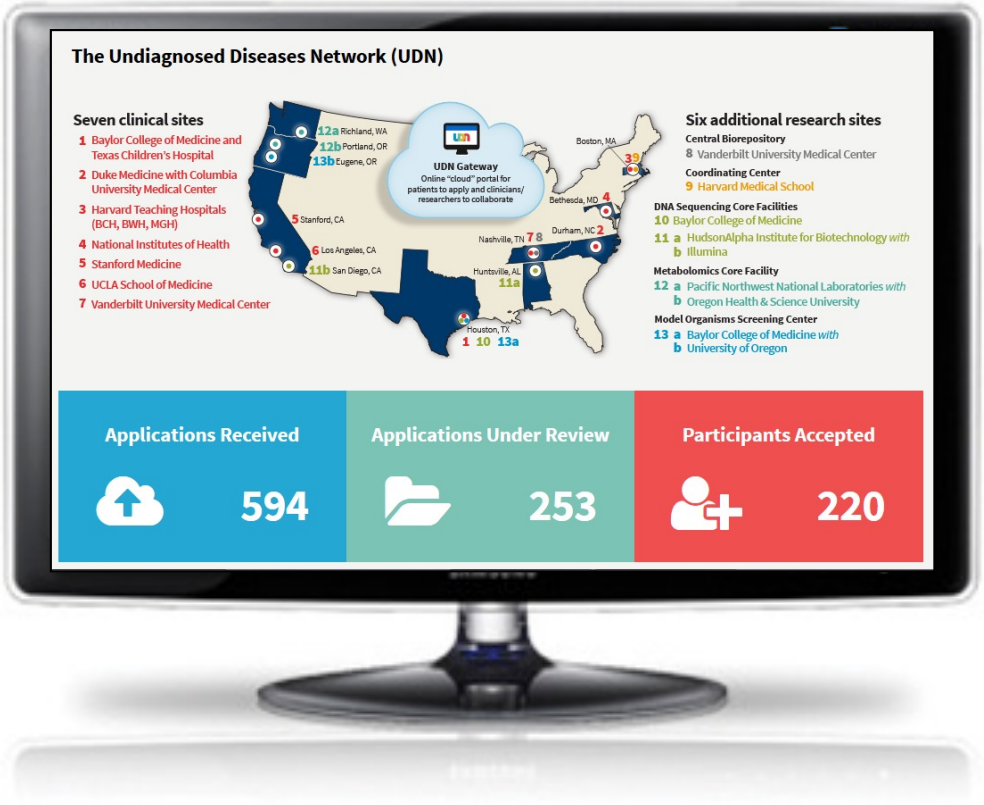
- Applications 4923
- Accepted 1956
- Evaluated 1525
- Diagnosed 467
- Patient exomes 457
- Patient genomes 1059

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

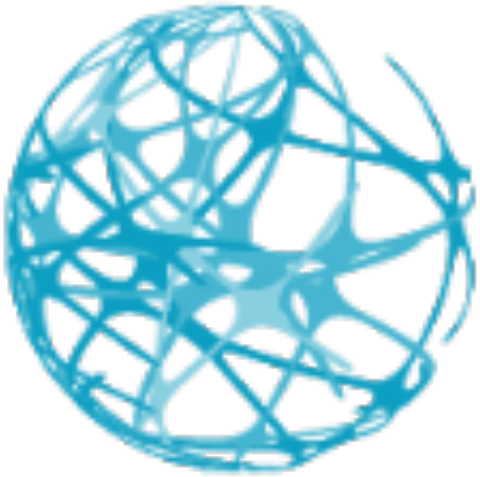


http://undiagnosed.hms.harvard.edu/apply/

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 – Jan/Feb 2022

UDNI Charter, Committees, Data Sharing Policy, Best Practices

New UDNI Initiatives

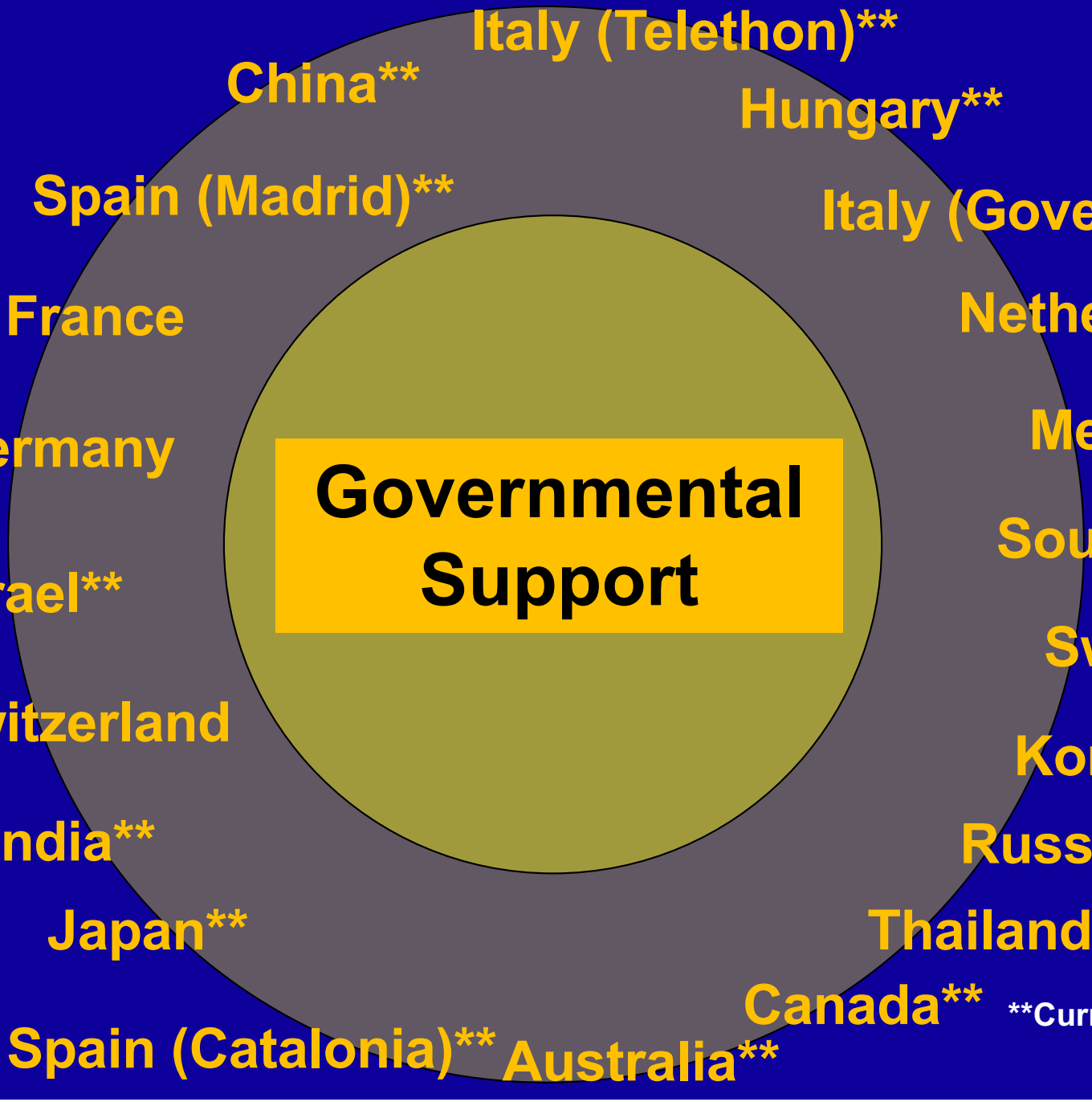
- Website facilities for datasharing

- Developing Nations Working Group

- Diagnostic Working Group

    - Reviews case records
    - Refers when possible
    - Young investigator involvement

The UDNI is a Global Network



**Currently Enrolling Subjects

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