The NIH Undiagnosed Diseases Program: Expansion to National and International Networks

REACT Conference

Bologna, Italy, March 7, 2018

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Clinical Director, NHGRI

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

Support from NIH Common Fund, Office of the Director

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

Kind and collaborative patients and families!

Personalized Medicine Starts with a Diagnosis

1. The NIH Undiagnosed Diseases Program (UDP)
   - Background
   - Discoveries & Common Diseases
   - Diagnoses/Mysteries
   - Treatments
2. Expansion to National and International Networks
3. Sharing

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
  - Many different specialties involved
  - Research oriented; not financially driven
- 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: ~1100
- Children: ~40%
- Neurological: ~50%
- Some diagnosis: ~30%
- Publications >80

UDP Investigations

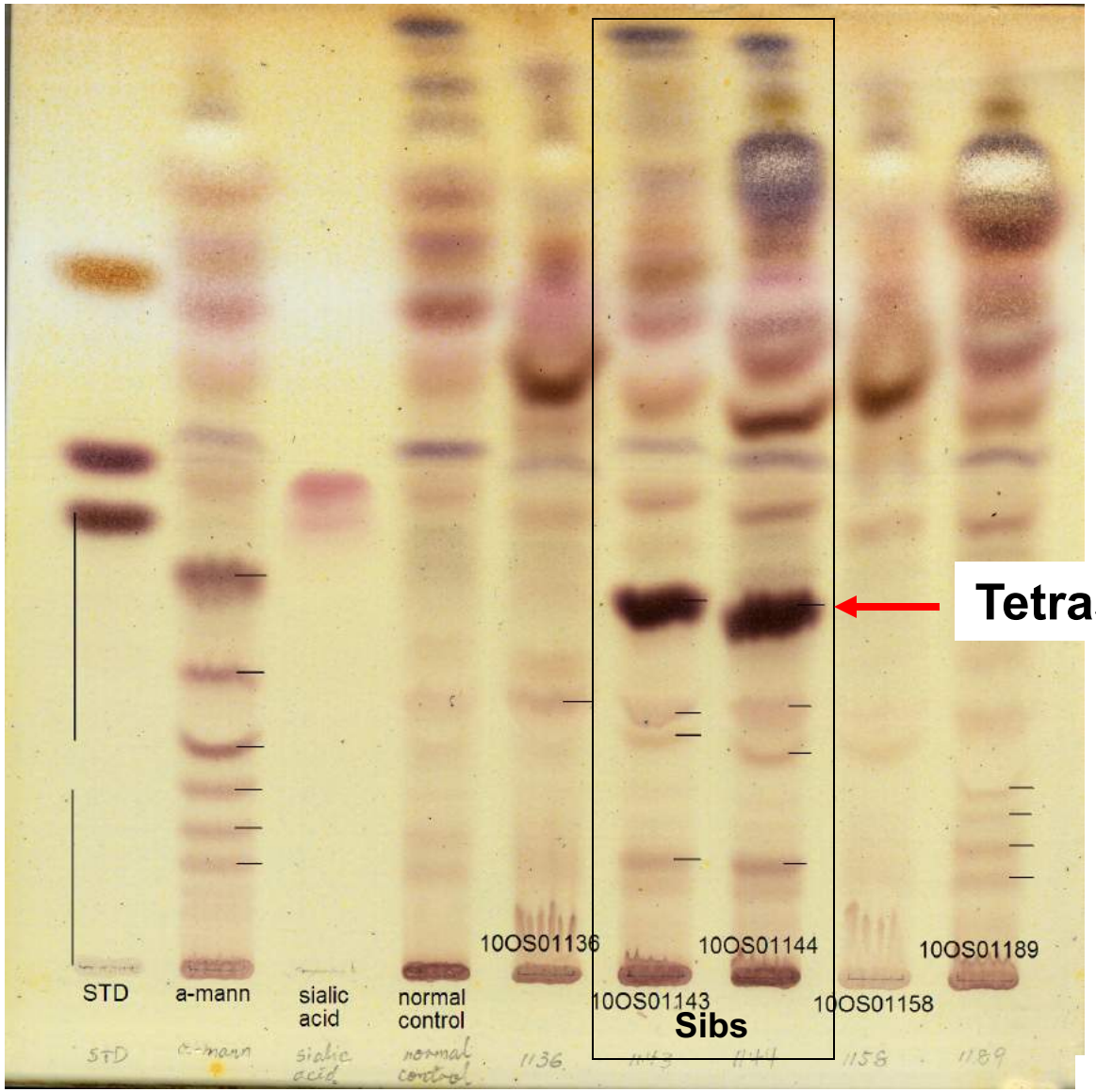
1. Customized (Personalized) patient phenotyping to rule out known diseases.
2. Genetic studies
   a. Commercial testing
   b. SNP arrays
   c. Exome sequencing
3. Functional studies (assays, model systems)

Discovery

10 year-old boy and 5 year-old girl

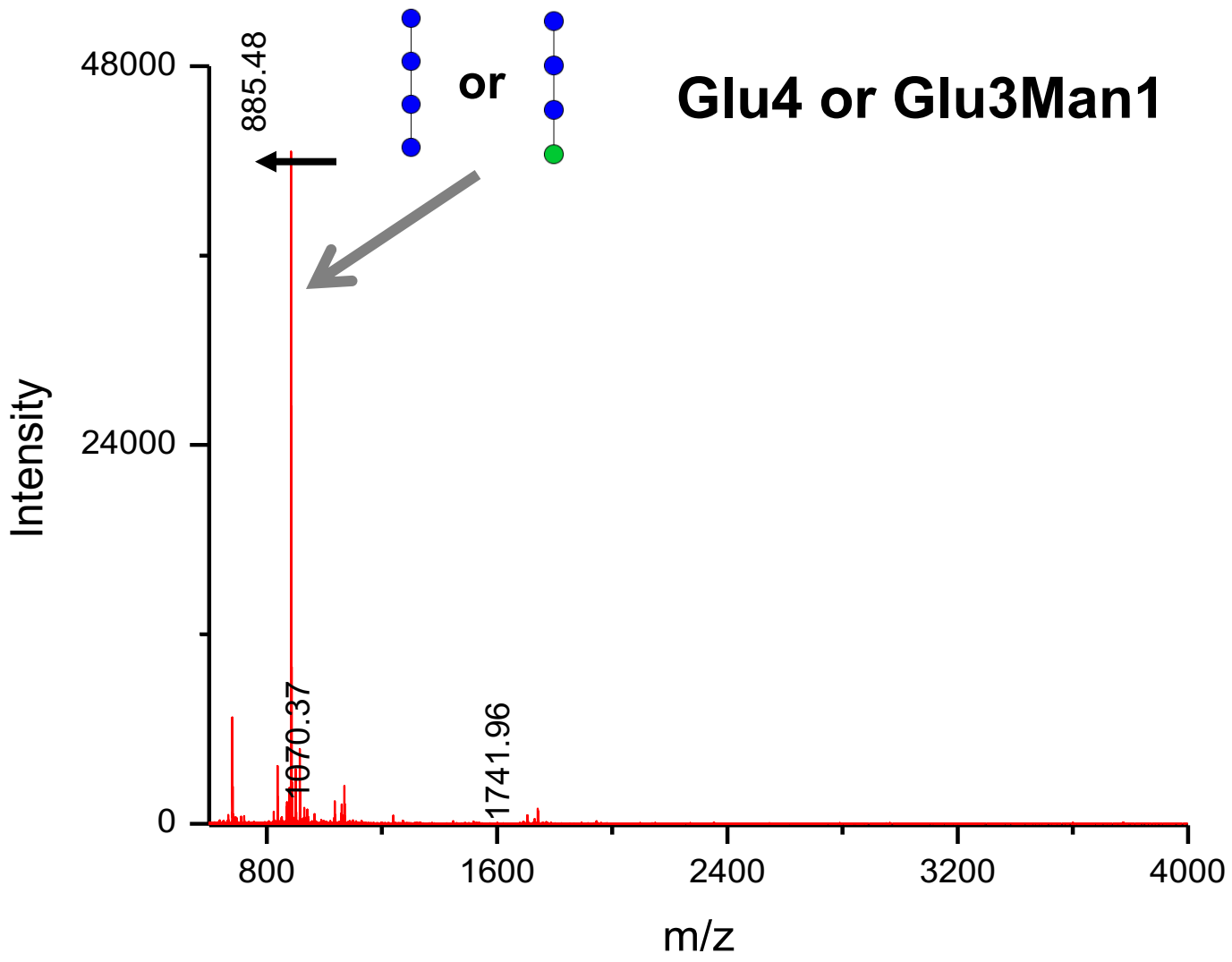
- Facial dysmorphisms, hypotonia, delays, hearing loss, nystagmus, seizures
- MRI: Diffuse atrophy, especially periventricular
- Labs normal, including transferrin IEF
- Urine glycan screening by MALDI (Dr. Miao He, Emory) confirmed hex4 band seen on TLC.

Oligosaccharide TLC



Dr. Miao He

Urine glycan MALDI profile



Dr. Miao He

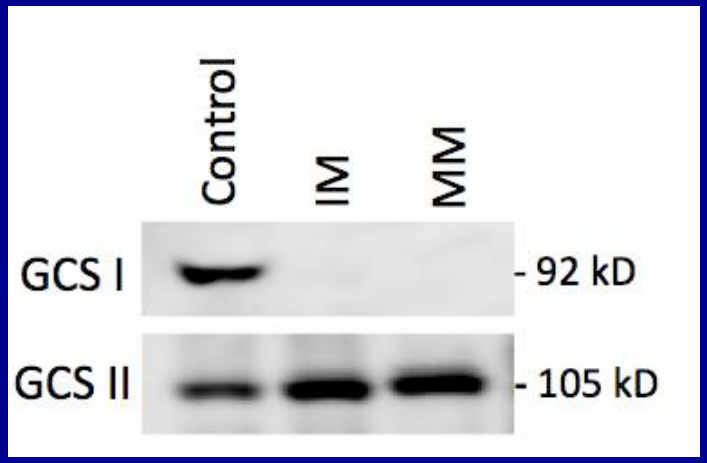
Deficiency of Glucosidase I?

- In N-linked glycoprotein synthesis, $Glc_3Man_9GlcNac_2$ is transferred to polypeptides in the ER.
- Glucosidase I is the first trimming enzyme, removing the terminal glucose.
- Further trimming allows for creation of complex oligosaccharide on N-linked glycoproteins
- Glucosidase I deficiency is Congenital Disorder of Glycosylation IIb (1 patient).

Mutation Analysis of Glucosidase I Gene

Both affected children are compound heterozygous for:

- **Exon 1: c.65C>A, p.A22E; c.329G>A, p.R110H**
- **Exon 2: c.370C>T, p.124Q>X**

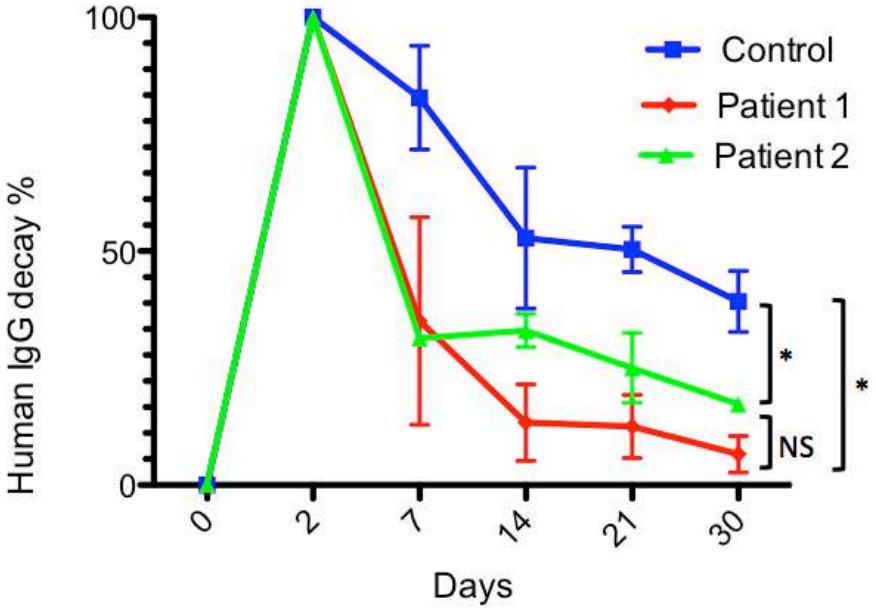


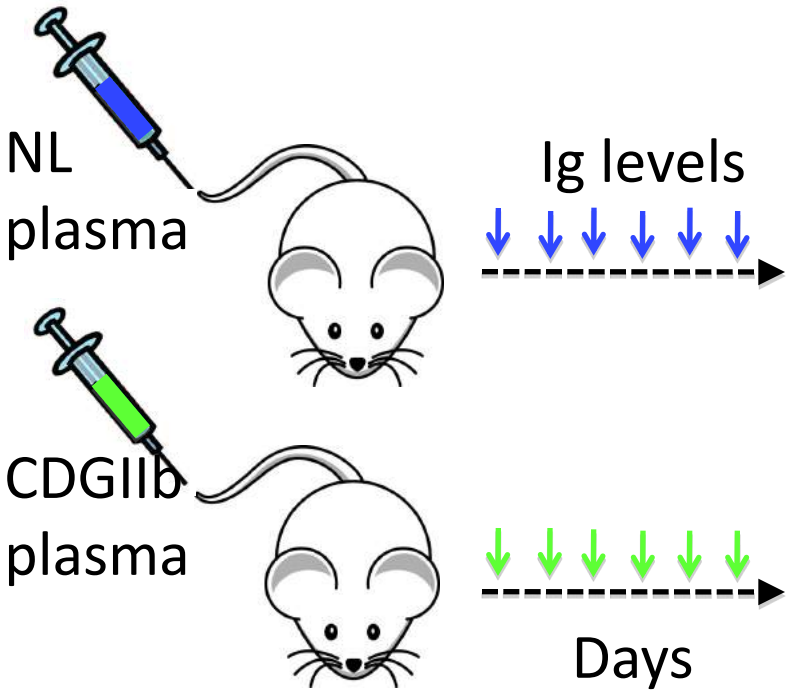
They are the 2nd and 3rd patients in the world with CDG IIb.

CDGIIb Patients

- Hypogammaglobulinemia
  - IgG 142 (504-1465 mg/dL)
  - IgA 18 (27-195 mg/dL)
  - IgM 25 (24-210 mg/dL)
- Why?
- IgG, lacking oligosaccharides in patient, is rapidly degraded.

Hypo/agammaglobulinemia Evaluation: IgG Half-life in RAG1 SCID Mice





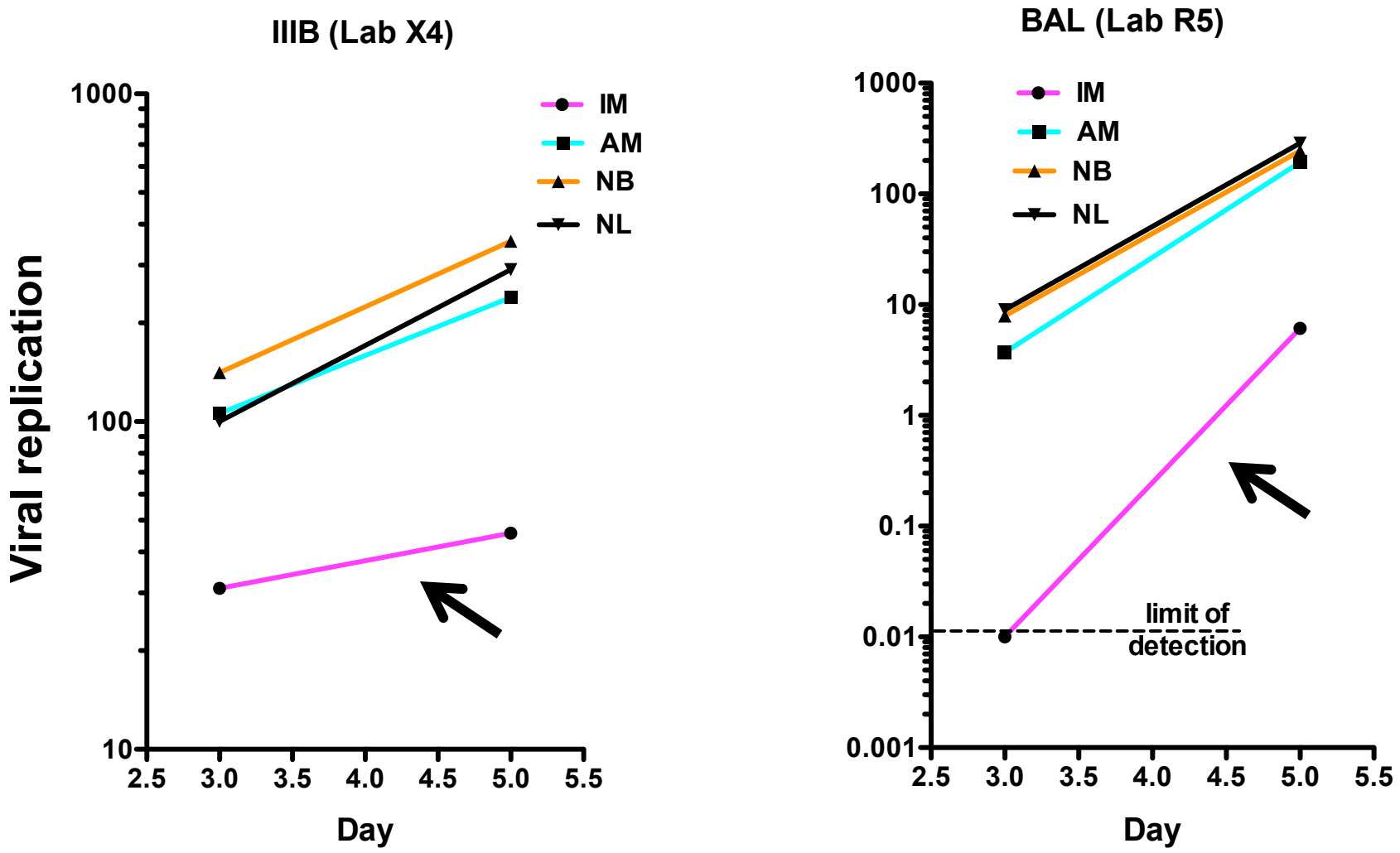
IgG half life: **Control 21 days**
Patient 6 days

Dr. Sergio Rosenzweig

Despite hypogammaglobulinemia, CDGIIb patients do not get infections!

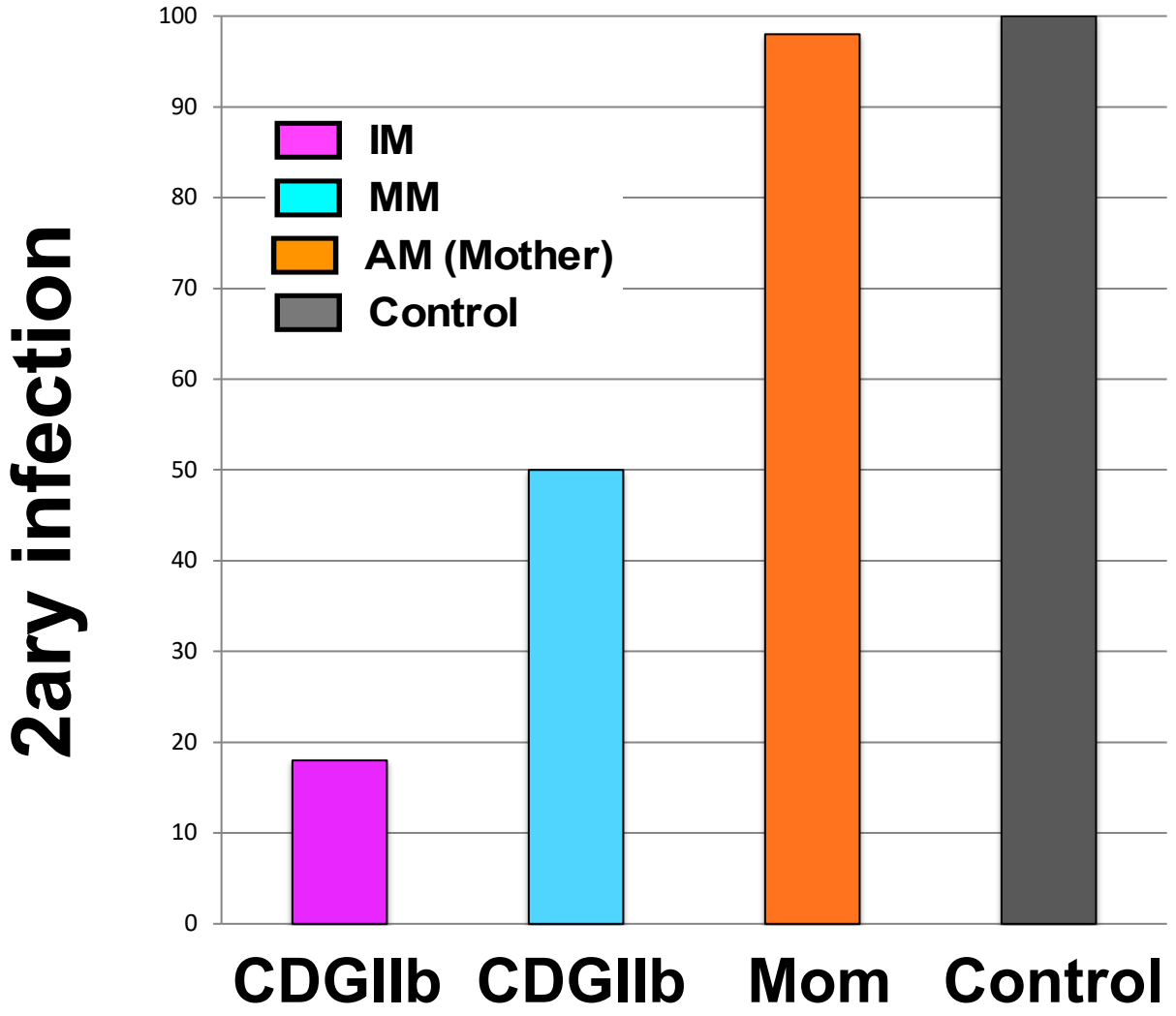
WHY?

CDGIIb cells could be infected with virus, but once infected, they produced much less virus.



Dr. Sergio Rosenzweig

And the virus produced is 50-80% less infective, as gauged by the ability to produce a secondary infection.



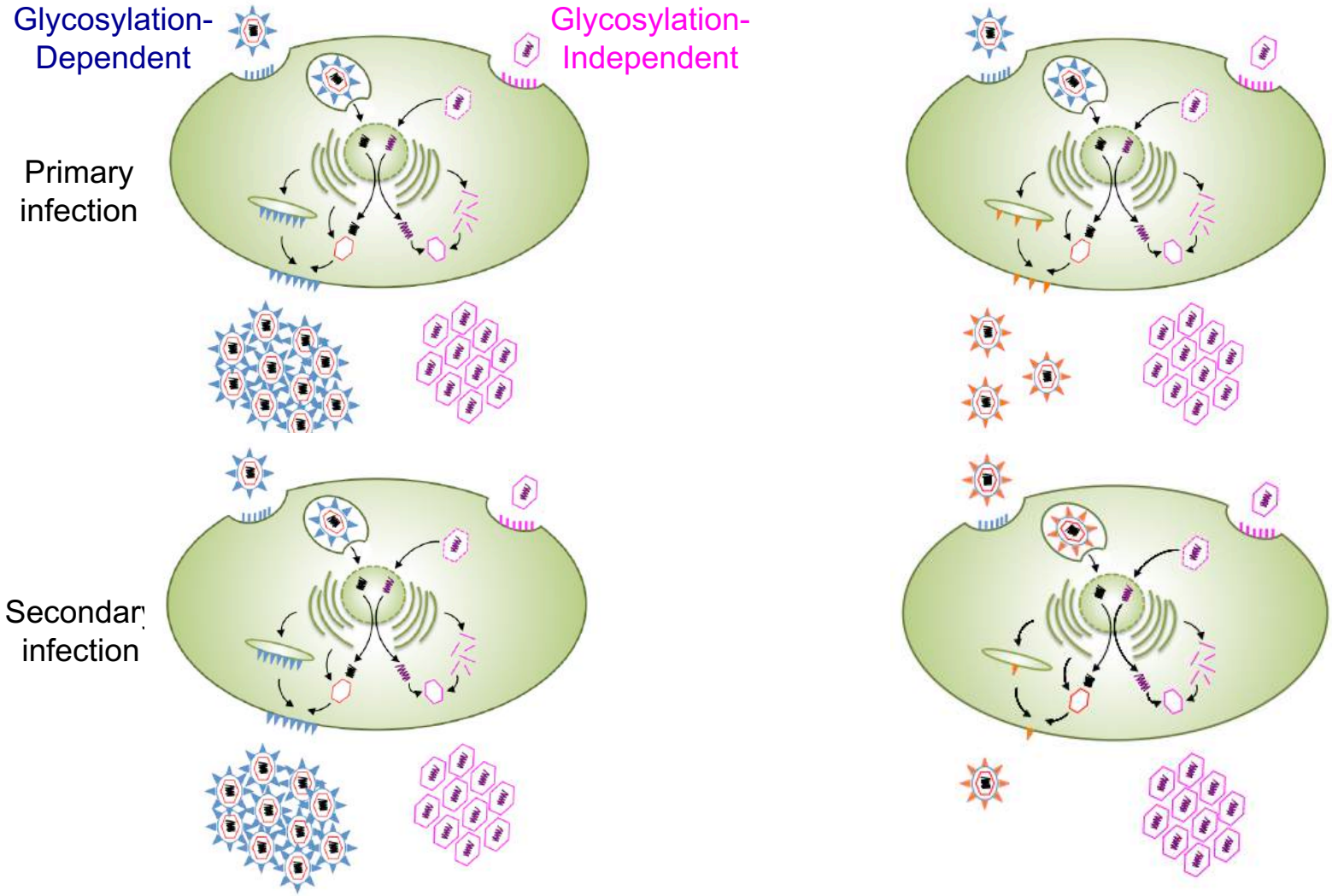
Dr. S. Rosenzweig

Dr. Sergio Rosenzweig

Viral susceptibility model

Infections in WT cells

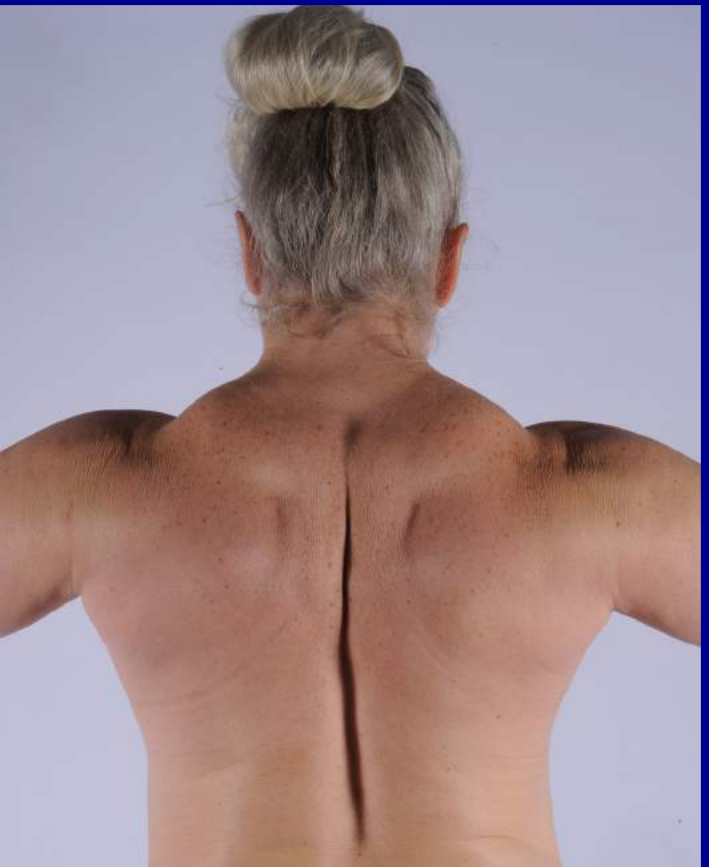
Infections in CDGIIb cells

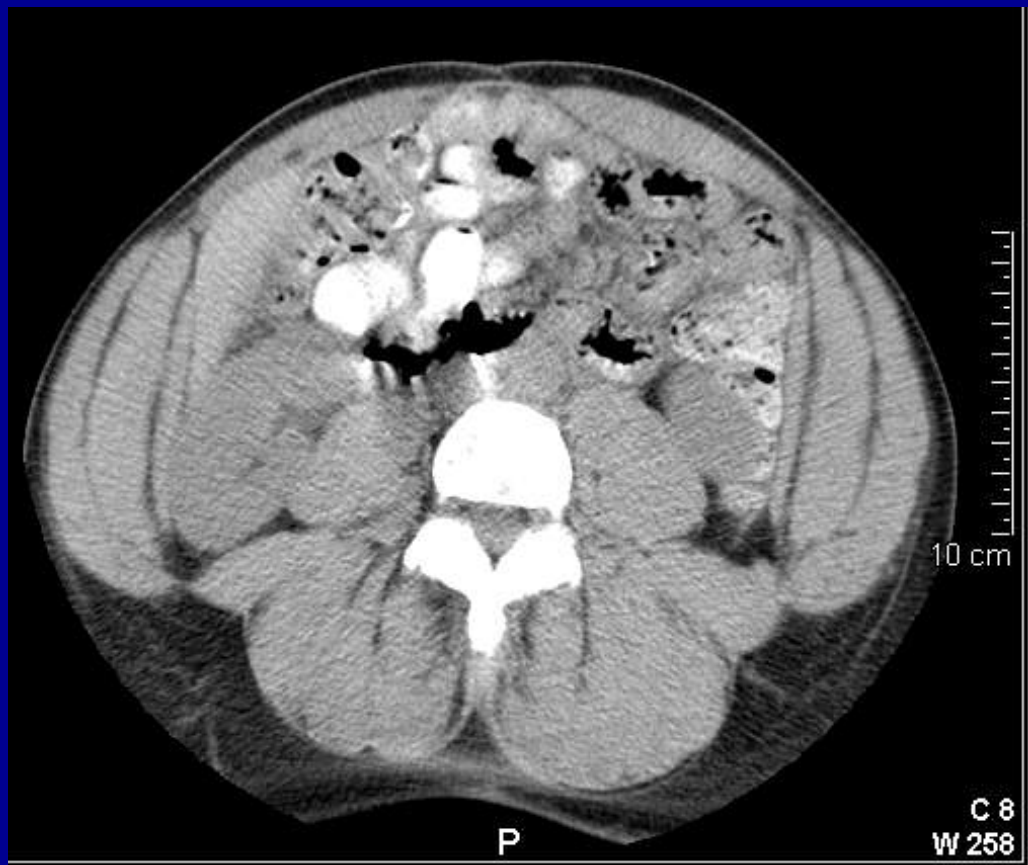


Diagnoses

52 Year old woman with increased muscle without increased strength

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



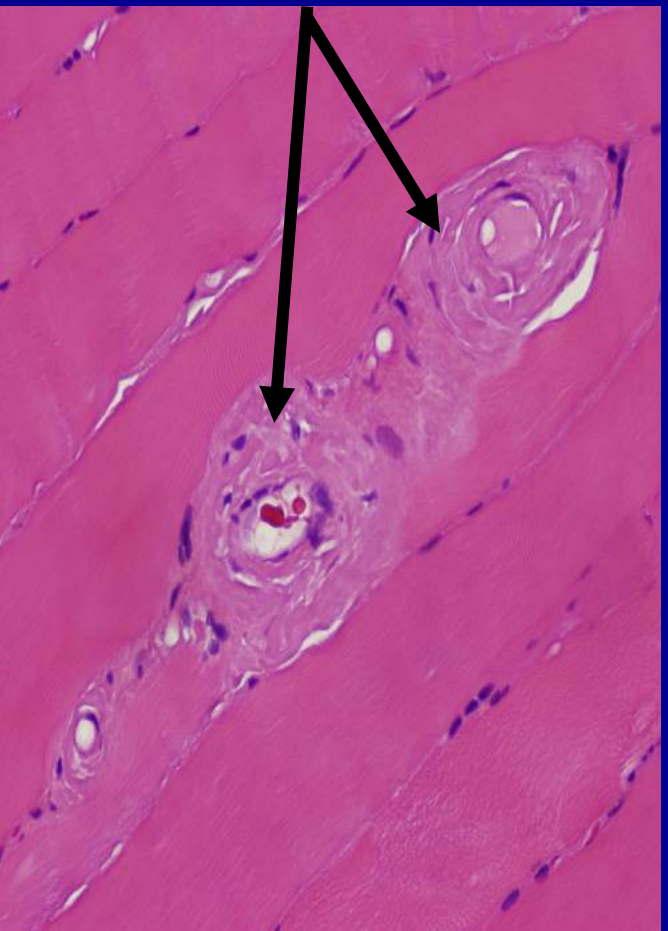


52 Year old woman with increased muscle:

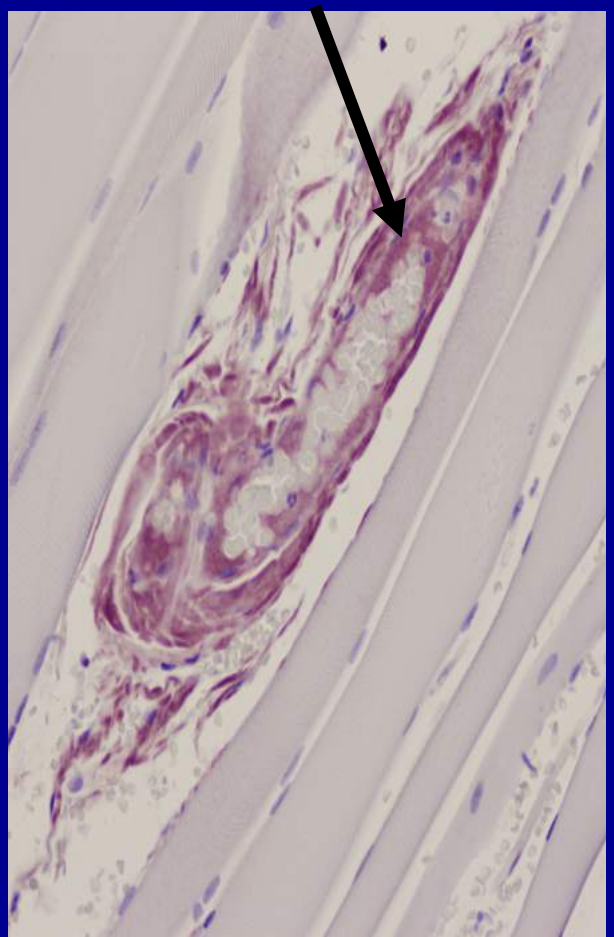
Amyloid Myopathy

Bone marrow: 10% plasma cells

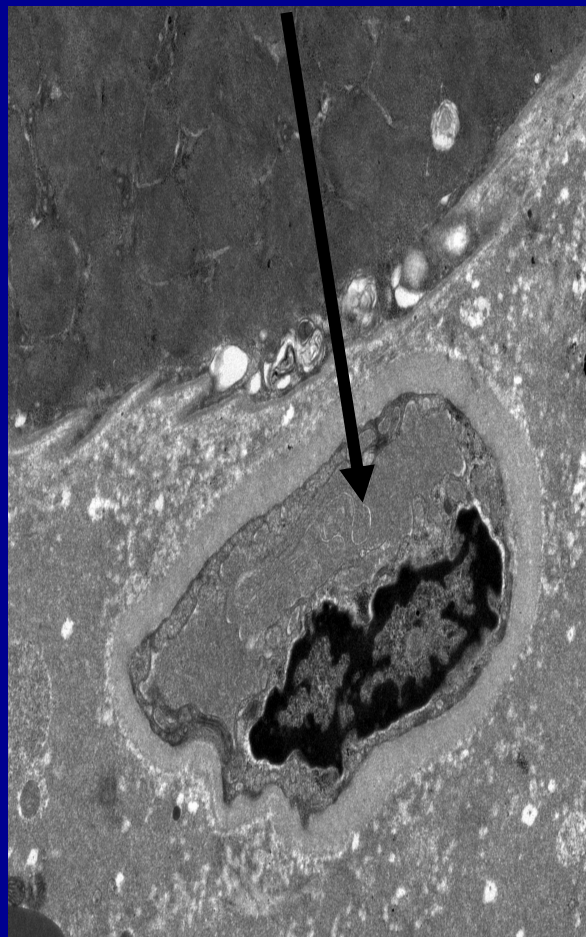
Thick vessel wall



Congo Red Stain



Protein aggregate



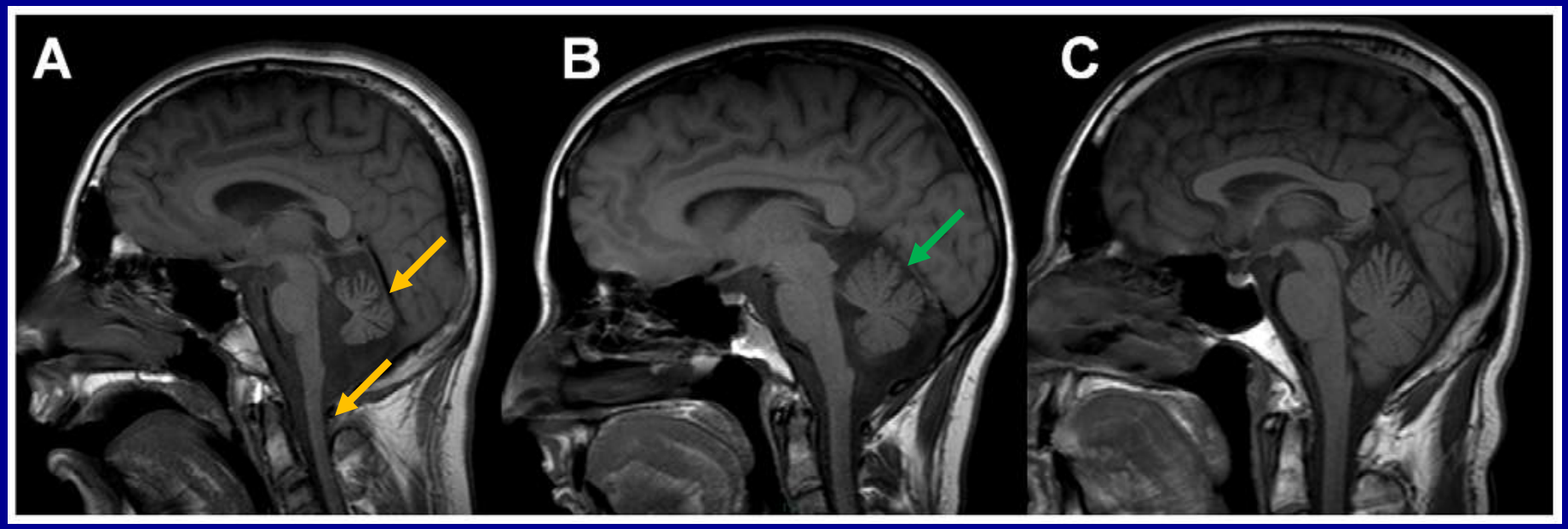
Outcome (Dr. Irini Manoli)

- 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.

Brothers age 13 & 14; parents first cousins

- One lost milestones at 18 mo & died at age 13.
- Other seen at NIH.
  - 14 mo: Ataxic; later myoclonic
  - 8 years: Seizures; stopped walking and talking.
- MRI: Cerebellar atrophy; small pons & medulla
- Labs negative; abn muscle mitochondria & cyt C oxidase

MRI Imaging: Spastic Ataxia-Neuropathy Syndrome



Proband Mother Father

Whole exome sequencing:

Model: *Parents first cousins*
Brothers homozygous
Parents heterozygous

Operation	Variants
None	120,469
1000 genome	59,482
SNP chip linkage	53,087
Homo recessive	11
Homo rec/not in db130	3

Homozygous T>C causing p.Tyr616Cys in the *AFG3L2* gene.

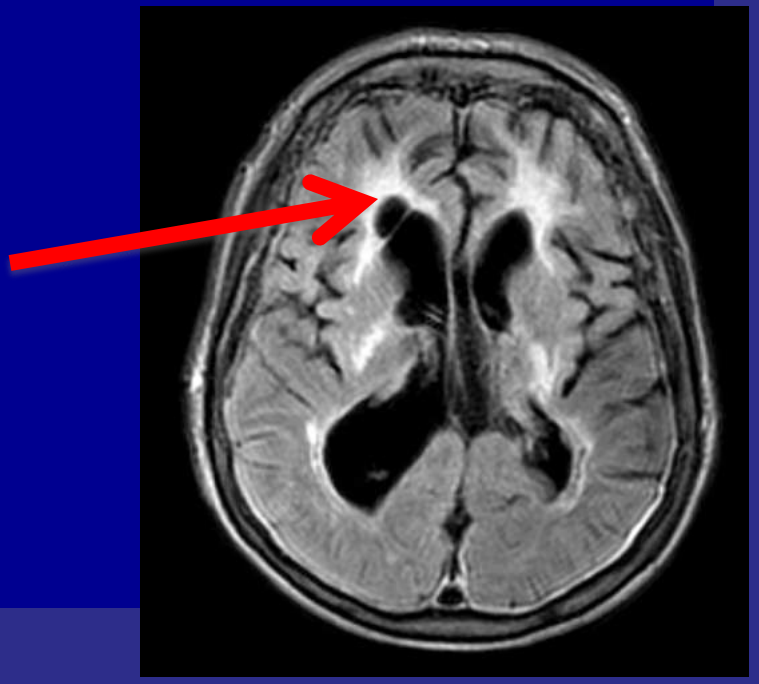
AFG3L2

- Mitochondrial m-AAA protease functions in axon formation; 2 isozymes
  - AFG3L2 and paraplegin (SPG7) hetero complex
  - AFG3L2 homo complex
- *AFG3L2*: Dominant Spinocerebellar ataxia (SCA28); no myoclonus or epilepsy
- Paraplegin: Recessive Hereditary Spastic Paraplegia (SPG7)
- First patient with bi-allelic muts in *AFG3L2*
- Expands the SCA28 phenotype to include progressive myoclonic epilepsy.

Hereditary Diffuse Leukodystrophy with Spheroids

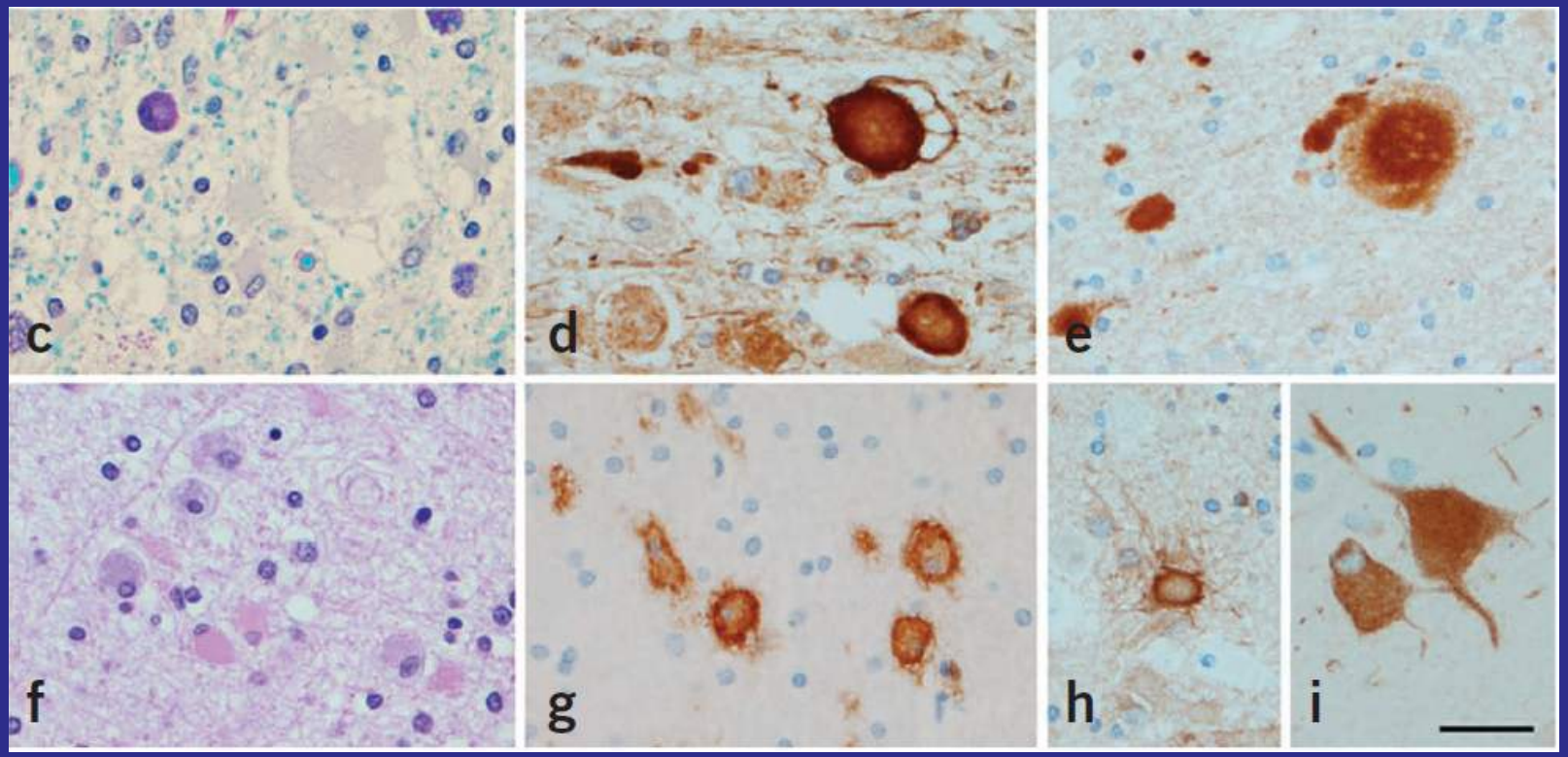
Described in 1984 in a large Swedish pedigree

- Pathological features
  - ✓ Enlarged ventricles as a result of WM loss
  - ✓ Diffuse astrogliosis
  - ✓ Axonal loss
  - ✓ Axonal spheroids
- Clinical Features:
  - ✓ Presenile dementia
  - ✓ Depression
  - ✓ Parkinsonism
  - ✓ Spasticity



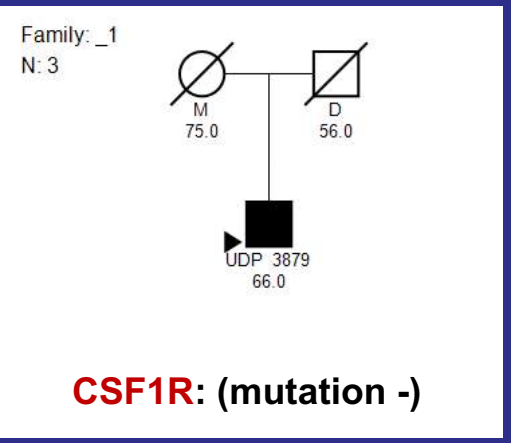
UDP_5688

Loss of CSF1R kinase activity causes Hereditary Diffuse Leukoencephalopathy with Spheroids

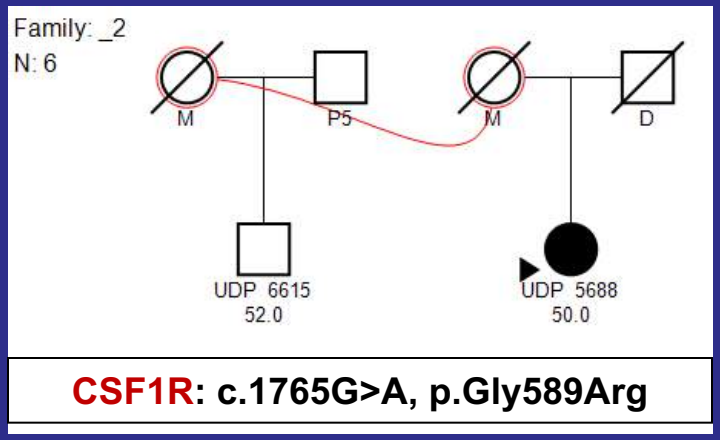


Rademakers R, Baker M, et al. Nature Genetics, 2011.

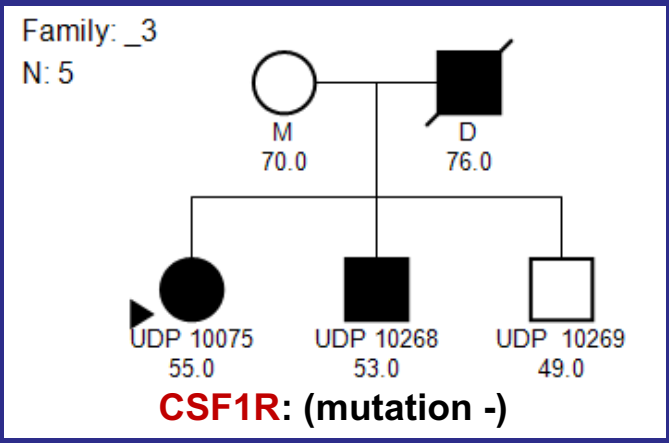
UDP Families with HDLS



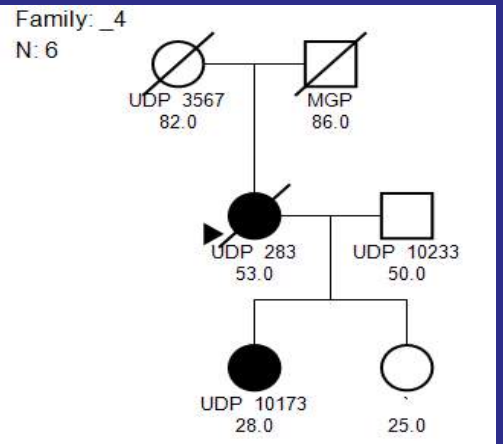
CSF1R: (mutation -)



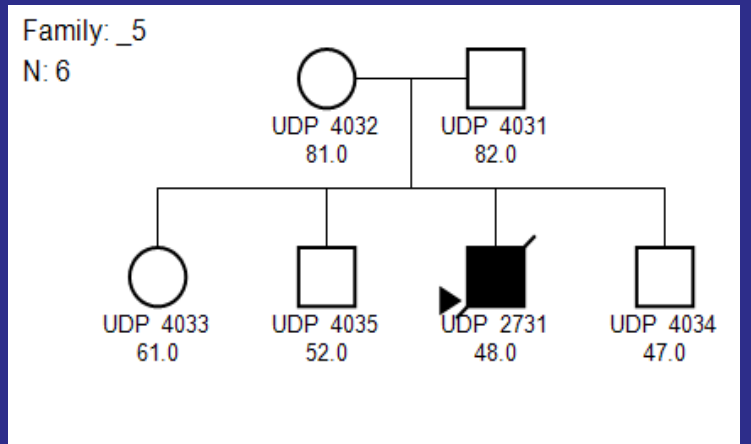
CSF1R: c.1765G>A, p.Gly589Arg



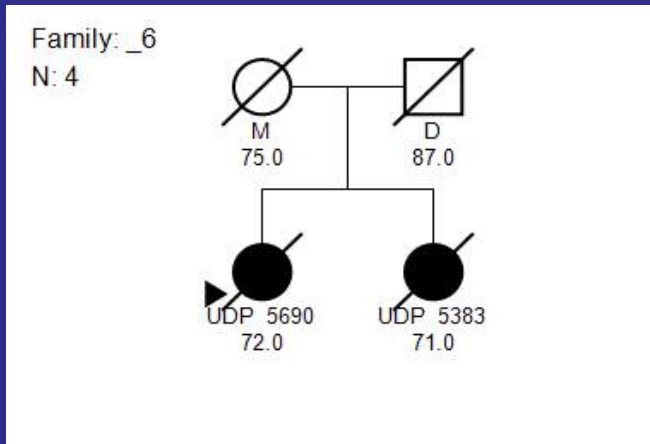
CSF1R: (mutation -)



CSF1R:c.2381T>C, p.Ile794Thr



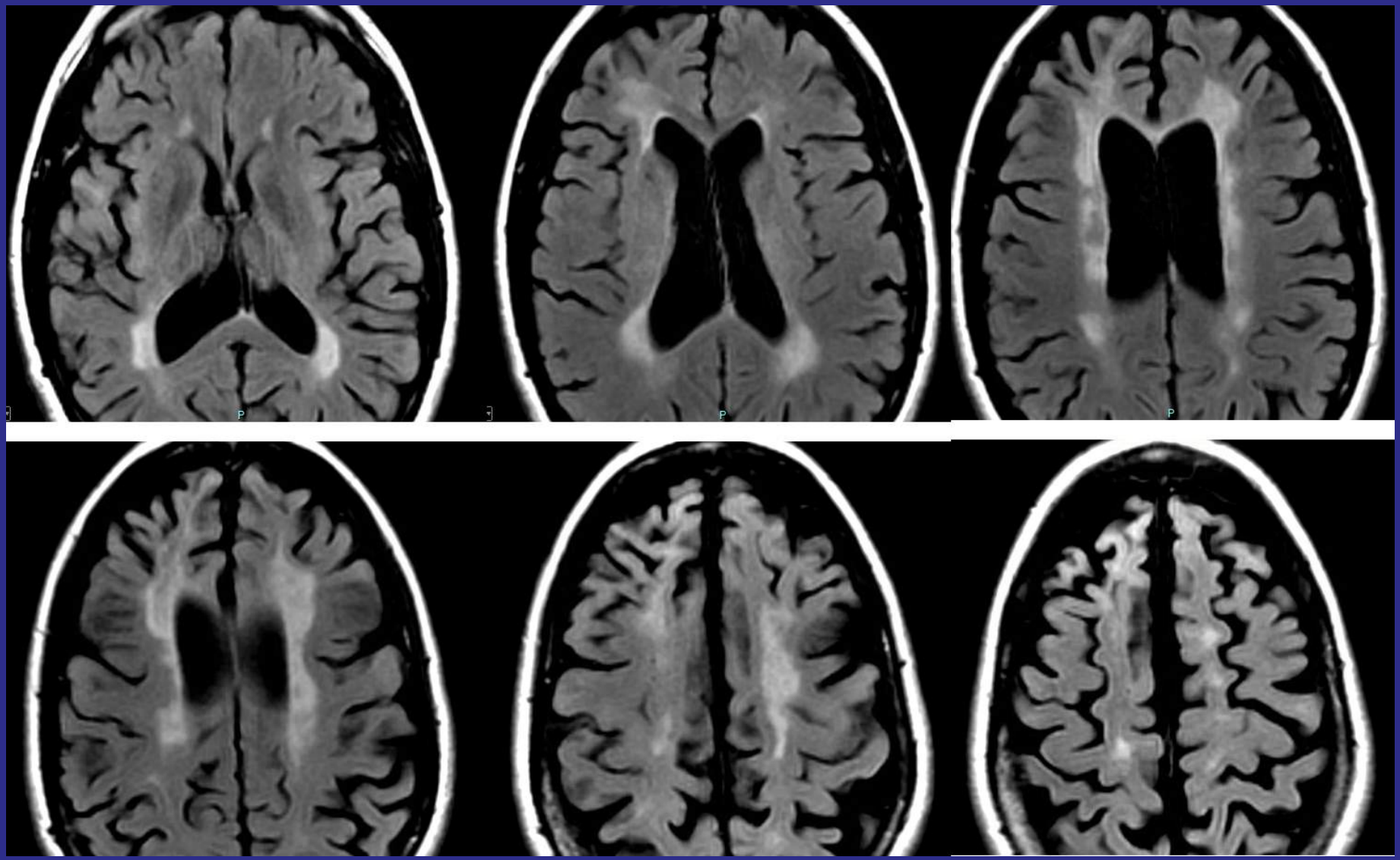
CSF1R:c.2512G>T, p.Val838Phe



CSF1R: (mutation -)

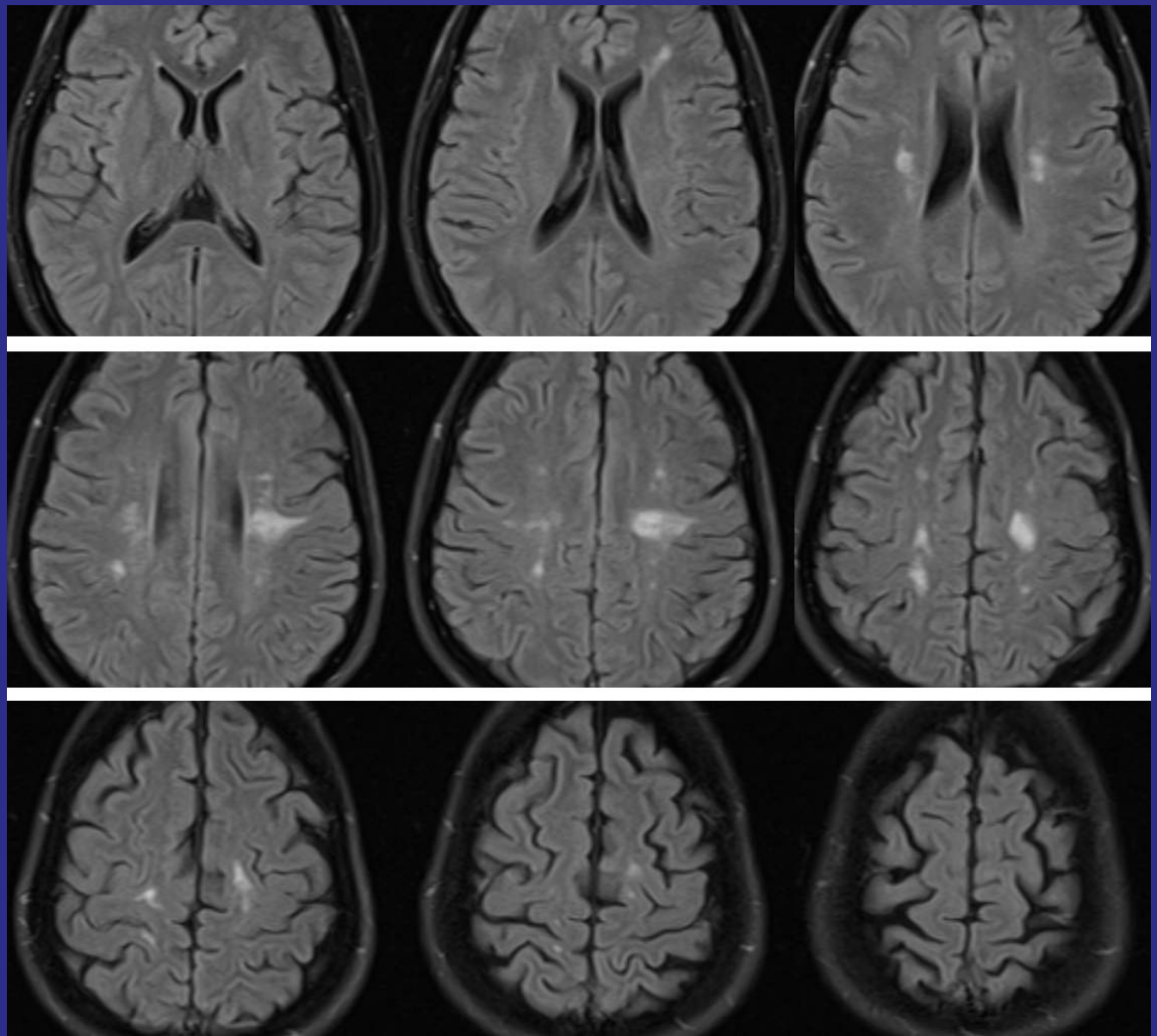
Courtesy of Camilo Toro, MD

UDP_283 Age 44



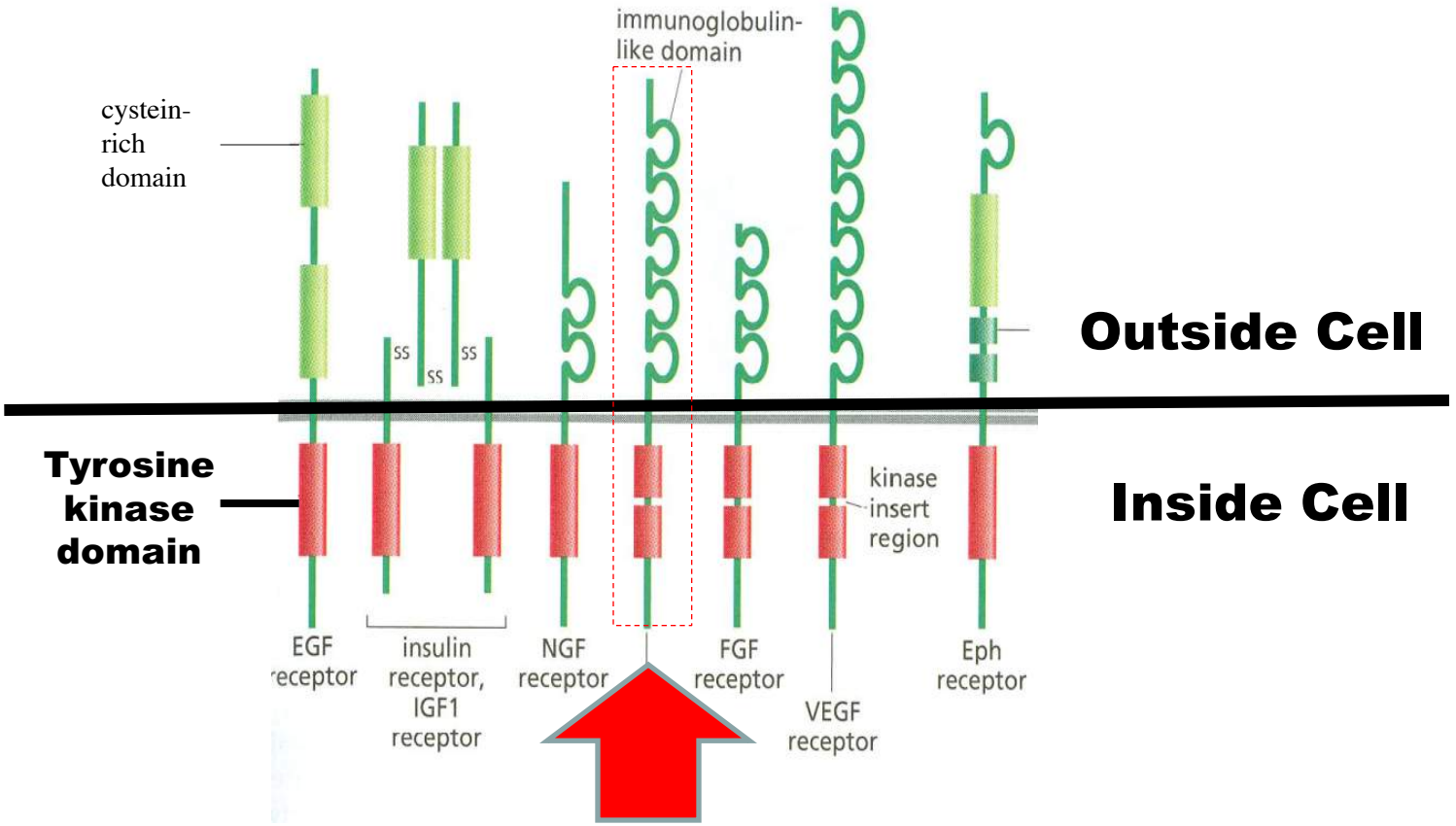
Courtesy of Camilo Toro, MD

UDP 283 Daughter (age 26) - Probably youngest patient ever



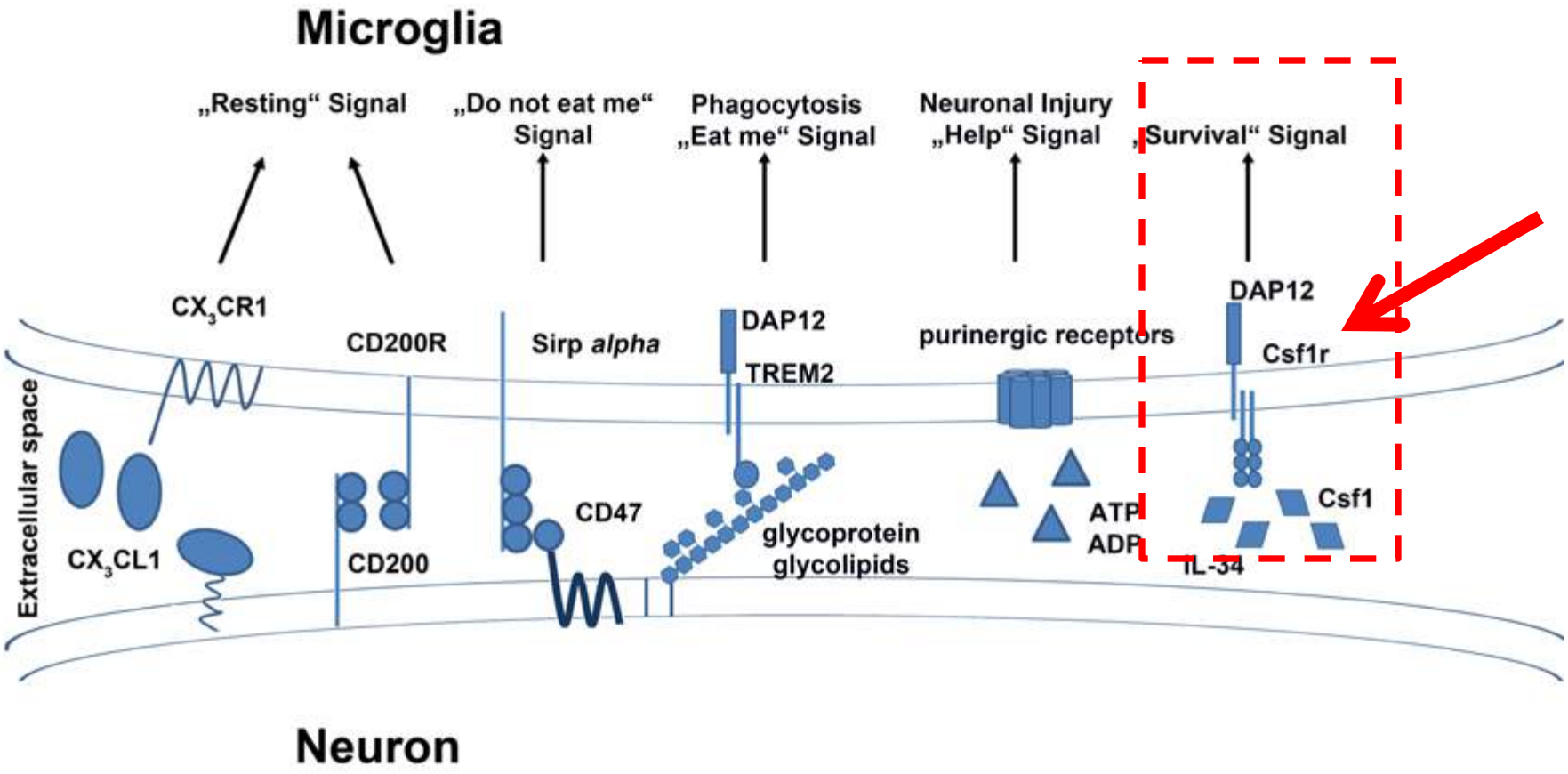
Courtesy of
Camilo Toro, MD

CSF1R – A Receptor Protein Tyrosine Kinase Responds to MCSF in Myeloid Cells.



Microglia derive from myeloid cells.
Neuronal-Microglial Signaling

1. Study this interaction when CSF1R is mutated.
2. Look for other mutations in the CSF1R pathway.



46 y.o. man with progressive neurological deficit (Left spastic hemiparesis and PBA)

- Negative family history
- Vacationed in Mexico as a child
- Age 14: Severe febrile illness weeks after trip to Mexico-- N/V, myalgia and diarrhea.
- Age 15: Numbness and tightness of left leg
  - Cystic lesions on cranial CT lead
  - Diagnosed as neurocysticercosis
  - Two courses of Praziquantel—No help
  - Off-balance with sports
- Progressive left spastic foot drop
- Recent Pseudobulbar affect (PBA)
- No seizures

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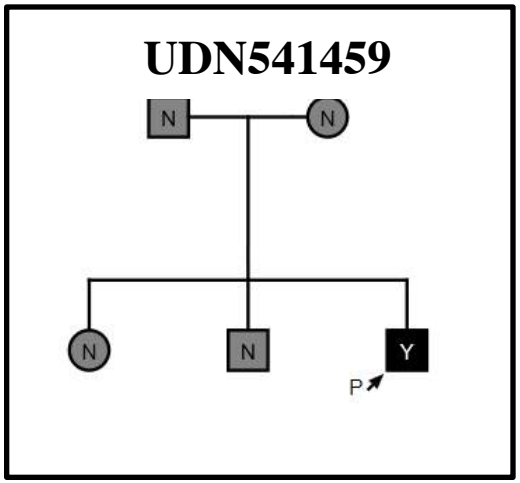

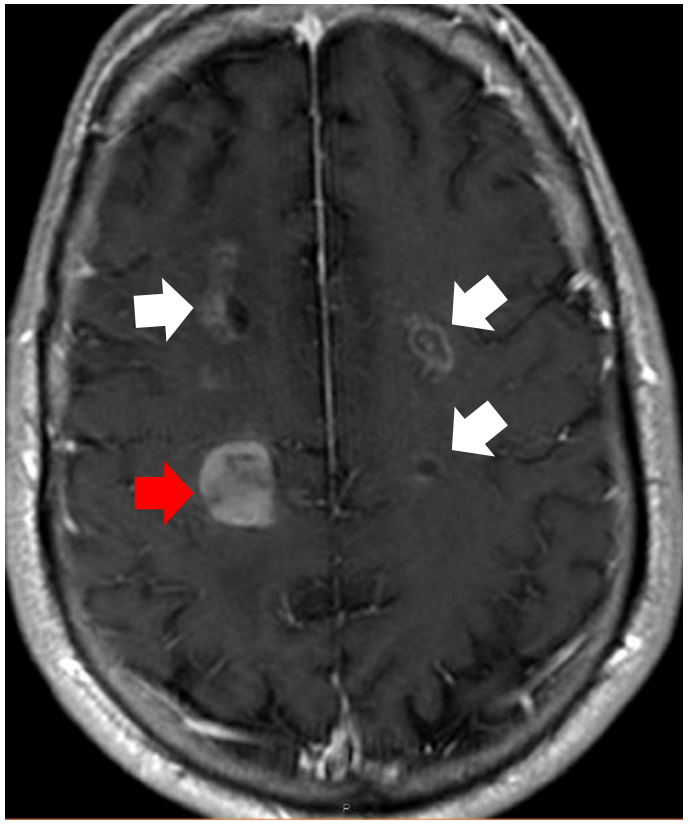

Multiple cystic and contrast enhancing brain lesions

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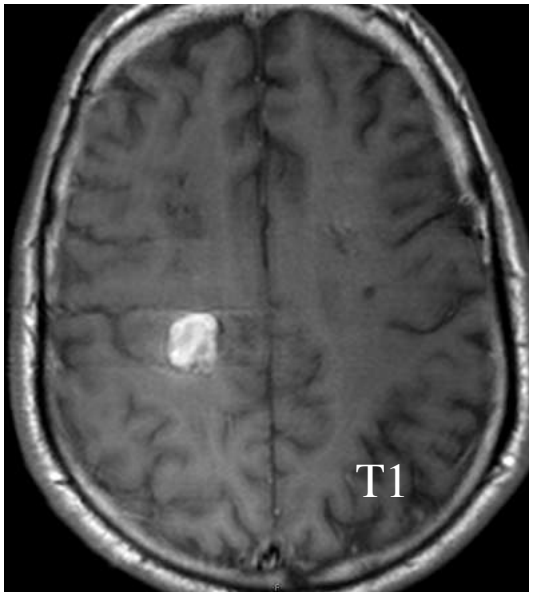
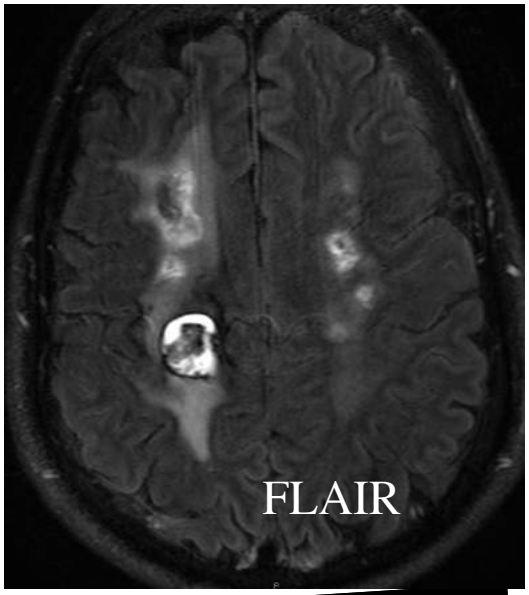
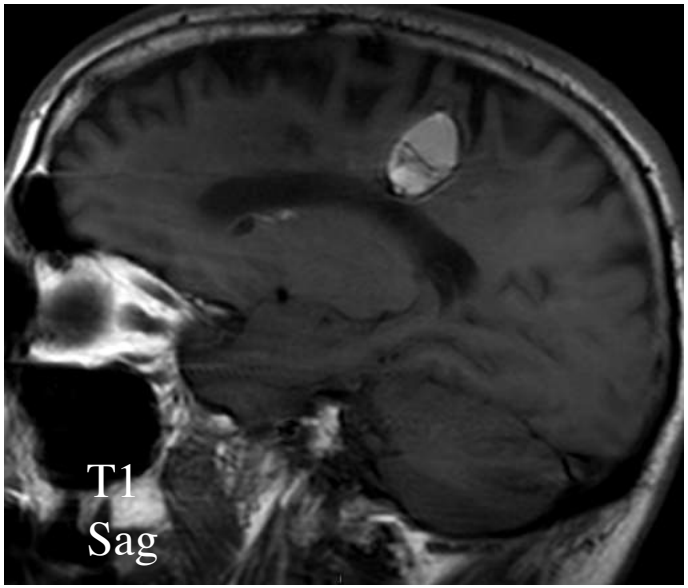
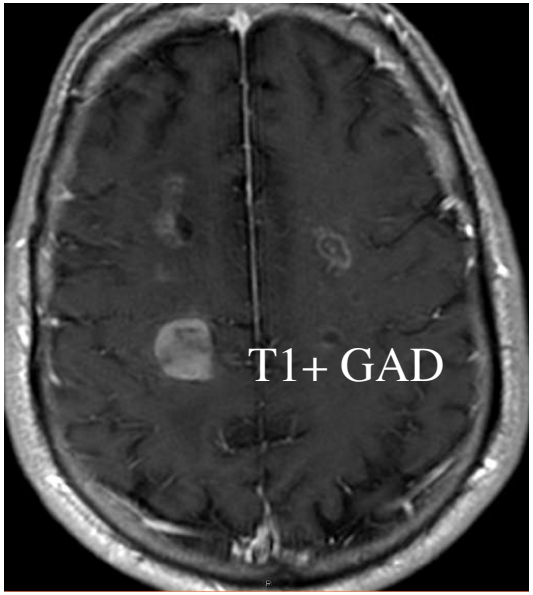
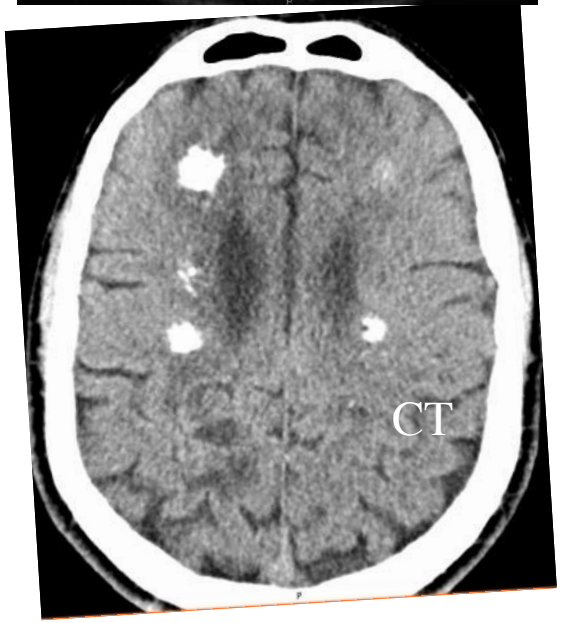
UDN541459

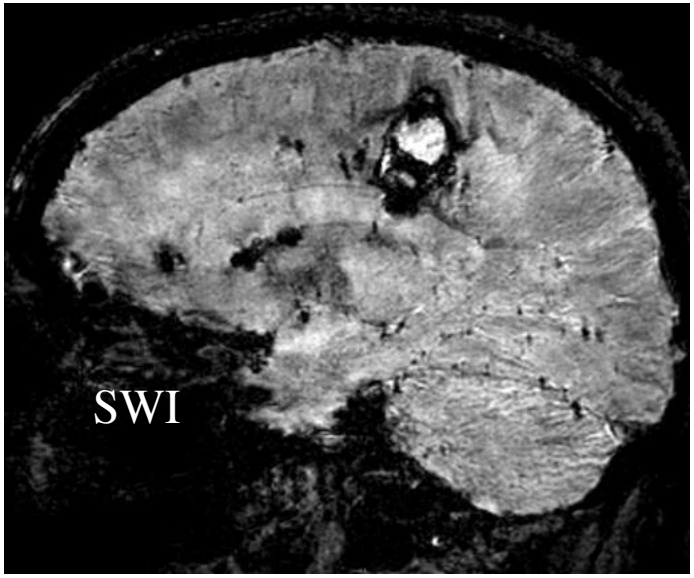












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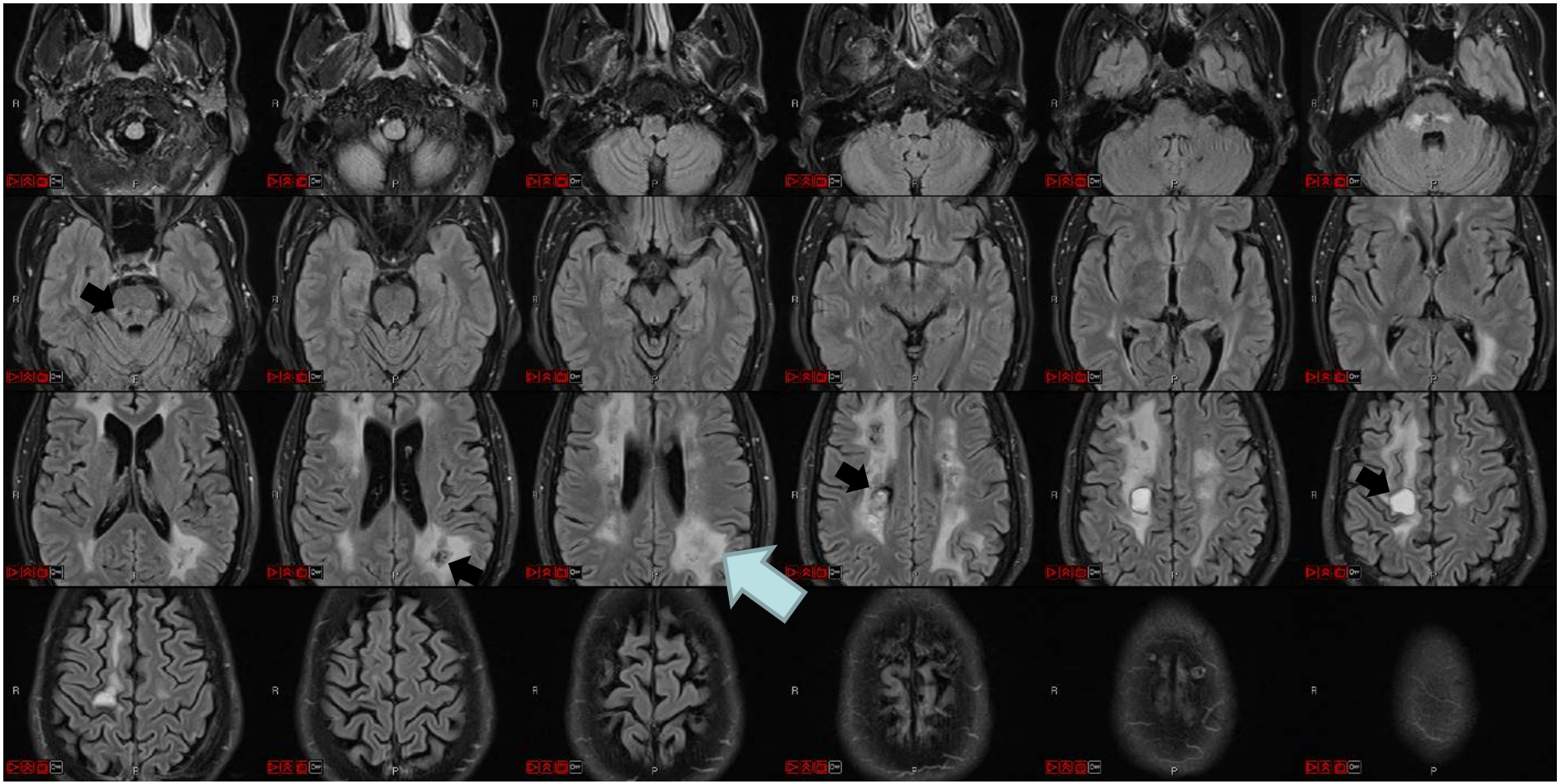

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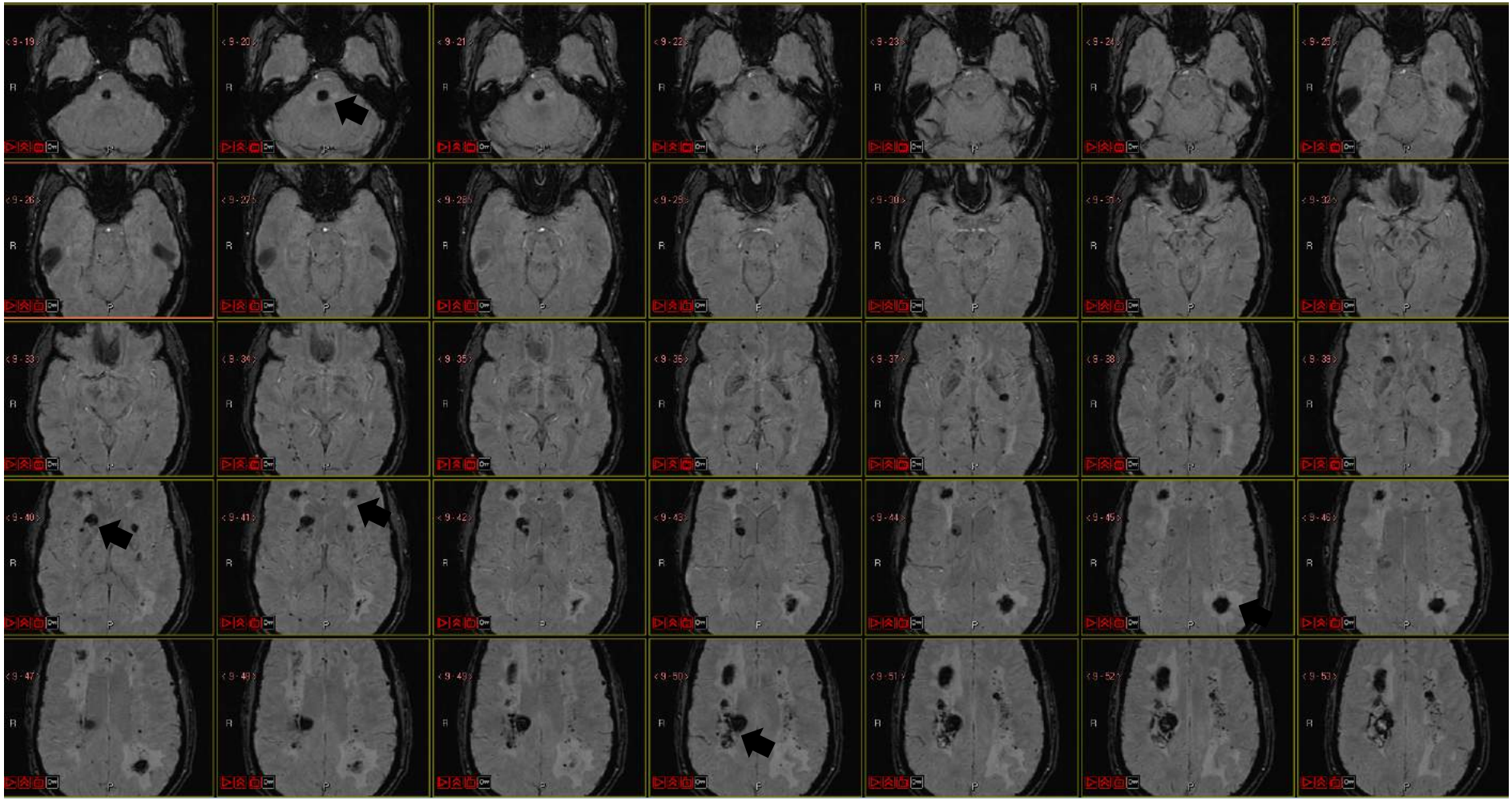

Evaluation (04/08/2016)

- Deep phenotyping
- Completely normal retina exam
- CSF:
  - No WBC
  - Normal Protein
  - Normal IgG Index
  - No Oligoclonal Bands (OCB)
  - Normal CSF lactate/CSF AAs/Neurotransmitters/CDC CC serology
- mtDNA sequencing -- Negative
- Quintet Exome Sequencing [*CTC1*, AGS (I-VII) genes, *PDGFBR*, *PDGFB*, *SLC20A2*, *CSF1R,* Leukodystrophy genes, etc.]
- Quartet WGS via UDN did not yield a good candidate

NIH Clinical Center
America's Research Hospital

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Camilo Toro, MD

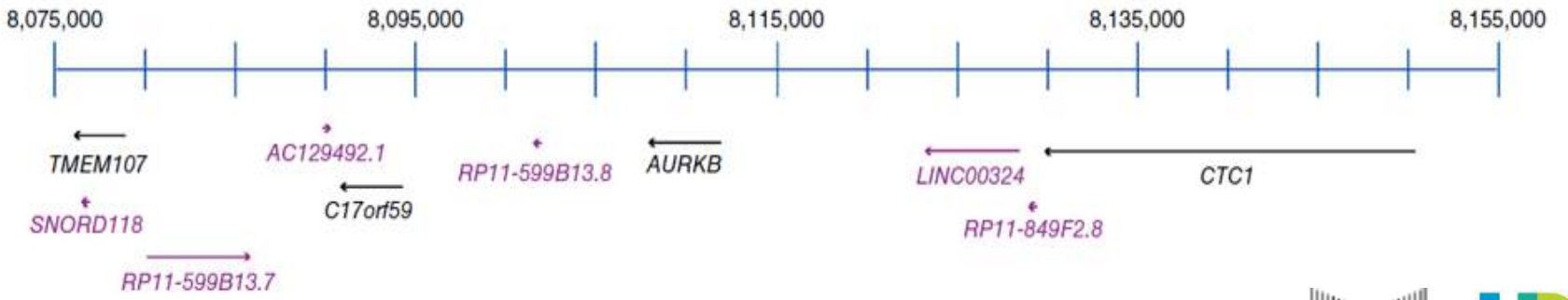
nature genetics

Mutations in *SNORD118* cause the cerebral microangiopathy leukoencephalopathy with calcifications and cysts

Received 14 September 2015; accepted 5 August 2016; published online 29 August 2016; doi:10.1038/ng.3661

NATURE GENETICS ADVANCE ONLINE PUBLICATION

17p13.1



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nature genetics

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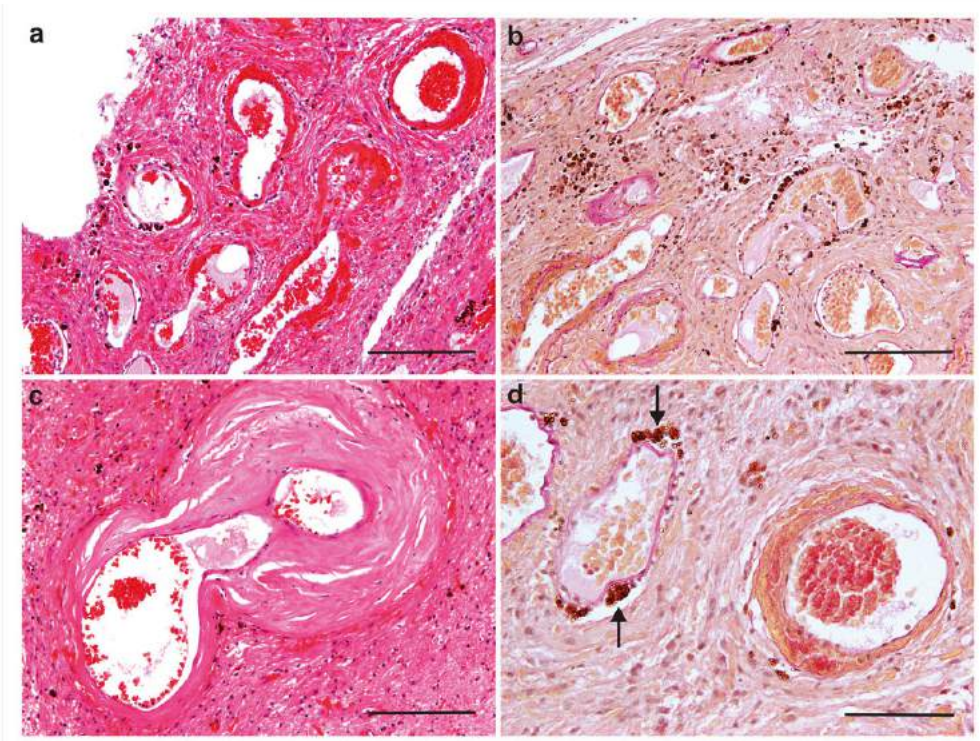
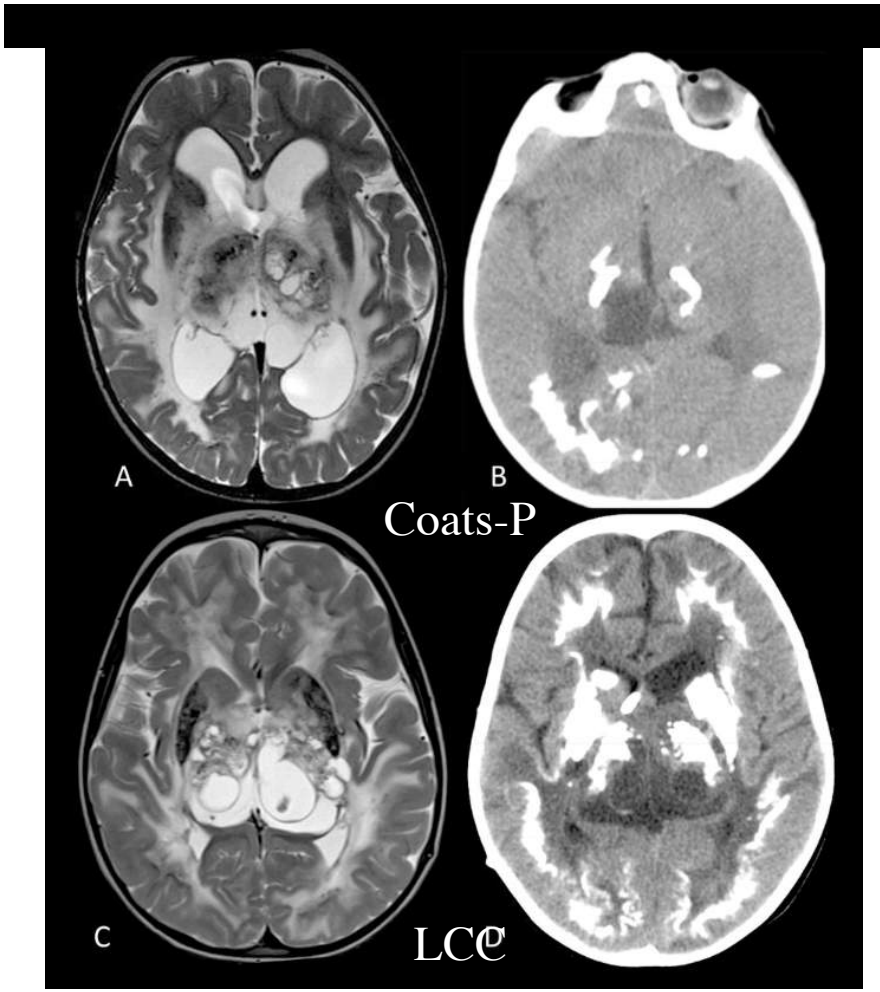


SNORD118-LCC is primarily a microangiopathy

MRI_T2 CT



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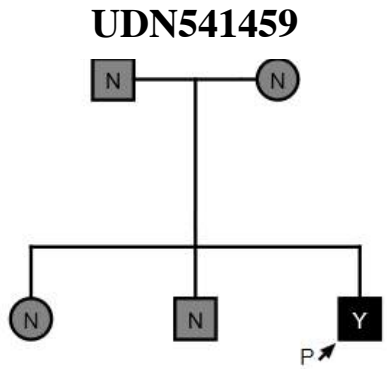
SNORD118 Sanger Sequencing



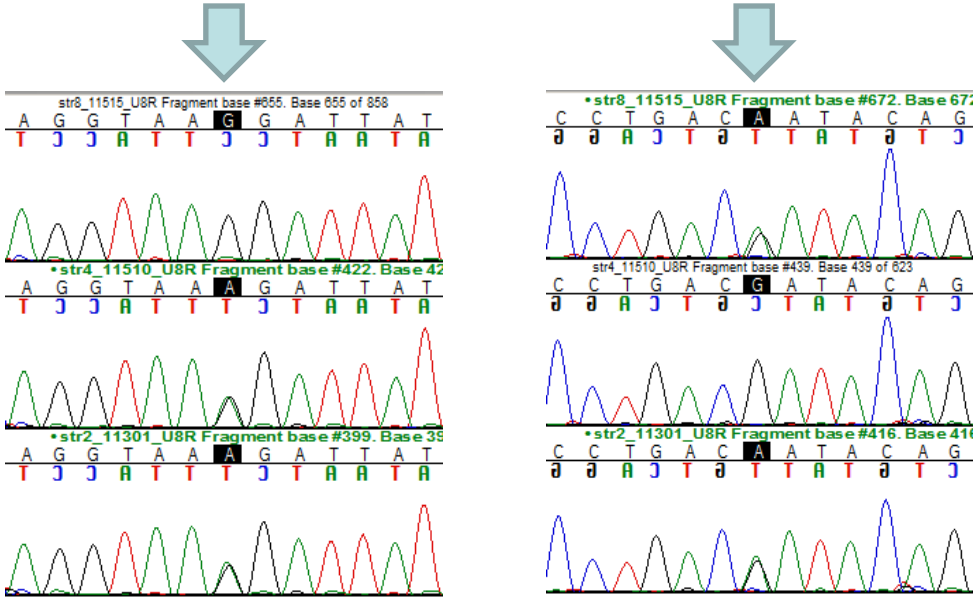
Variant: 17:8076904 G / A

Variant: 17:8076887 G / A

Father

Mother

Proband



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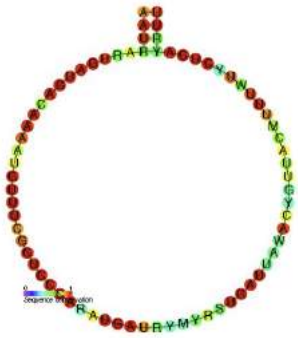
Small nucleolar RNAs (snoRNAs)

Definition: Small RNAs that guide chemical modifications of other RNAs, mainly rRNA, tRNA, and snRNA.

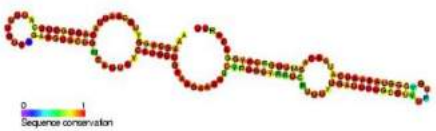
Two main classes of snoRNAs
- **C/D box** (methylation)
- **H/ACA box** (pseudo-uridylation)

  Mature rRNA Contains
  - ~115 methyl group modifications
  - ~ 95 pseudo-uridylation reactions

Important for ribosome biogenesis
Implicated in some disease states
- Paternal deficiency of HBII-85 C/D box small nucleolar RNA cluster implicated in forms of Prader-Willi Syndrome
- **But…mutations in snoRNAs had never been known to directly cause a Mendelian disorder**



C/D box



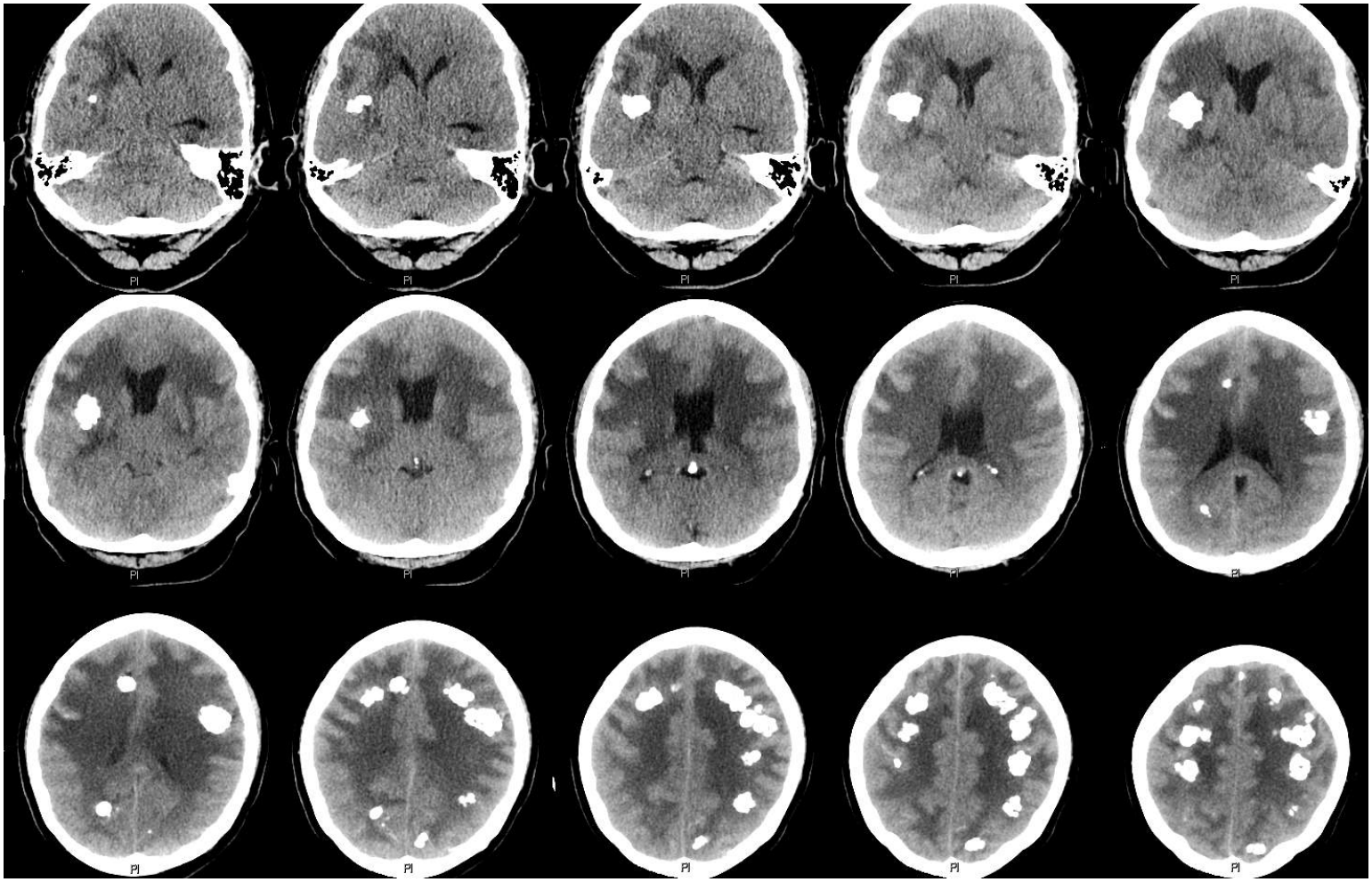
H/ACA

Undiagnosed Diseases Network

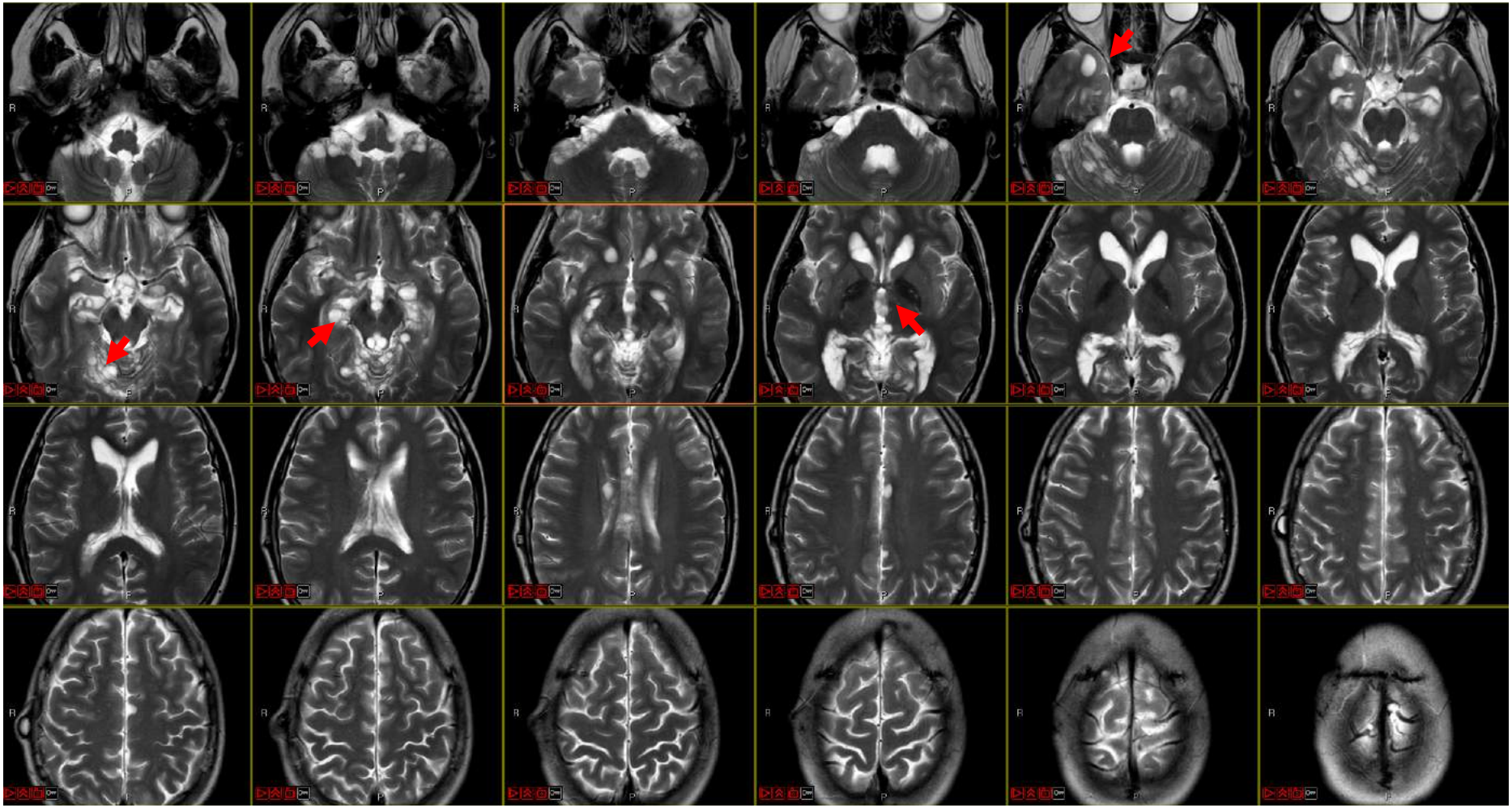
Camilo Toro, MD

There are still other undiscovered causes of Leukoencephalopathy, Calcifications and Cysts.

51 year-old woman with seizures



21 year-old male with seizures, hydrocephalus, & many cystic lesions



Rare Diagnoses - 1

- 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 - 2

- 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 - 3

- 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 - 4

- Congenital Disorder of Glycosylation type 2b (2^{nd} and 3^{rd} cases in world)
- Adducted Thumb-Clubfoot Syndrome & *CHST14* mutations (1^{st} case in U.S.)
- Spinocerebellar ataxia, myoclonic epilepsy & *AFG3L2* muts (1^{st} AR case)
- Autosomal Dominant Leukodystrophy & *LMNB1* duplication (~10 in world)
- 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

- 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

- 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-Goutierres due to *RNASEH2B* muts
- SPG11, NPC1, STIM1, GARS, A-T, NGLY1, MNGIE, CAV3

More Diagnoses

- 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

- 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

Recent Diagnoses

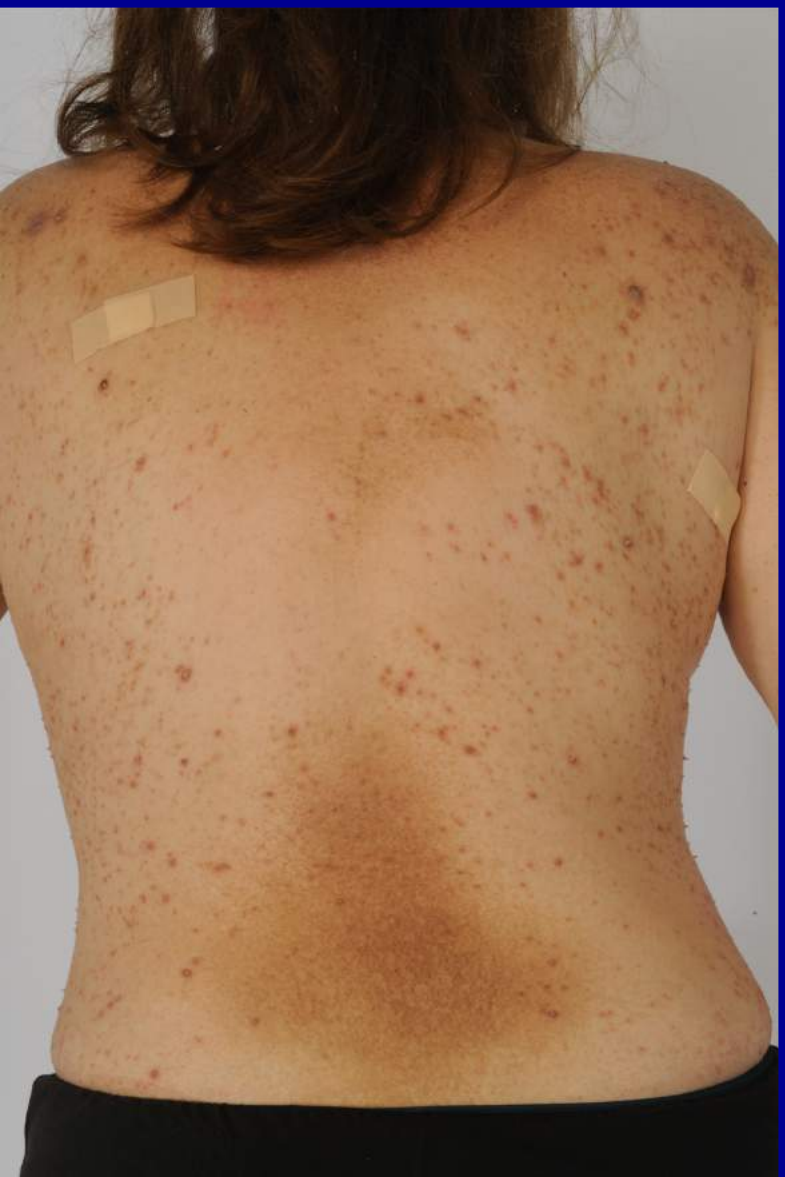
- Leukodystrophy & spheroids with CSFR1 muts
- Leucoencephalopathy, Calcifications, and Cysts due to SNORD118 mutations
- Oculodentodigital Dysplasia due to GJA1 mutation
- Dysmorphisms & delays due to TRAF7 mutation
- Microcephaly, dysmorphisms, autism spectrum due to CTNNB1 de novo
- Dysmorphisms &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

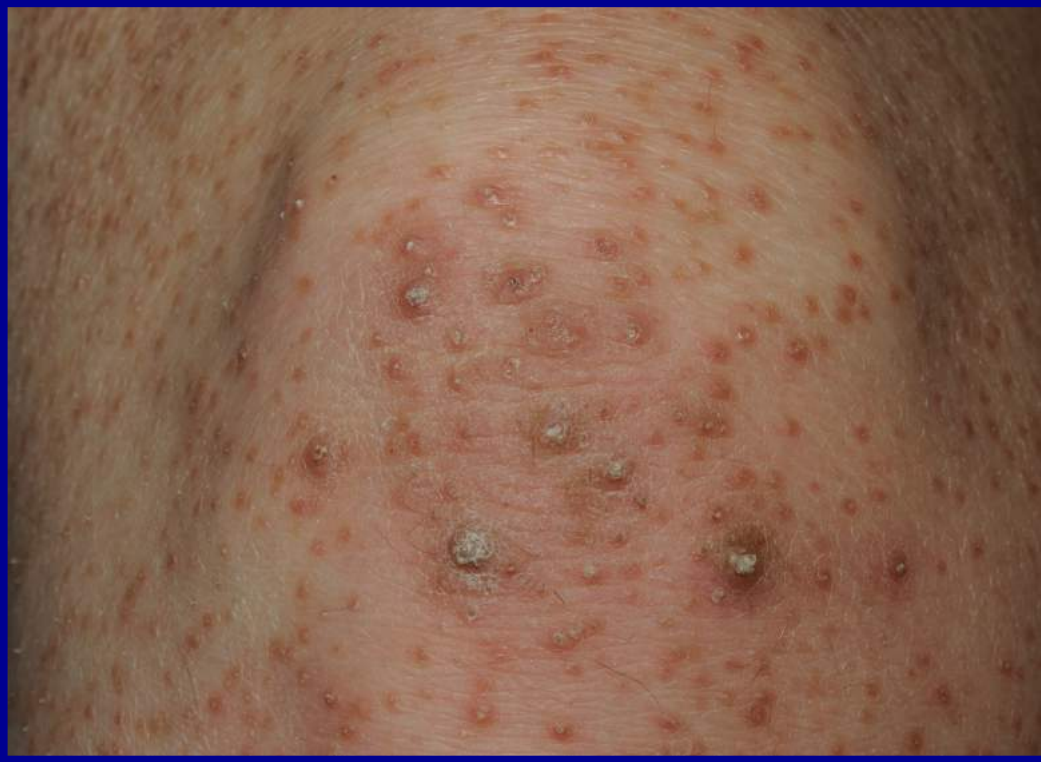
Recent Diagnoses

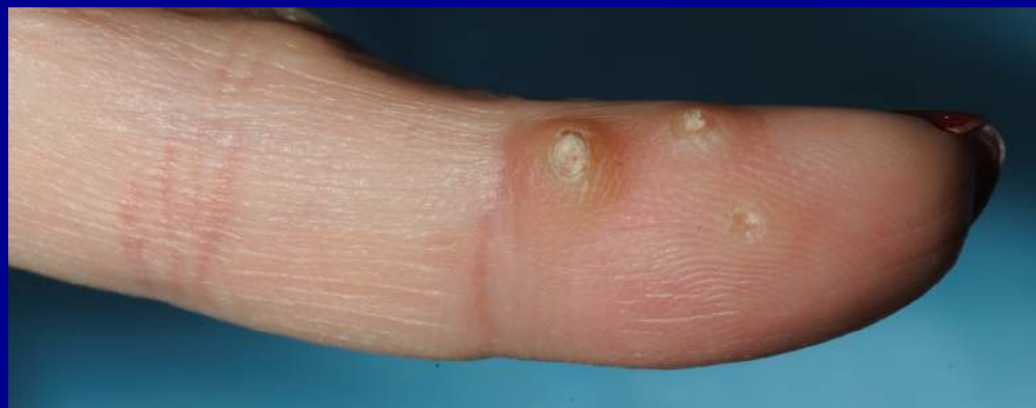
- Tremor and spasticity due to GAN de novo mutation
- Connective tissue and GI disorder due to TUBB2B de novo
- Mitochondrial disorder due to MTATP6 mutation
- Kleefstra Syndrome due to EHMT1 de novo

Mysteries

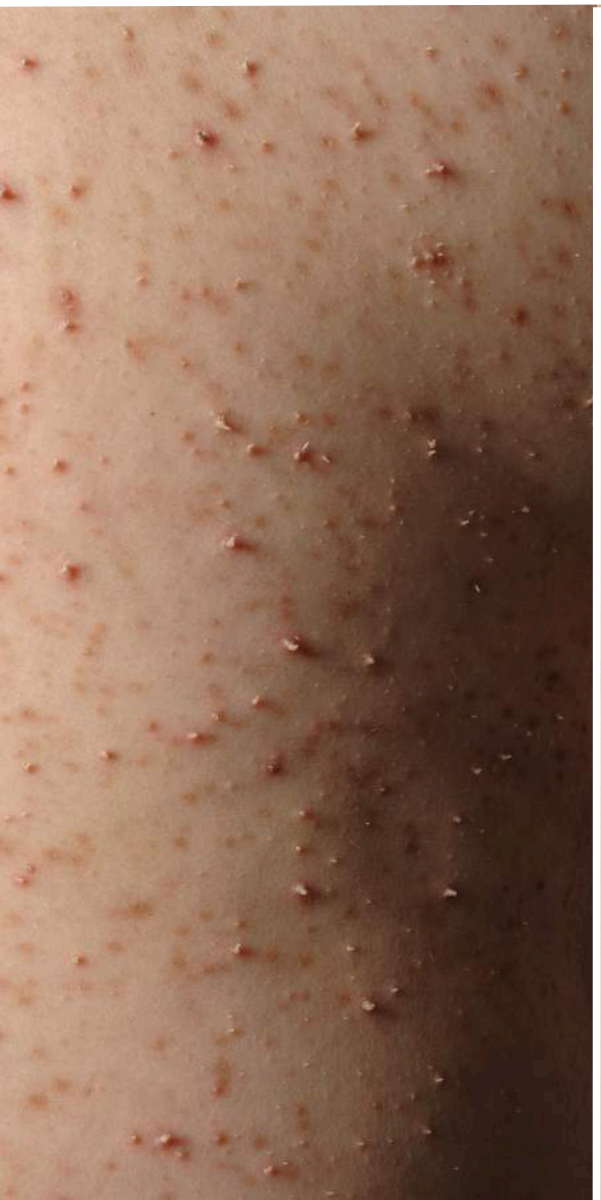
56 y-old woman with itchy, painful rash for 5y

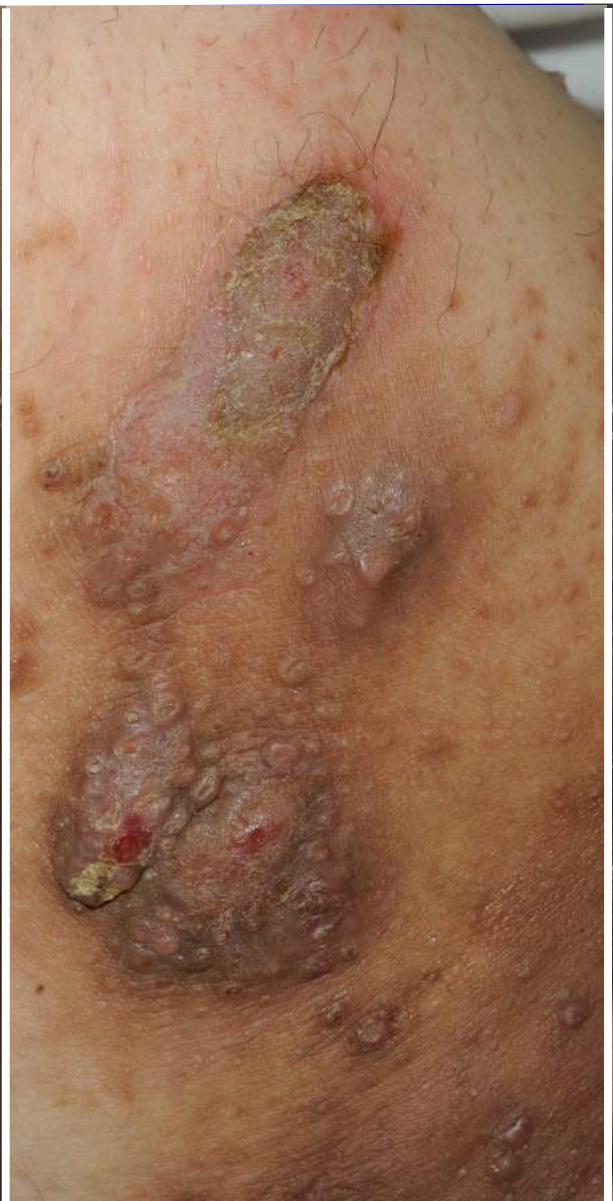


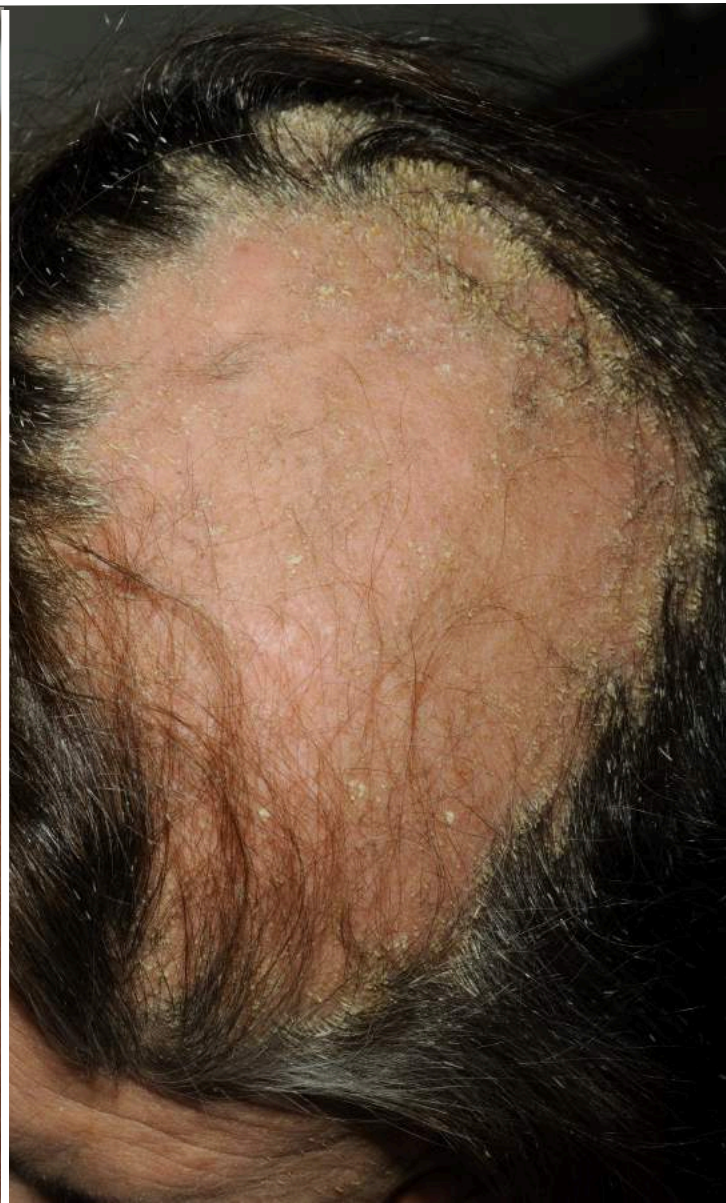




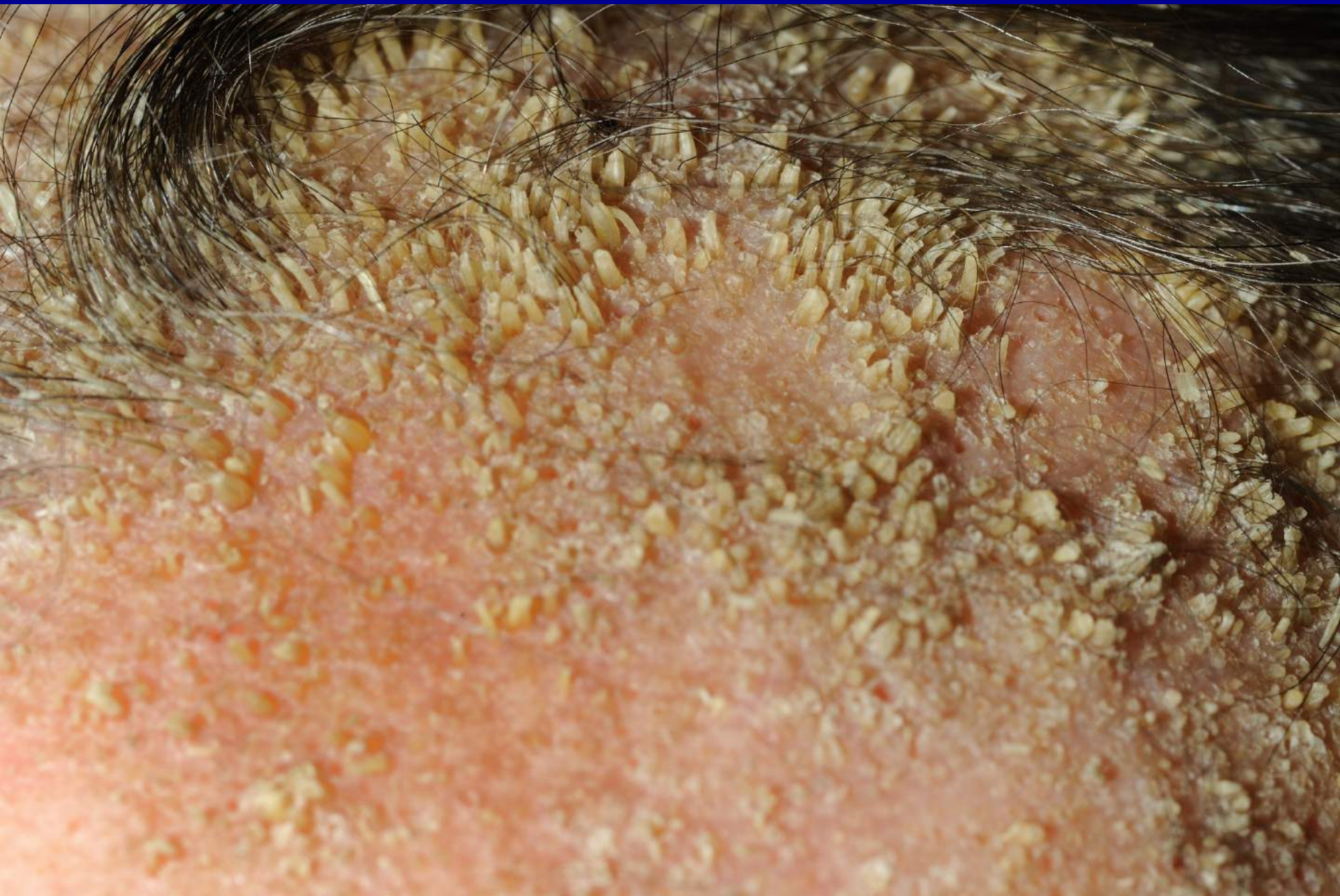
Rash worse with retinoid + PUVA 2 y ago

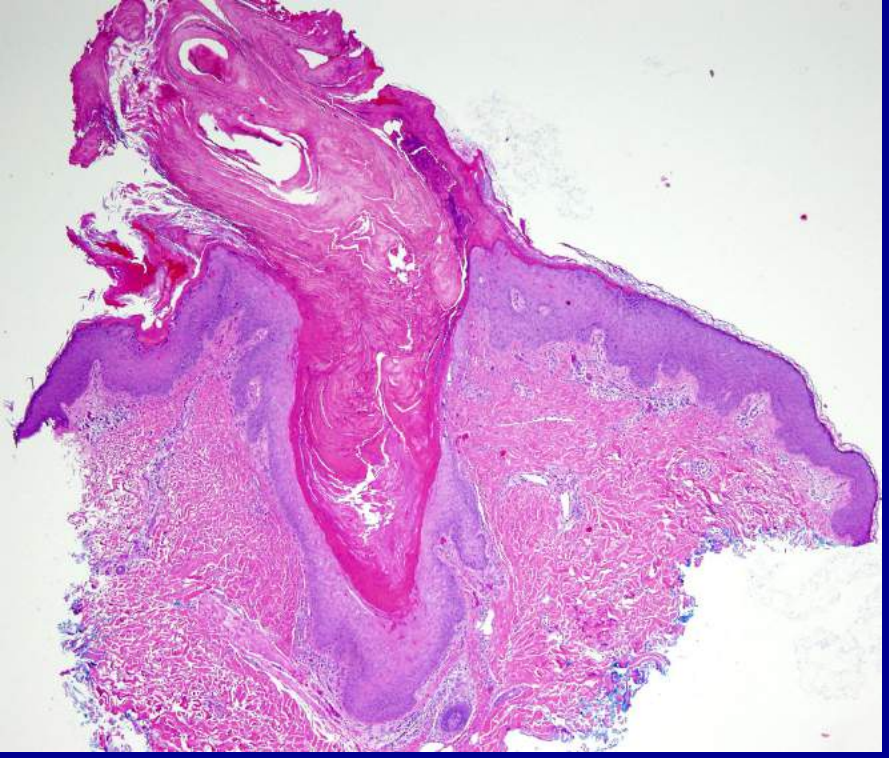


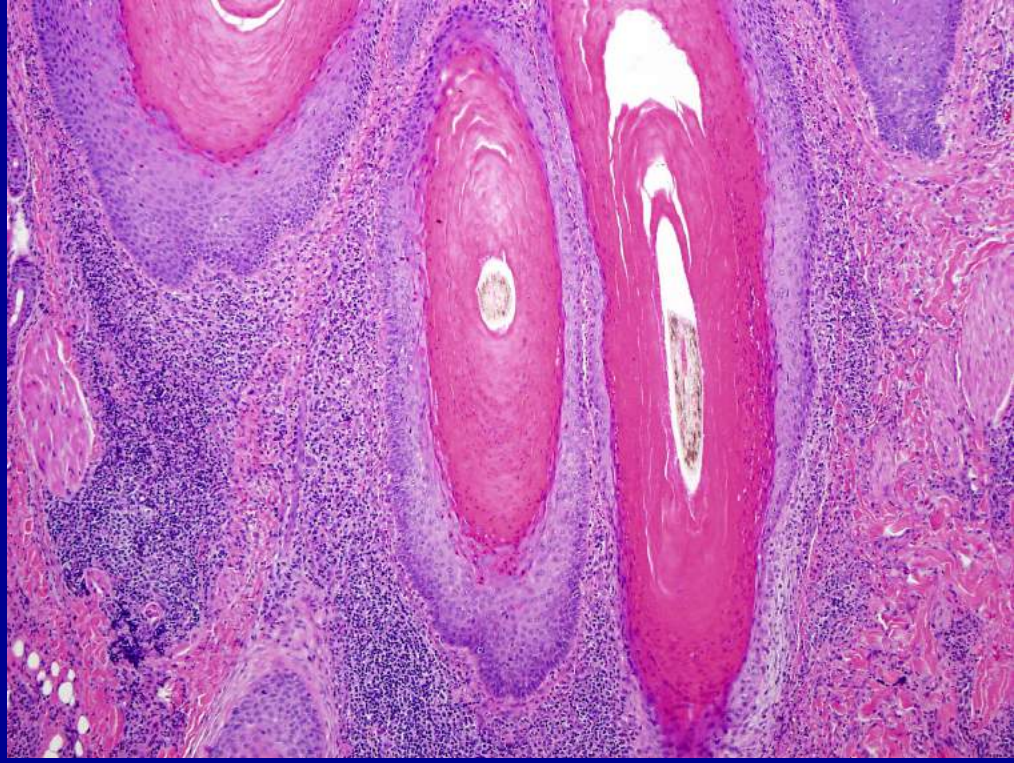


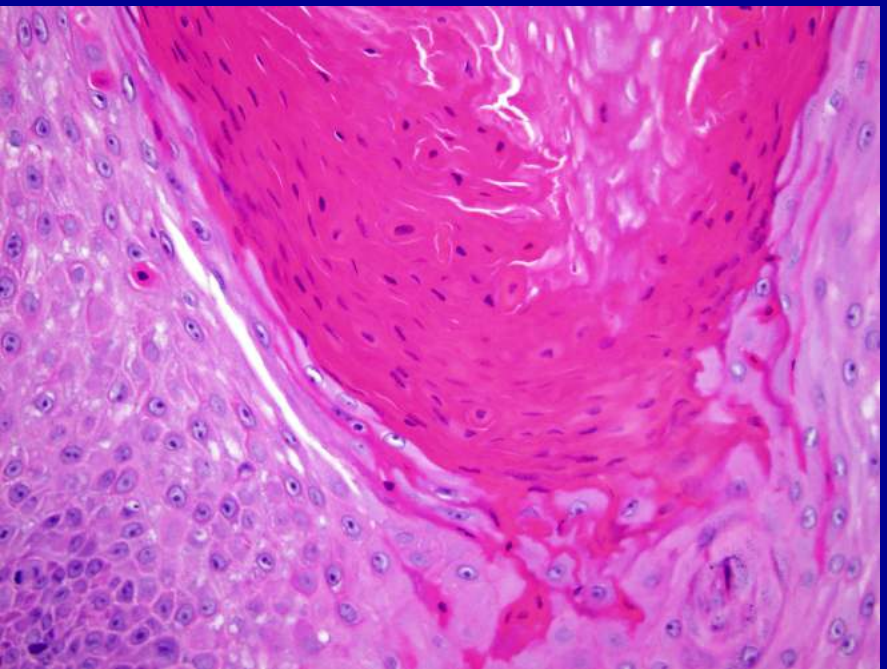


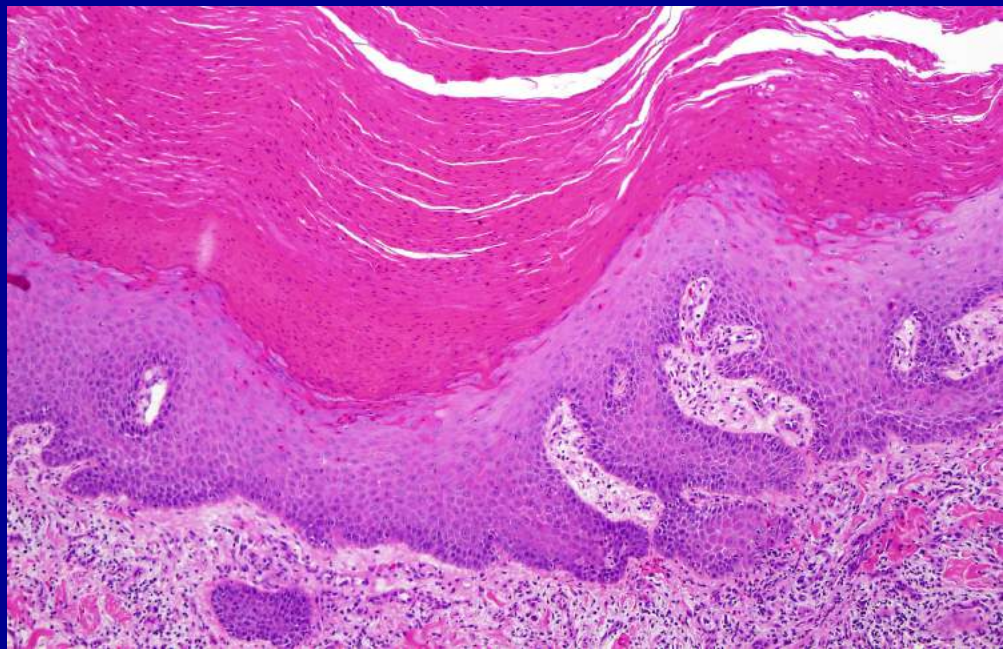
Scalp Lesions

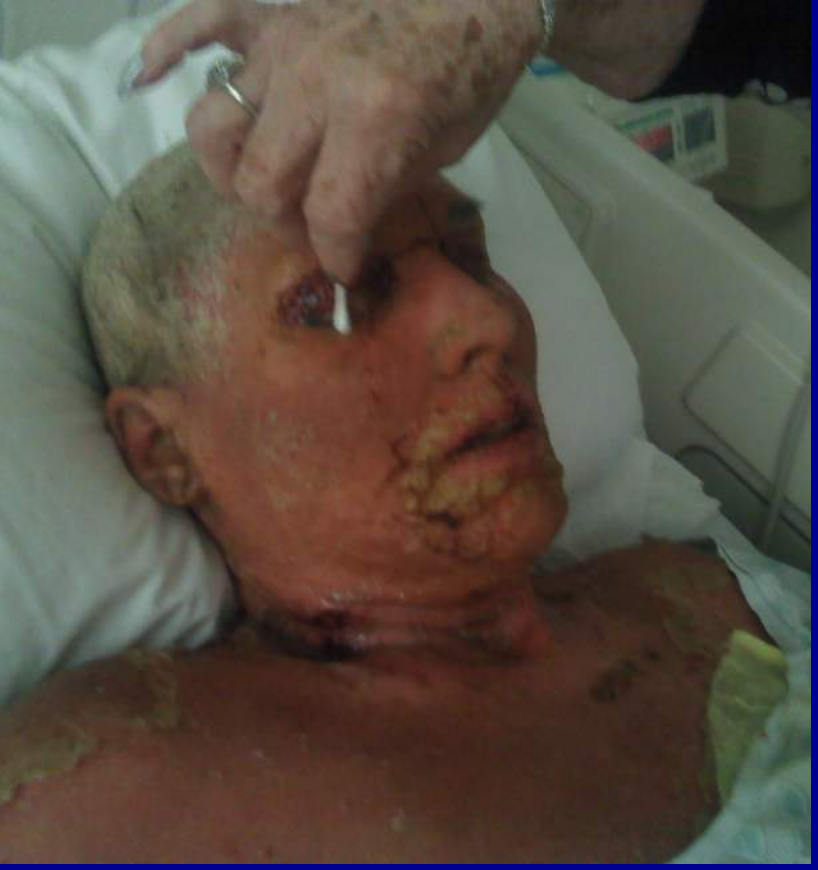


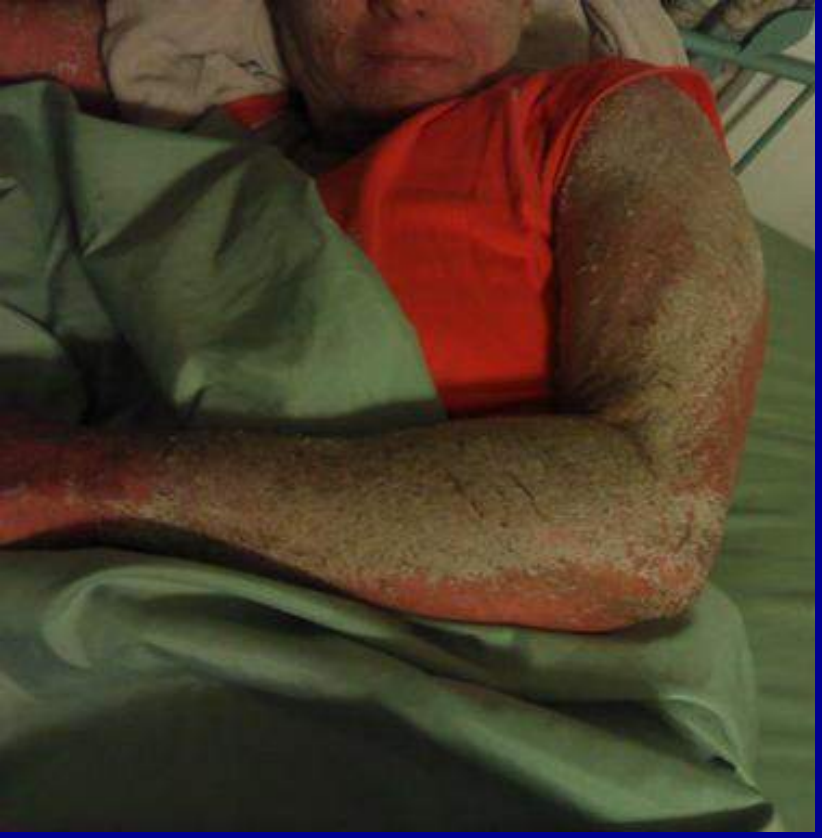


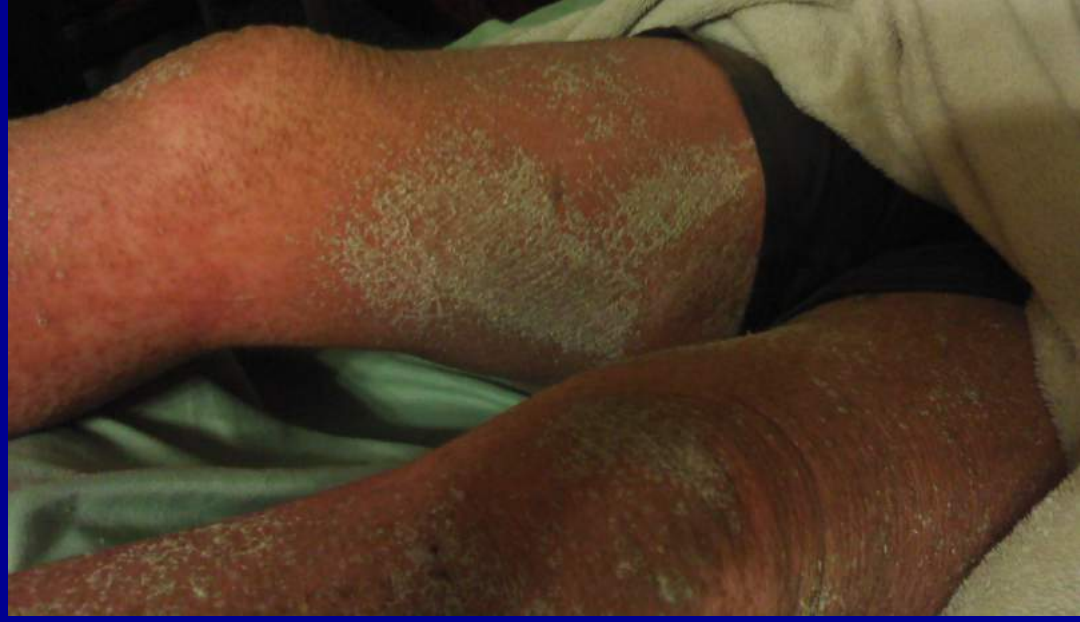


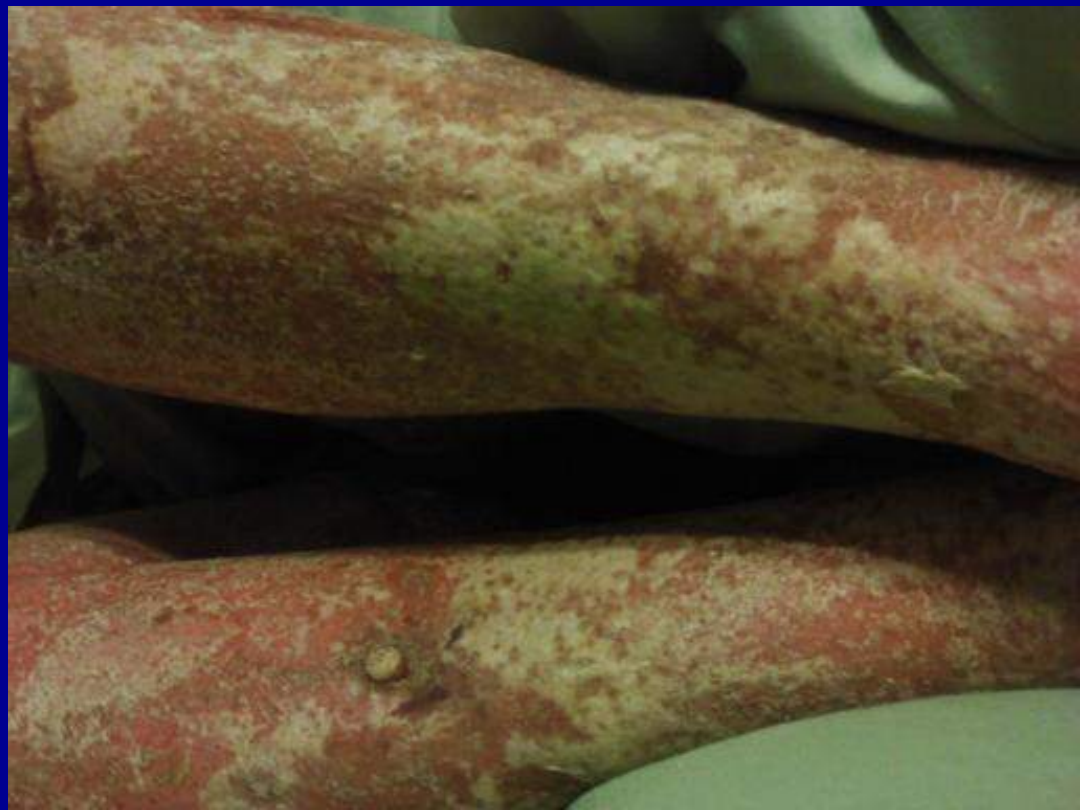




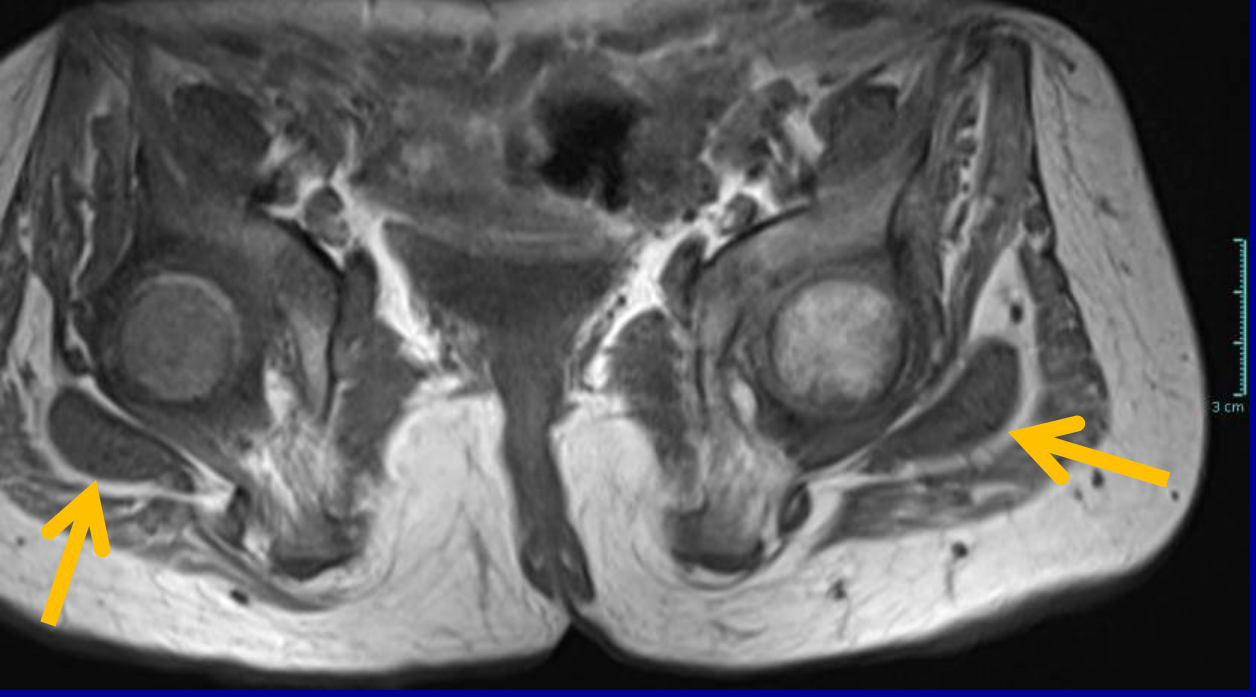




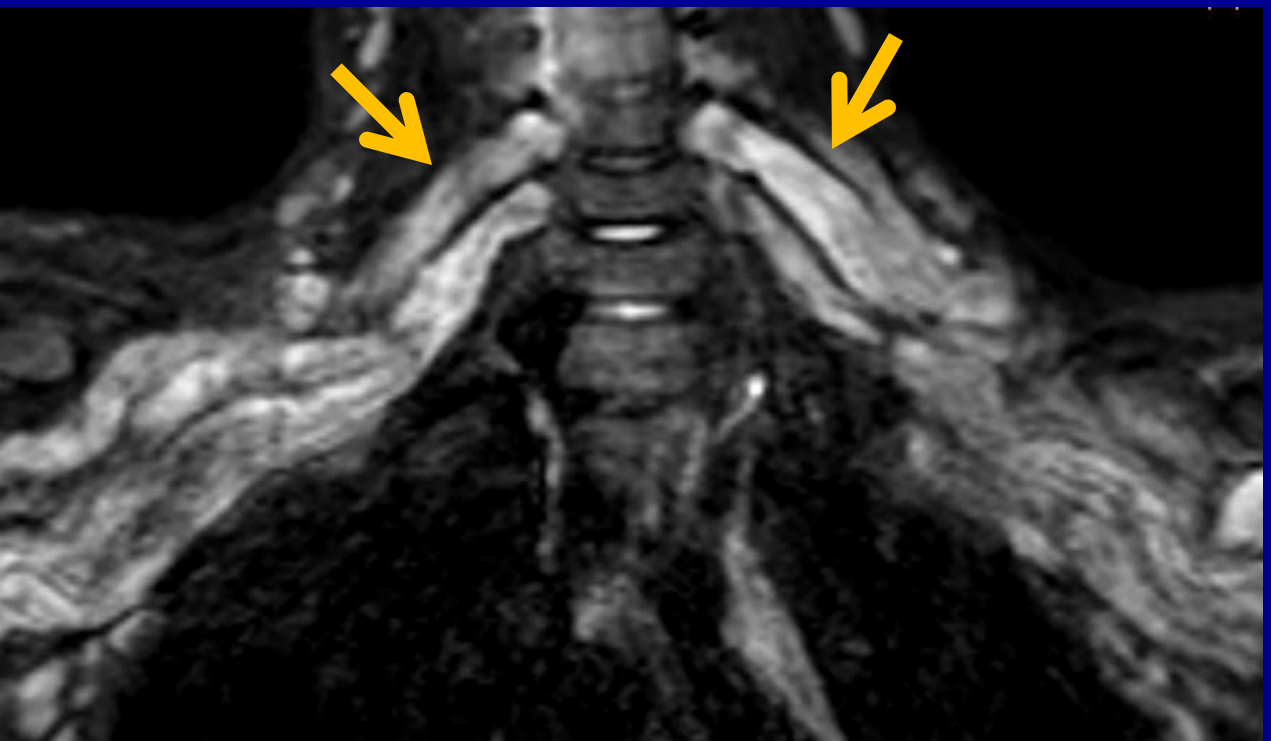




UDP #2179 - 7 year old with hypotonia

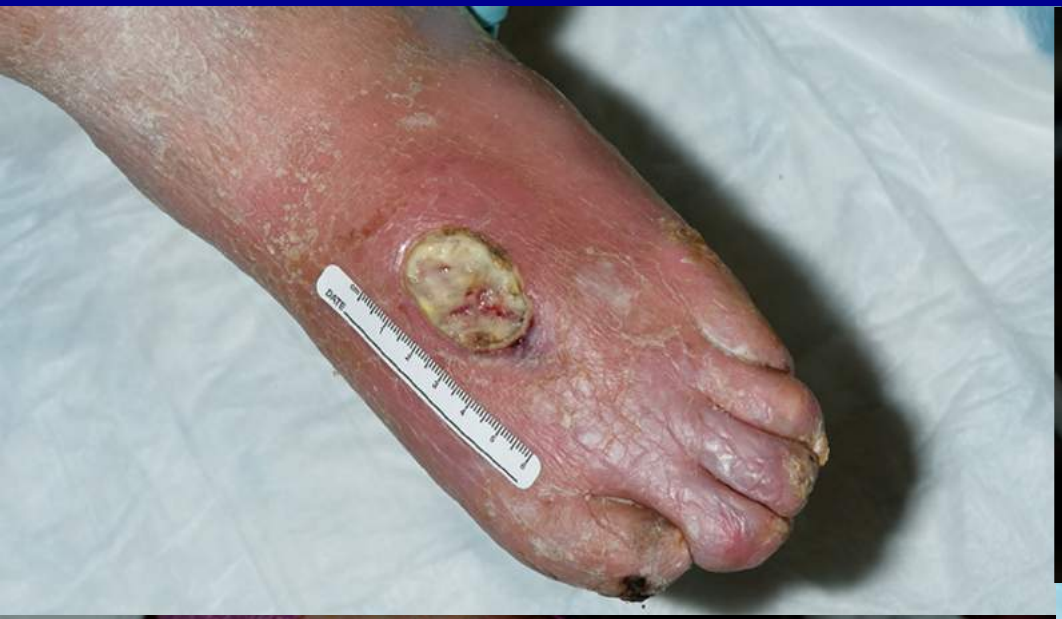


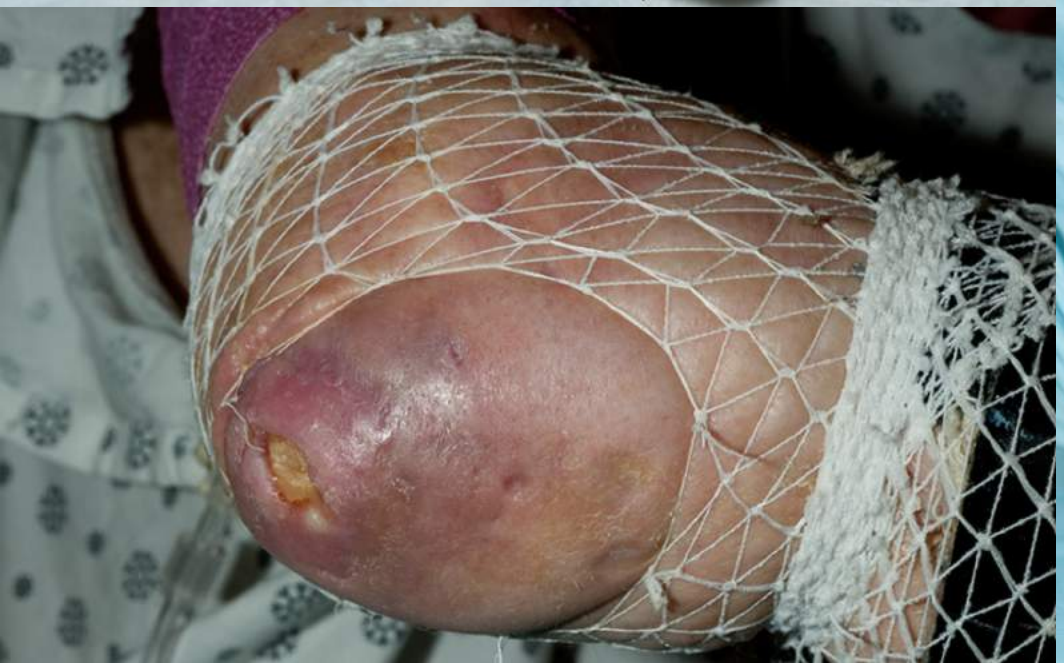
Sciatic Nerves

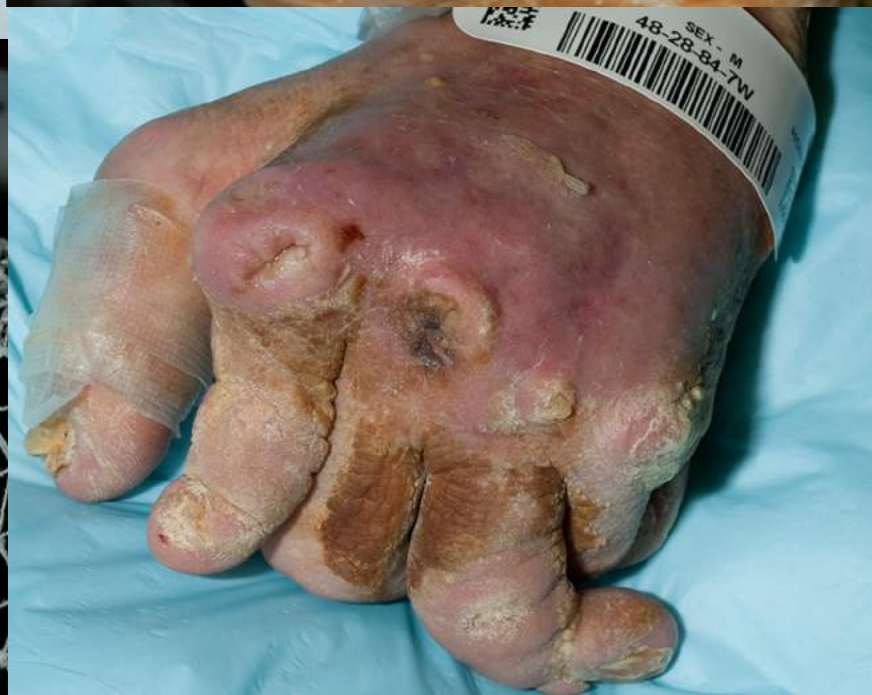


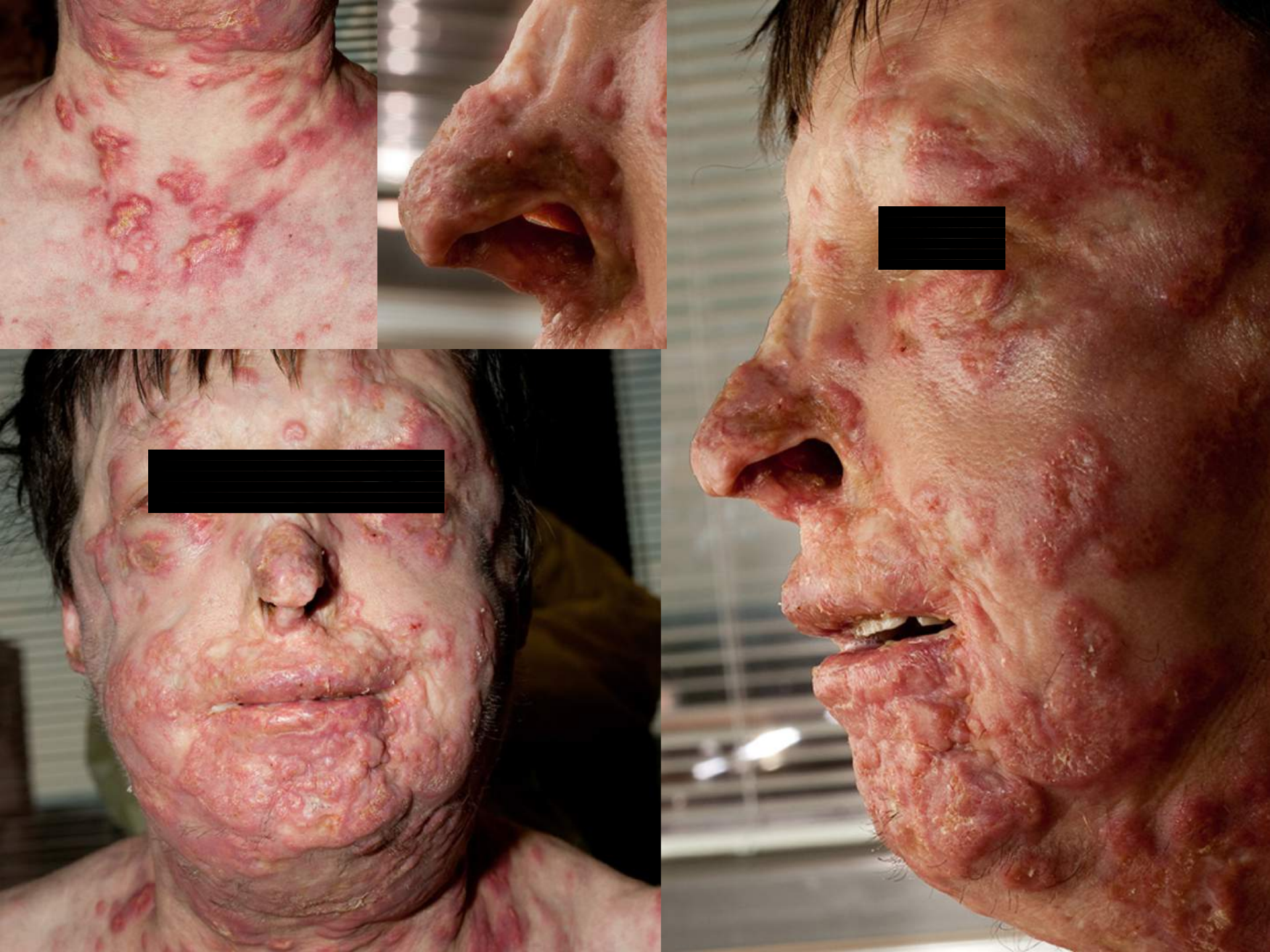
Brachial Plexus

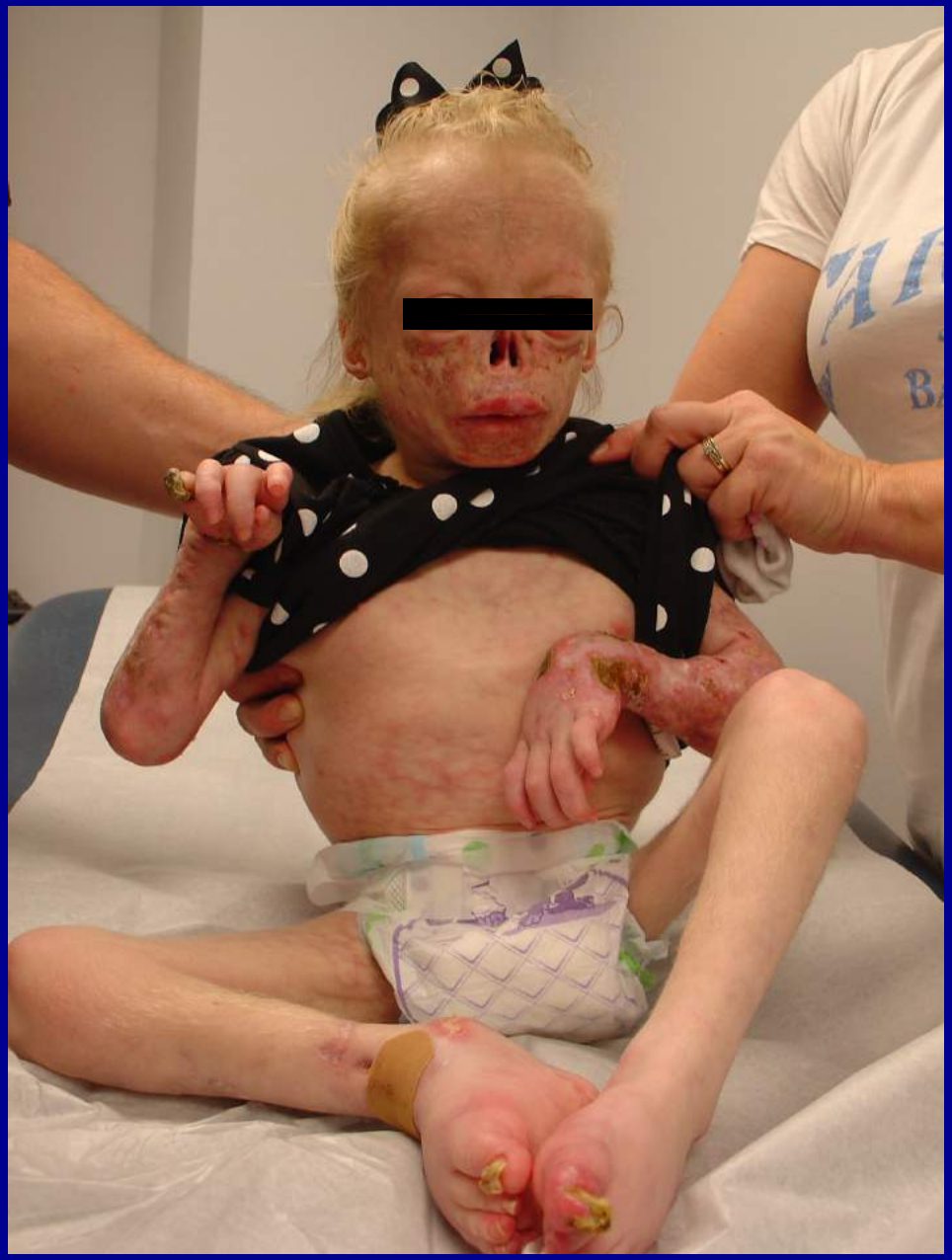
34 year old man with inflammatory lesions











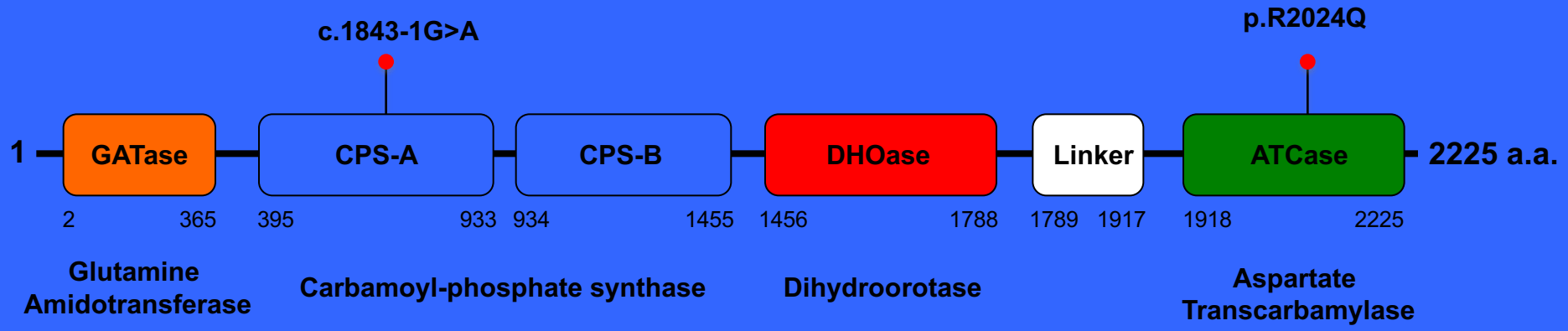
Individualized Treatment

UDP 4003: 6 year old boy with:

- Mild delays in fine motor, speech
- Abnormal Glycan screen of urine
- Compound heterozygous, damaging mutations in *CAD*

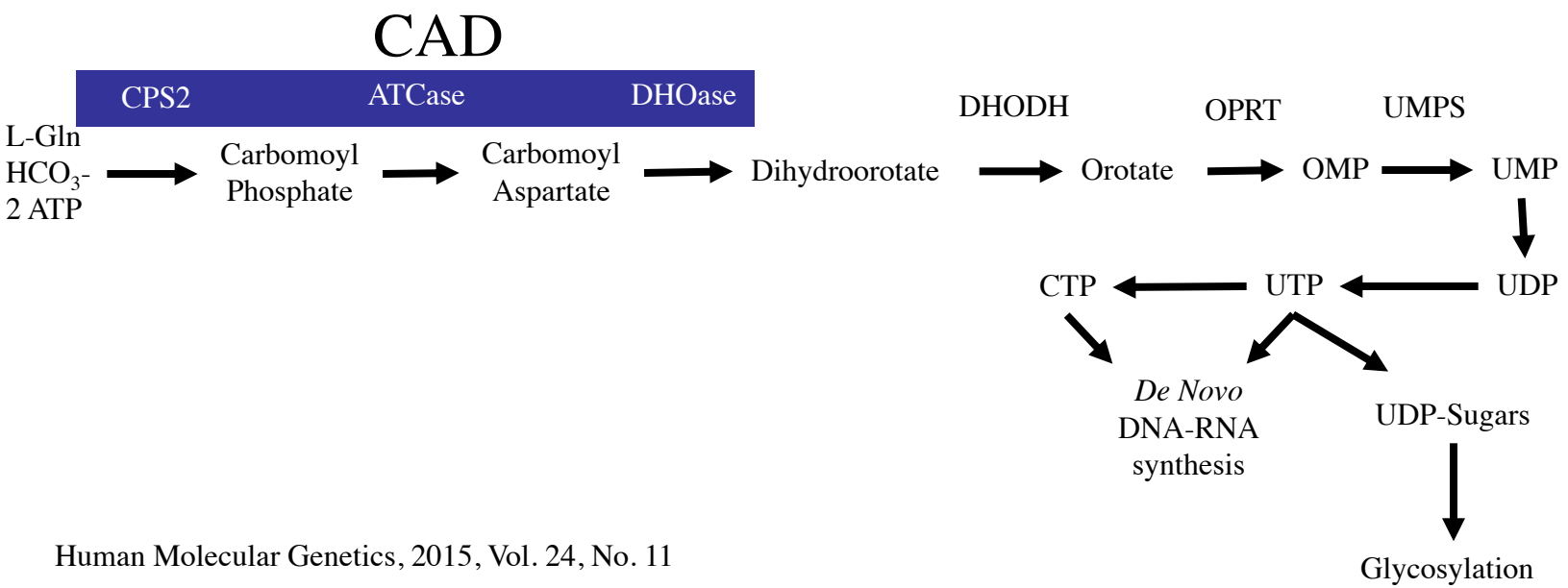
L. Wolfe, T. Markello, C. Tifft

The *CAD* Gene



CAD is a multifunctional CYTOPLASMIC enzyme in the pyrimidine synthetic pathway.

Activated Sugar Synthesis



Human Molecular Genetics, 2015, Vol. 24, No. 11

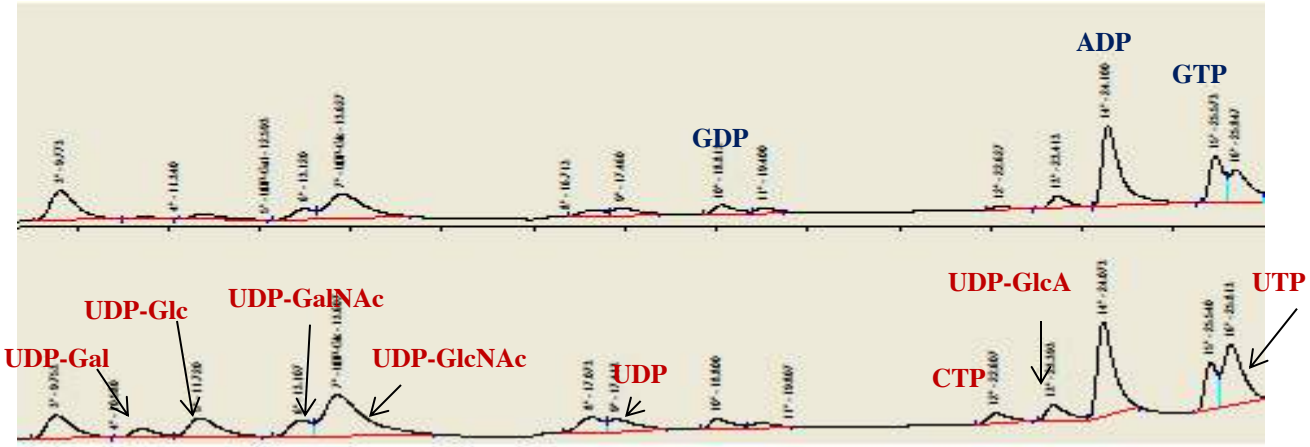
B. Ng, H. Freeze

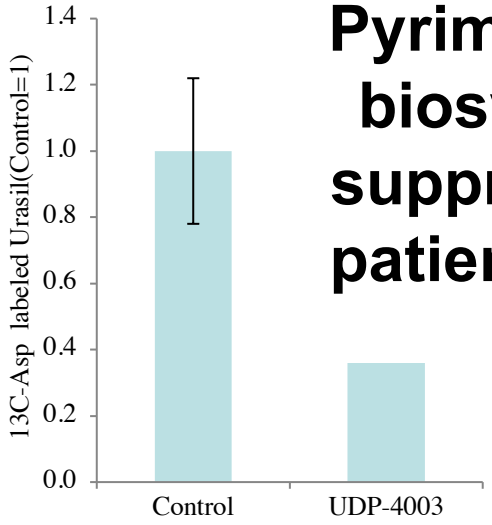
CAD deficiency results in decreased orotic acid, uridine, and UDP. N-linked glycosylation requires UDP-sugars to produce glycoproteins and glycolipids. Hence, CAD deficiency is a novel type of Congenital Disorder of Glycosylation.

In fibroblasts, UDP4003 shows lower levels of UDP-sugars and UTP; rescued by uridine feeding.

UDP4003

Control





Pyrimidine de novo biosynthesis was suppressed in UDP patient (UDP-4003).

3 other cases; treat with Xuriden.

B. Ng, H. Freeze

Conclusions: Phenotyping and Sequencing in Precision Medicine

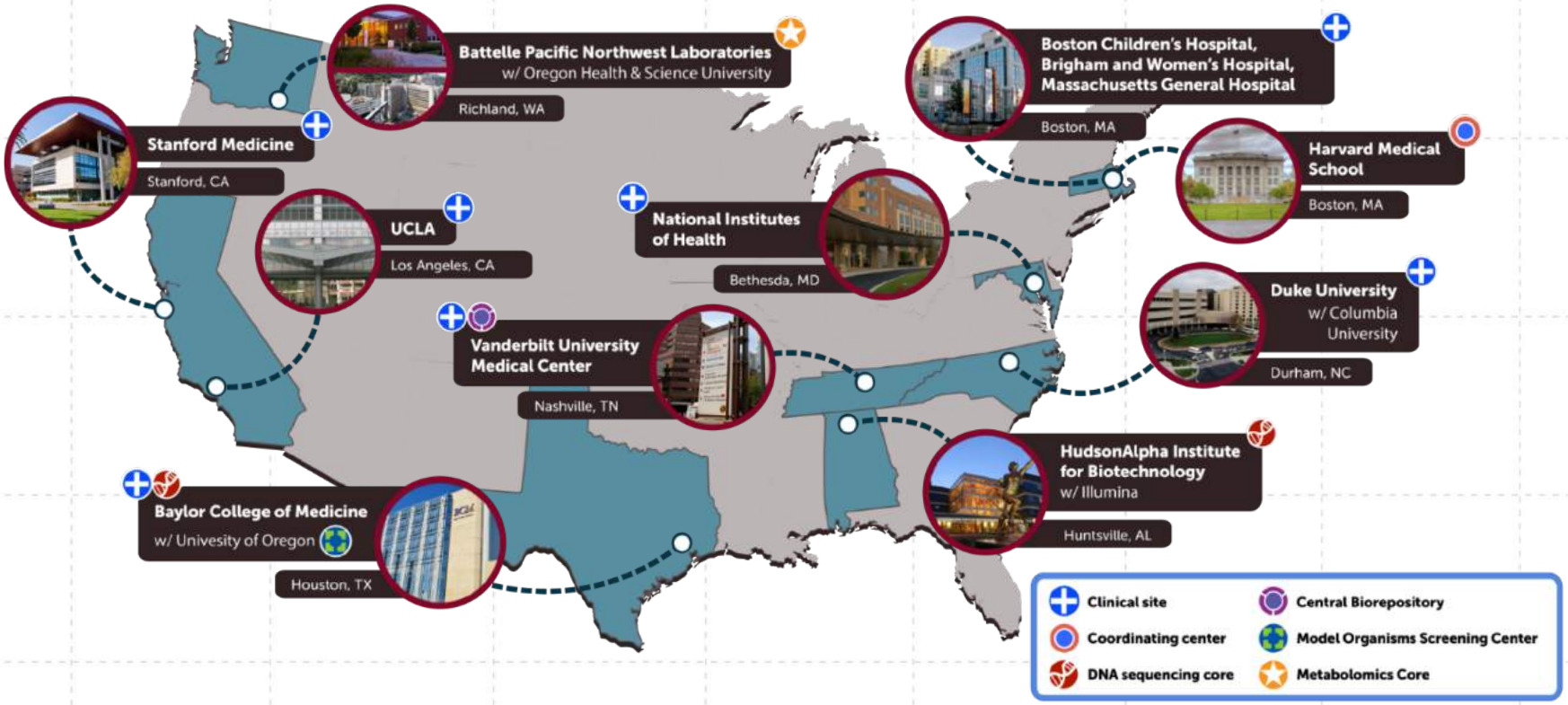
- Strong phenotyping is critical.
- Half of UDP diagnoses are NOT based on NGS; ¼ of UDN diagnoses are NOT.
- Functional studies are rate-limiting for new disease discovery.
- Sharing of sequence and phenotypic data is essential.
- Undiagnosed patients are desperate.

Increasing Access: The UDN

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

Undiagnosed Diseases Network

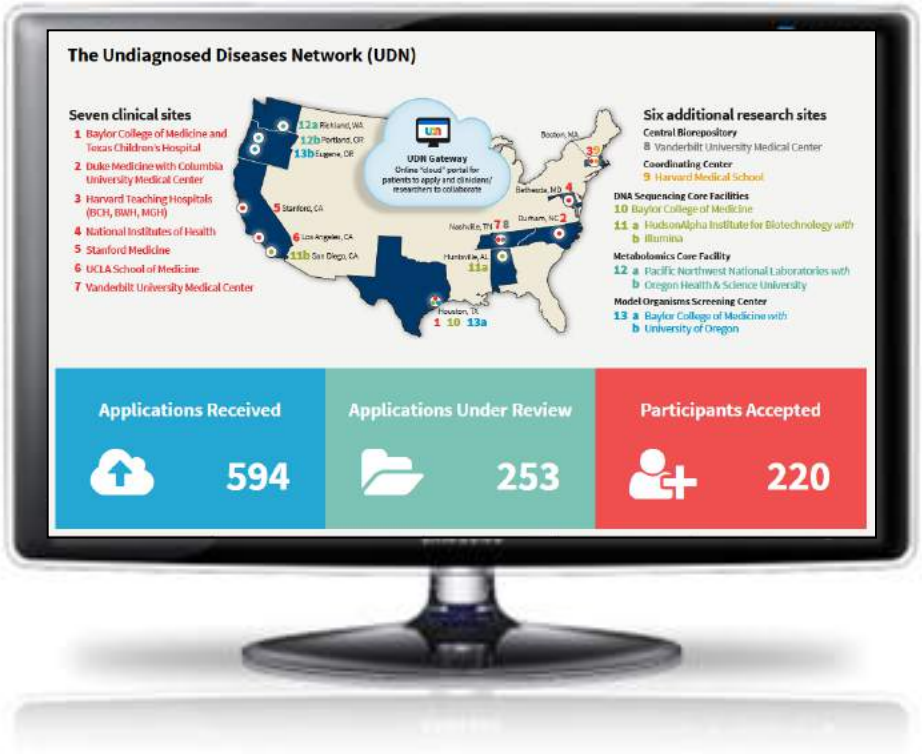
Seven clinical sites, a coordinating center, two DNA sequencing cores, a metabolomics core, a model organisms screening center, and a central biorepository



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.

The UDN Gateway

Click "Apply" button on any UDN website for more information



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

Other UDPs in the USA:

U. Alabama-Birmingham
Emory
Mayo
U. Utah
Others…

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.

Website:

http://www.udninternational.org/

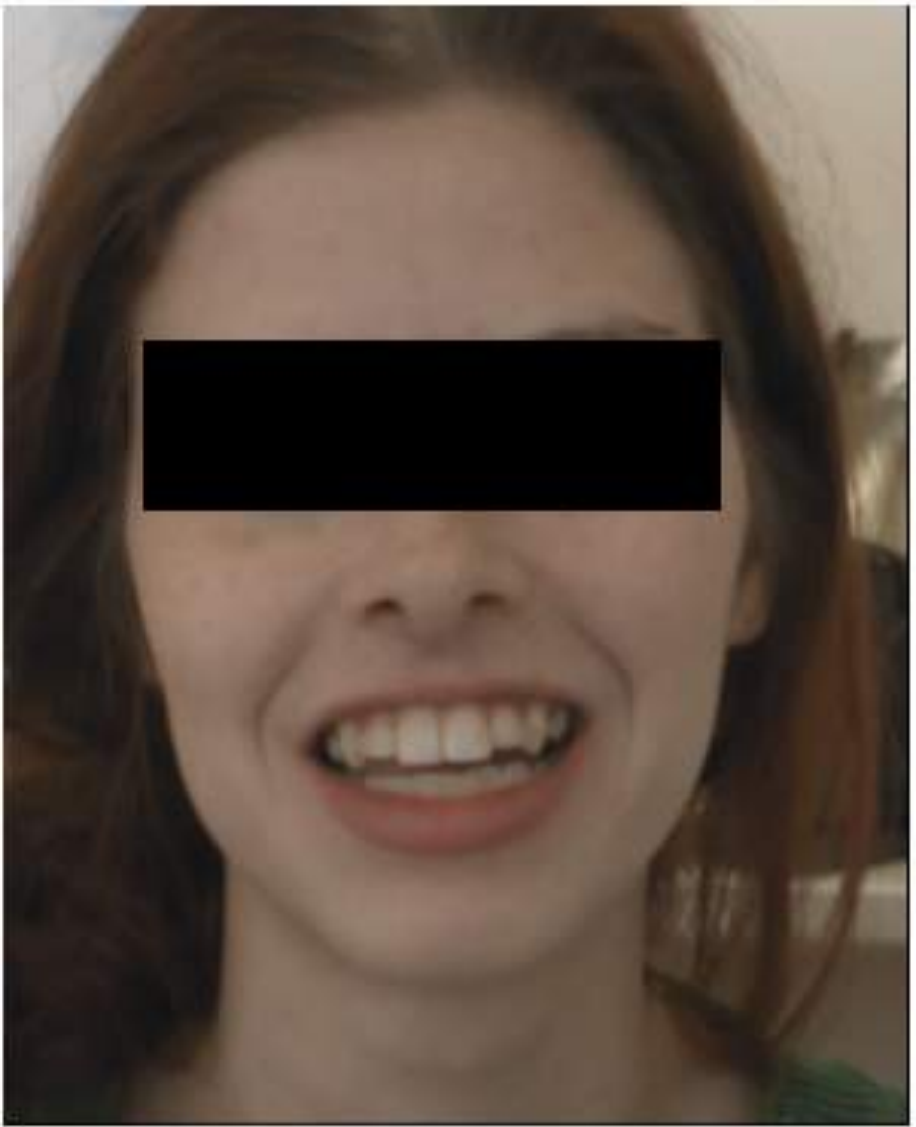
UNDI Conferences in:

Rome
Budapest
Vienna
Tokyo
Stockholm (Klee Membership)

UDPs in Other Countries Including:

- Japan (AMED;IRUD)
- Western Australia
- Austria (Vienna)
- Italy
- China (Shanghai)
- Stockholm, Sweden

22 year old woman with dystonia – diagnosed by sharing

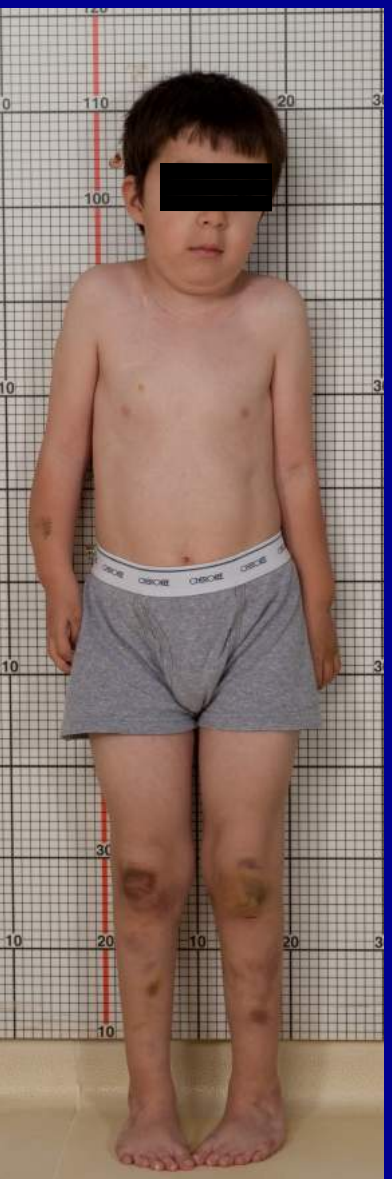


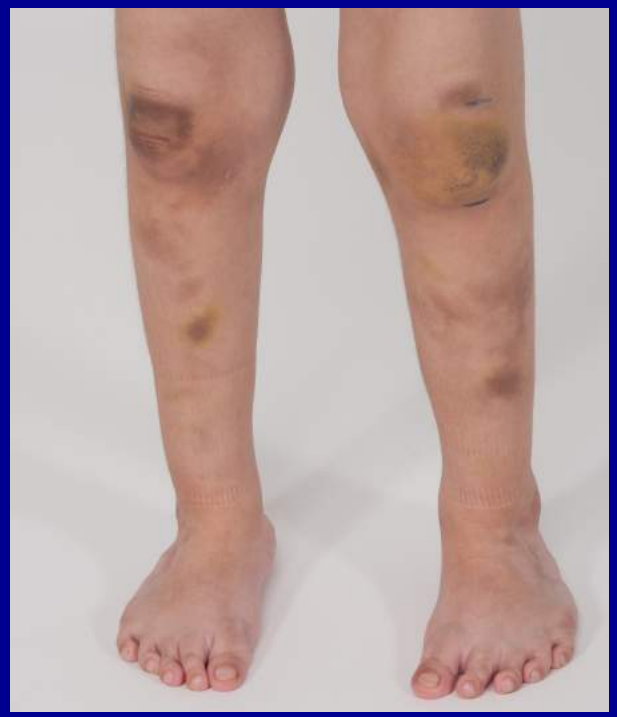
- ✓ Abnormal pen gripping
- ✓ Right foot deformity and twisting with gait
- ✓ Involuntary tongue movements
  - ✓ Speech
  - ✓ Swallowing
  - ✓ Nutrition
- ✓ Histone lysine methyltransferase deficiency

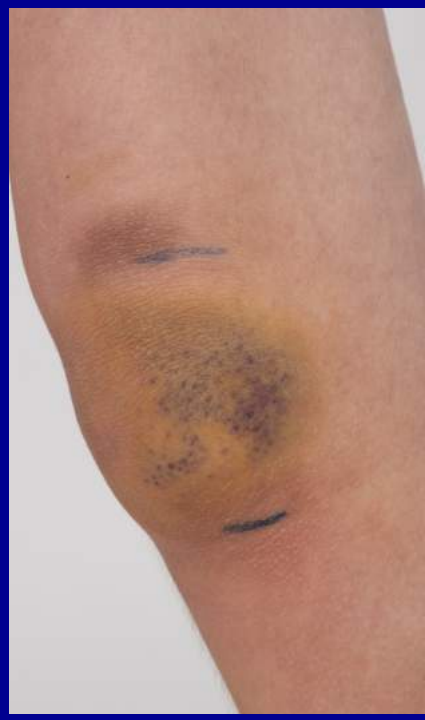
Pharmaceutical Company Interest in Personalized Medicine

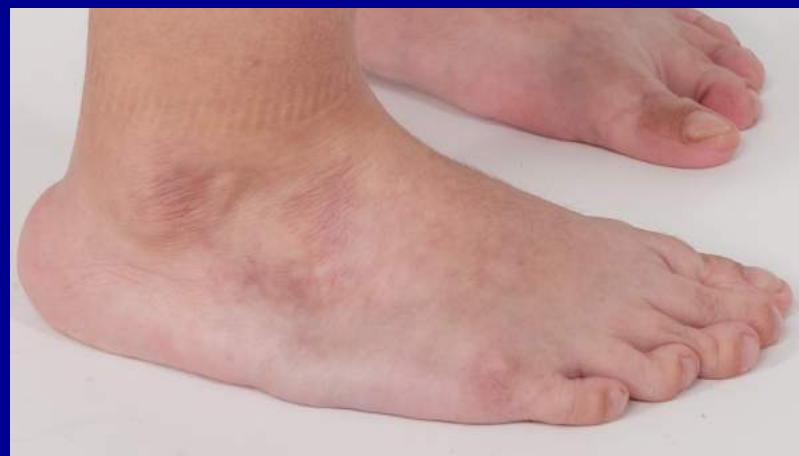
- Study UDP new disease patients' genetic defects
- Find new pathways and druggable targets for common diseases
- Assured that the pathway is functional in humans and pathological when defective

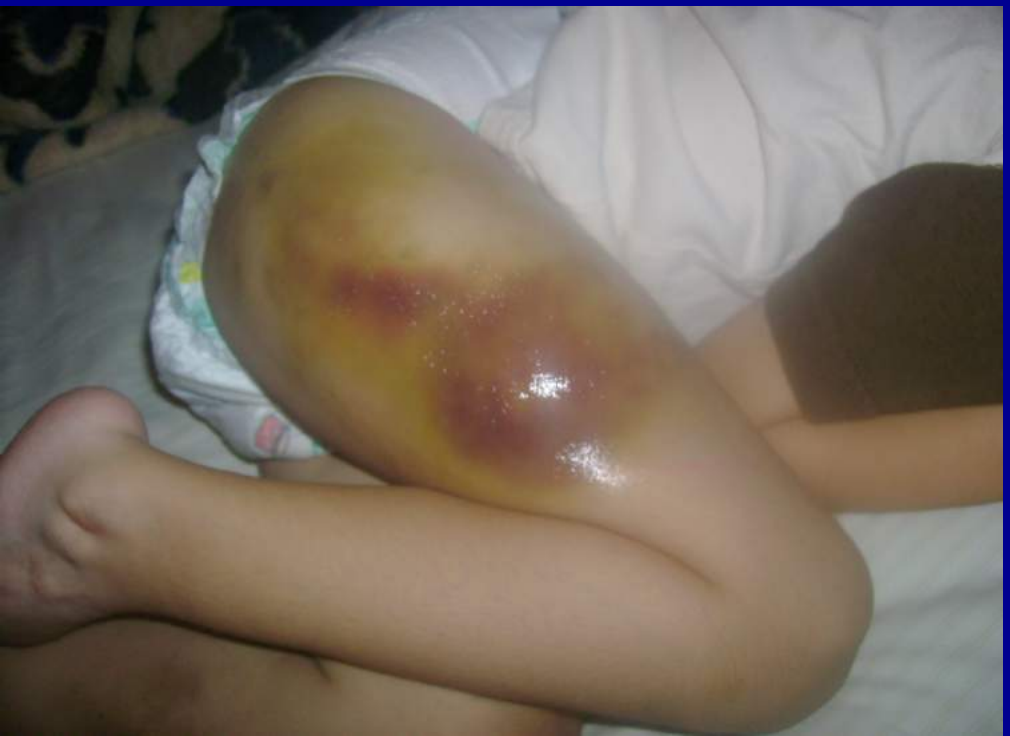
5 year old boy with congenital anomalies, hypotonia, delays, coagulopathy





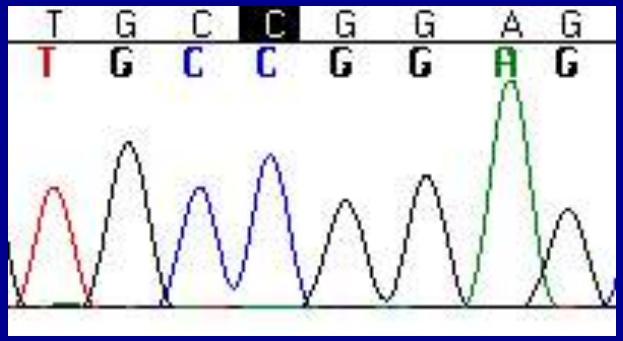






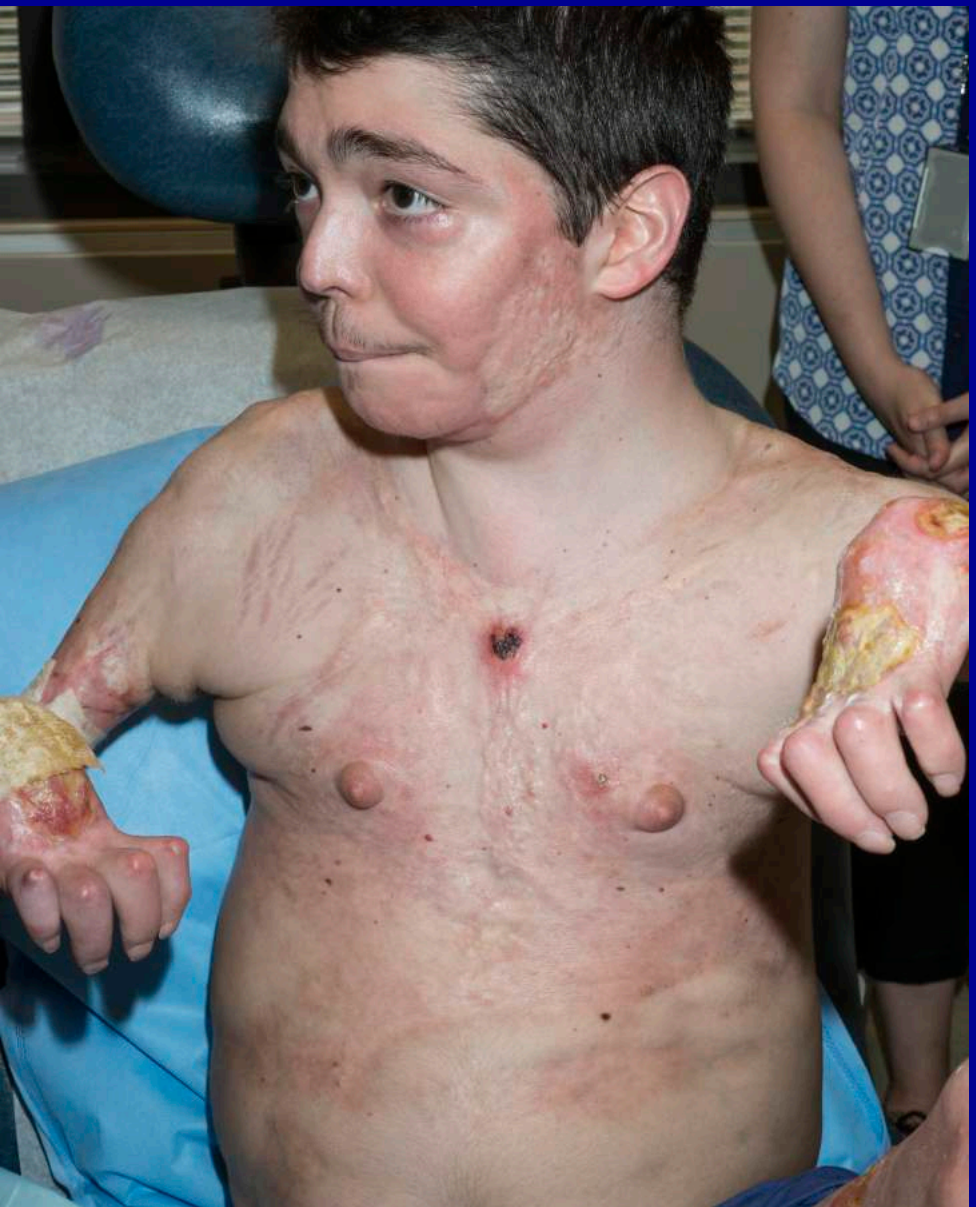
1. Parents are 1st cousins once removed.
2. There are two normal sibs
3. SNP array shows 26 Mb of homozygosity unique to the patient (72 genes).
4. One gene fits clinical picture: *CHST14*.
5. Patient homozygous for G>C in exon 14.

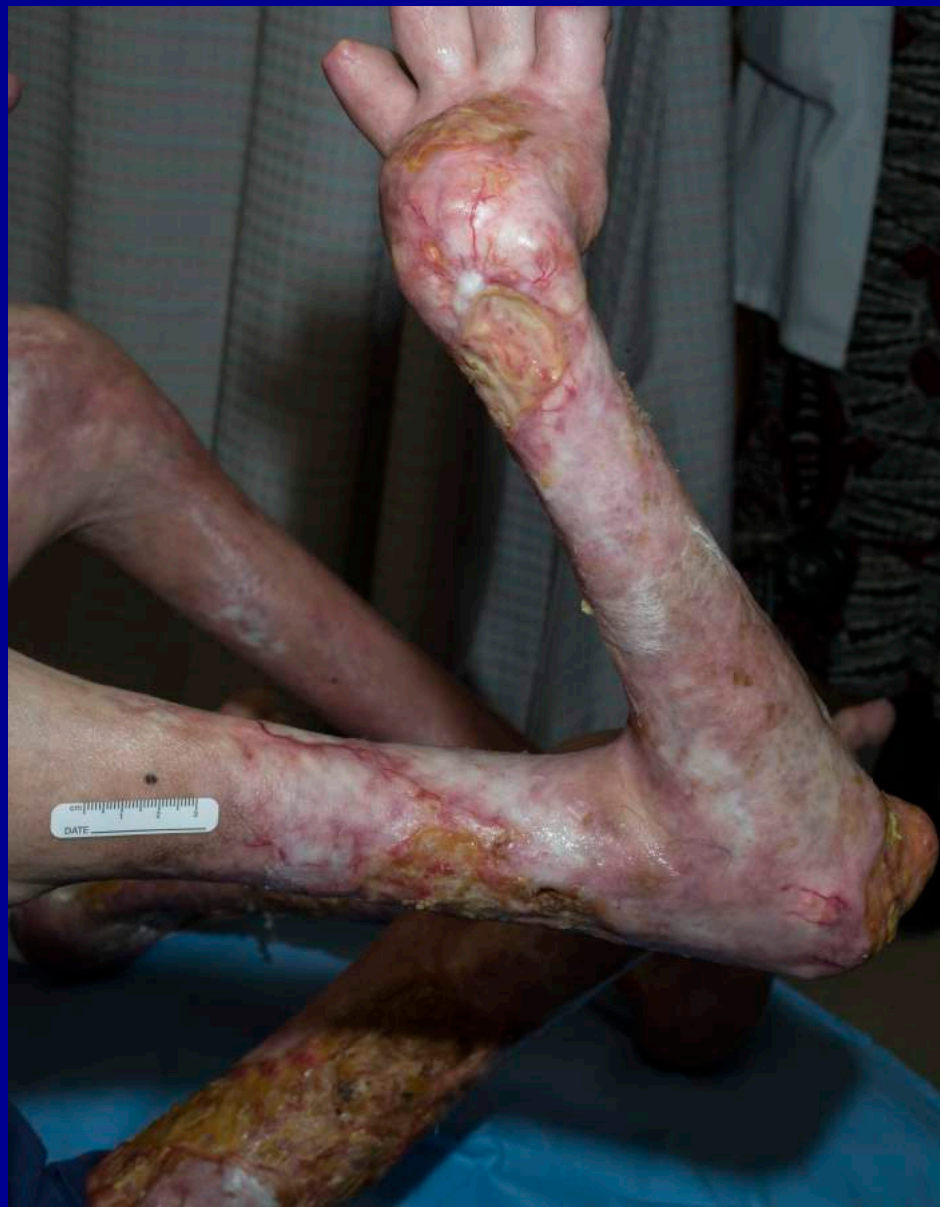
R216P



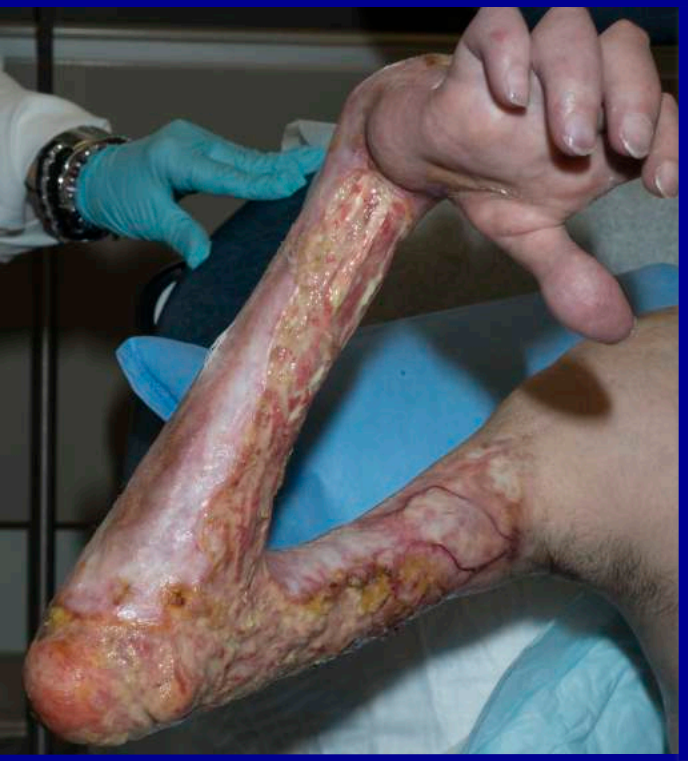
6. *CHST14* encodes dermatan-4-sulfotransferase 1; DS is fibrinolytic.
7. Hematoma treatment with DS/other?

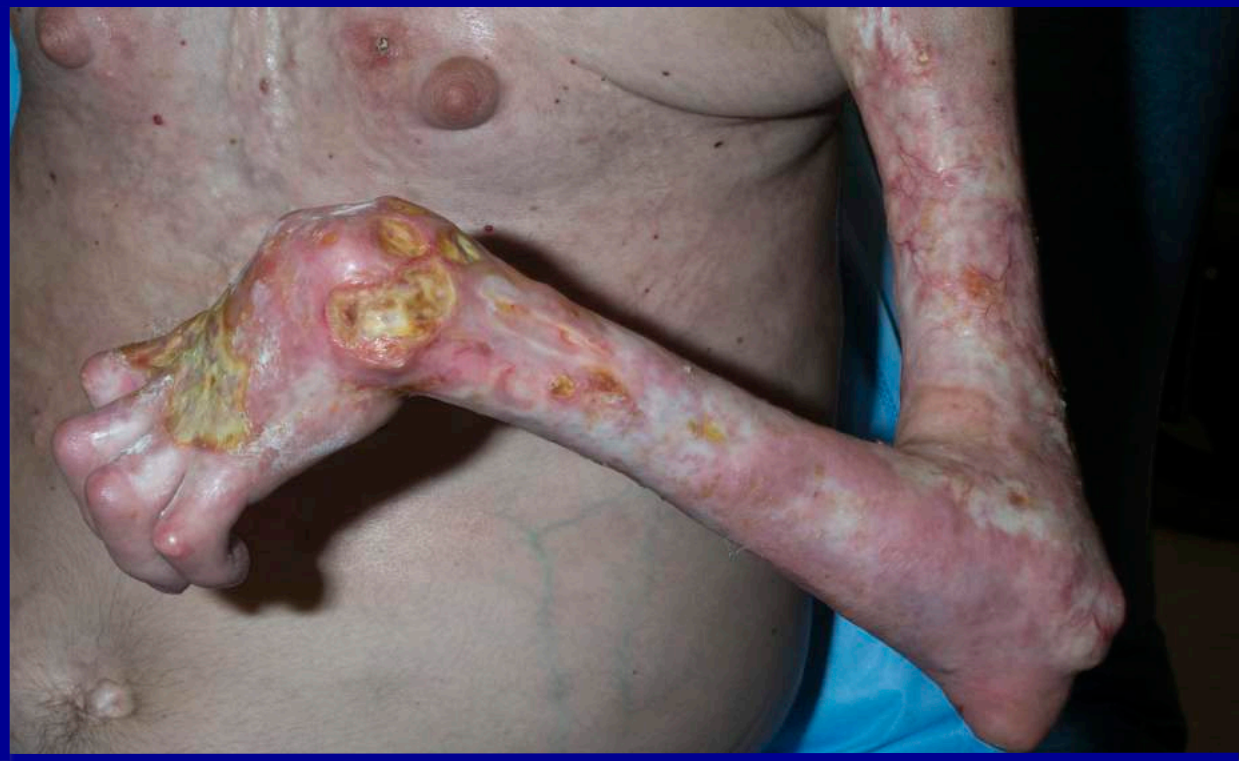
UDP 10237-25 year old man with contractures and skin ulcerations

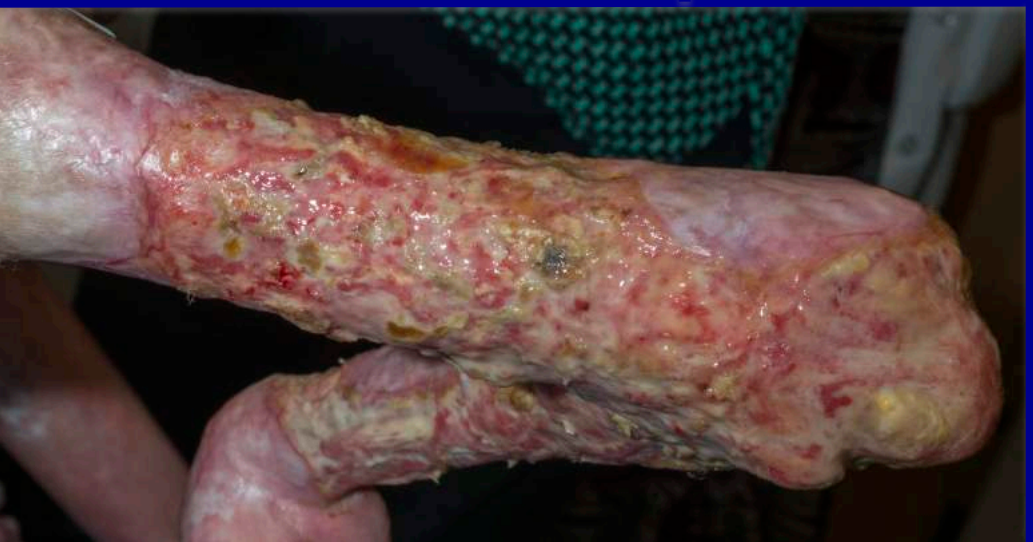


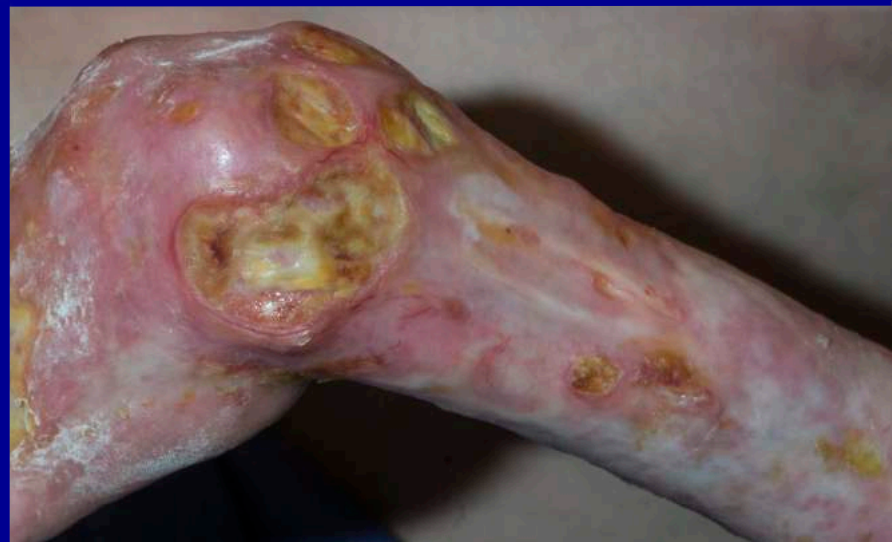


Upper Extremities

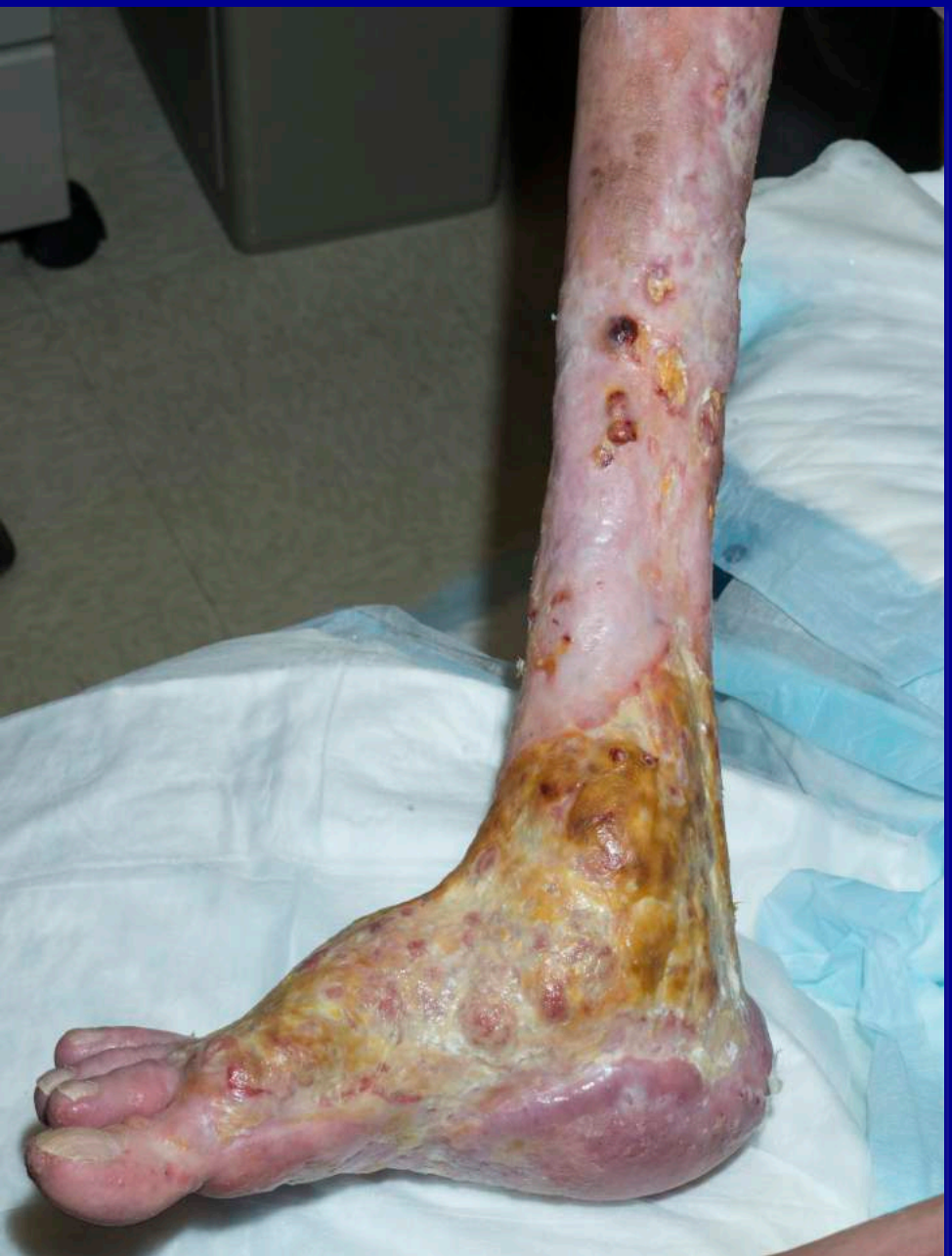


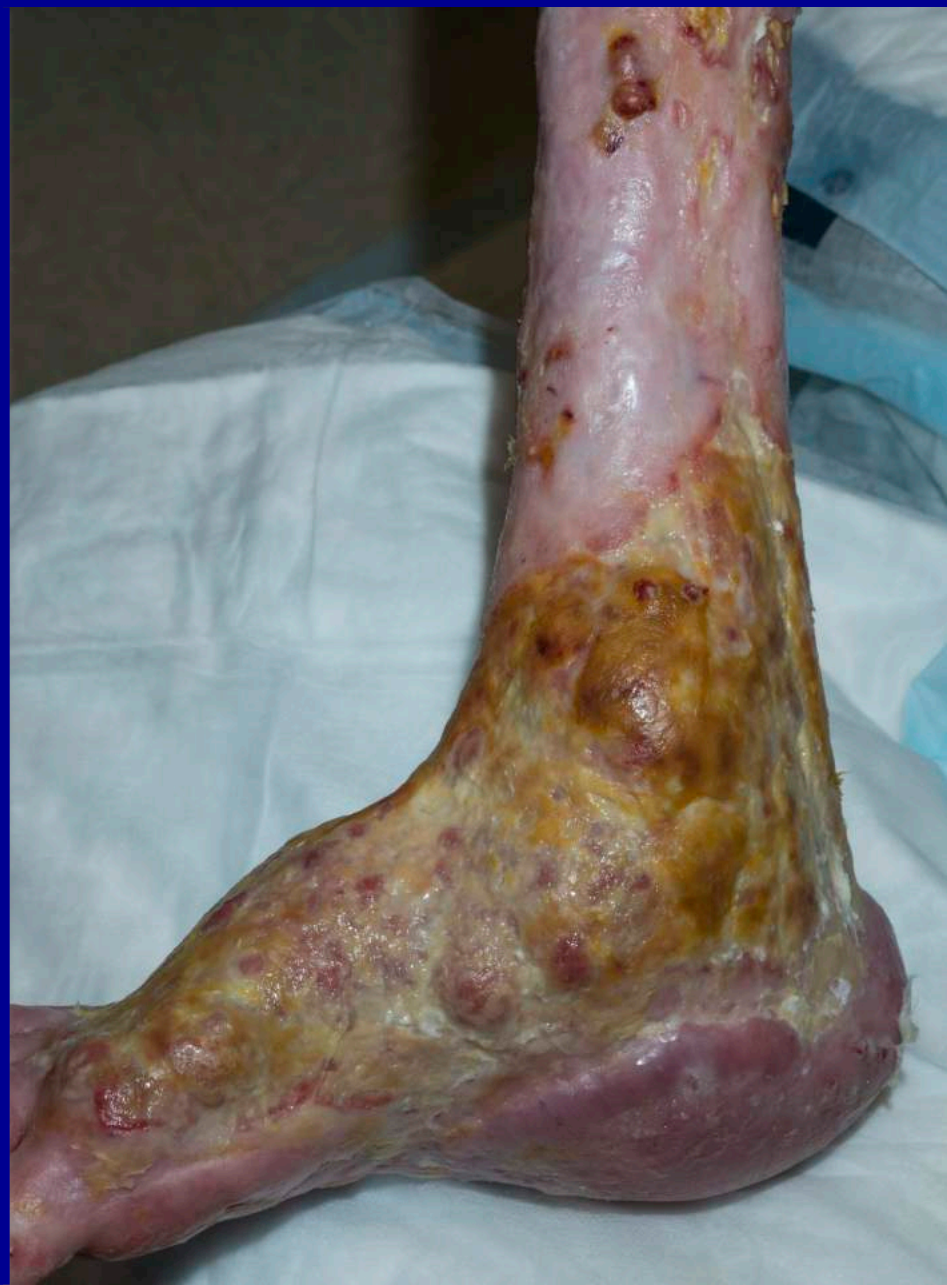




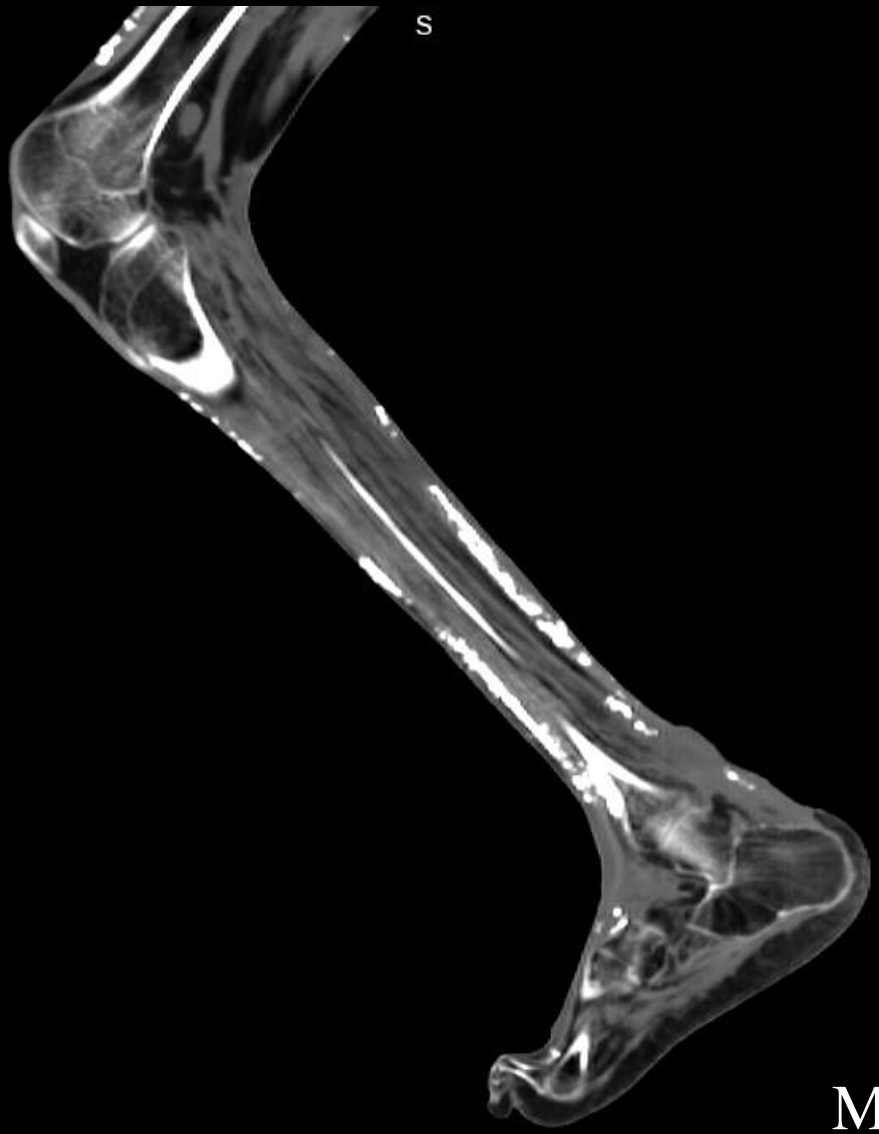


Lower Extremities

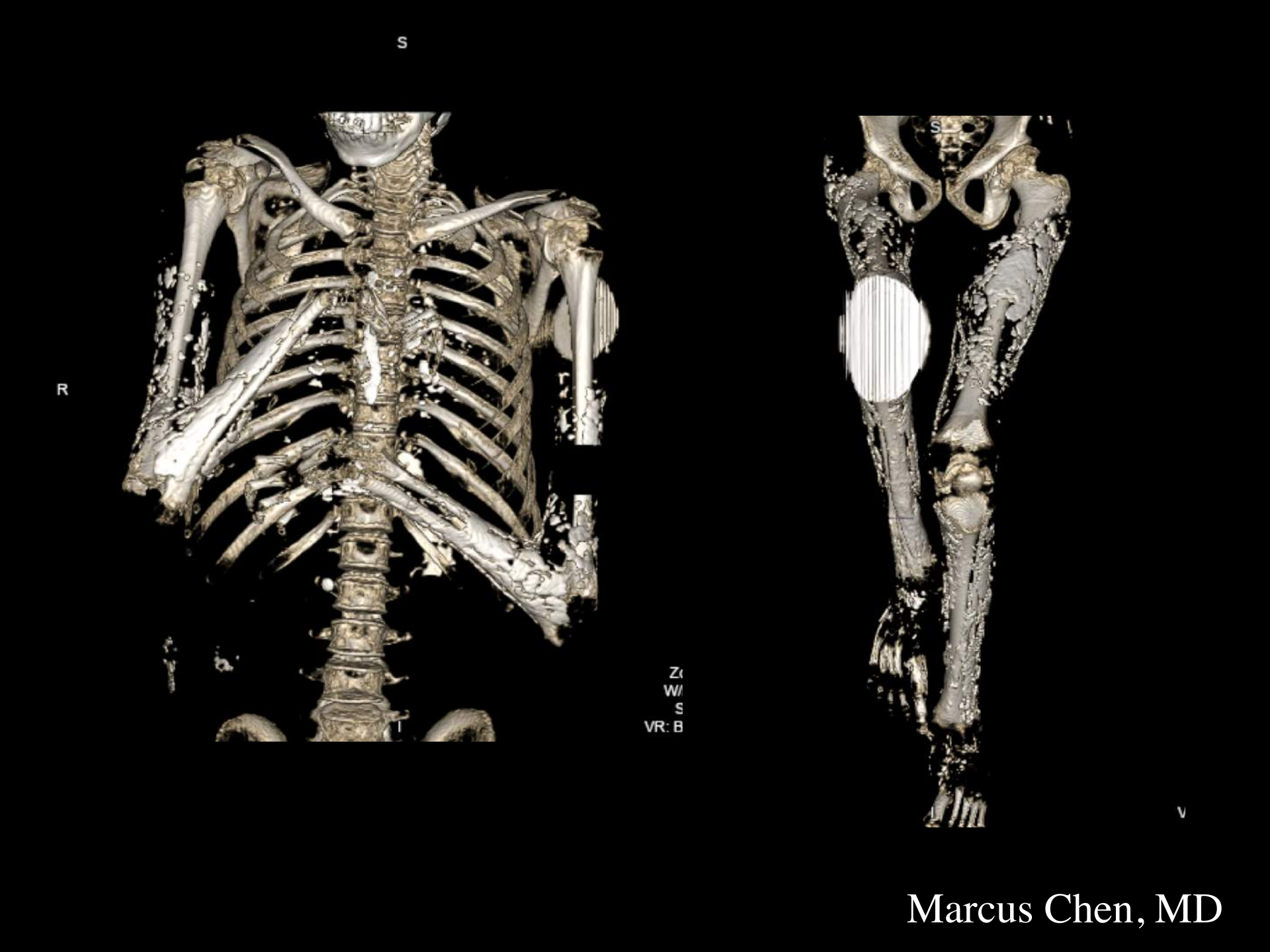




Left lower extremity



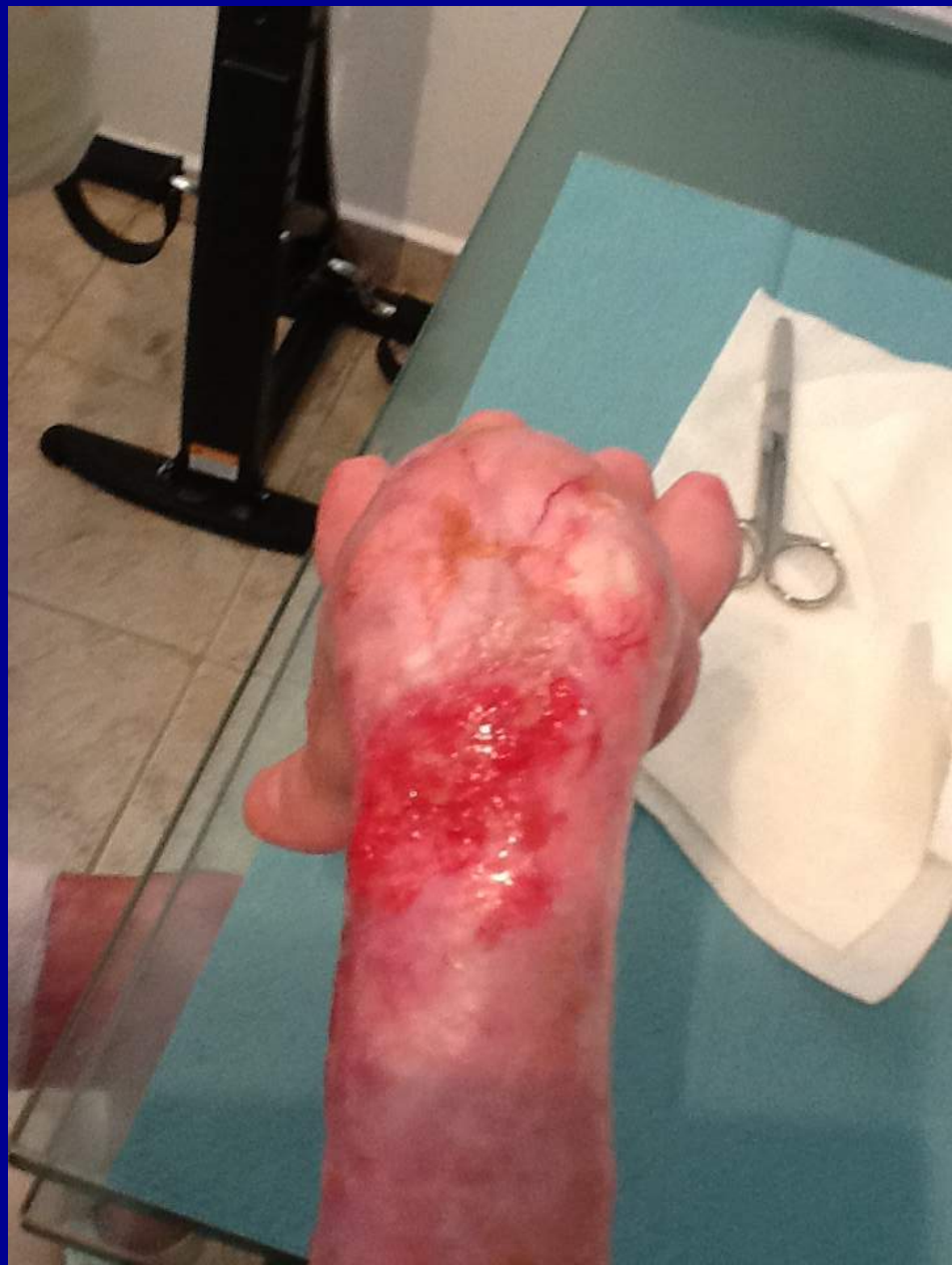
Marcus Chen, MD


S
R
S
Marcus Chen, MD

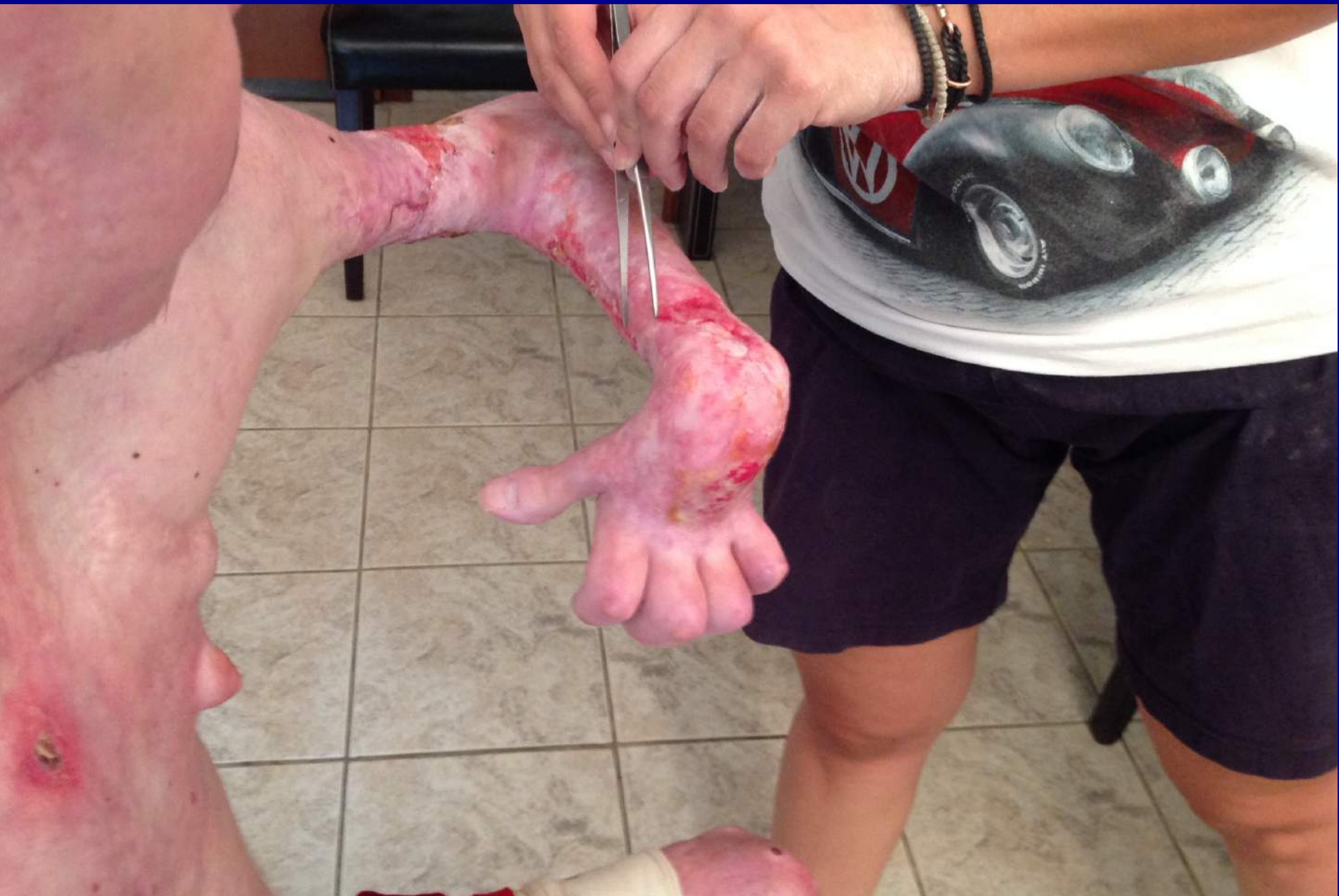
UDP 10237

- No diagnosis, but because the calcifications could be stimulating an inflammatory reaction, Dr. Lisa Rider (Rheum) suggested topical sodium thiosulfate.
- The thiosulfate salt of calcium is 250-10,000 times more soluble than the phosphate salt.

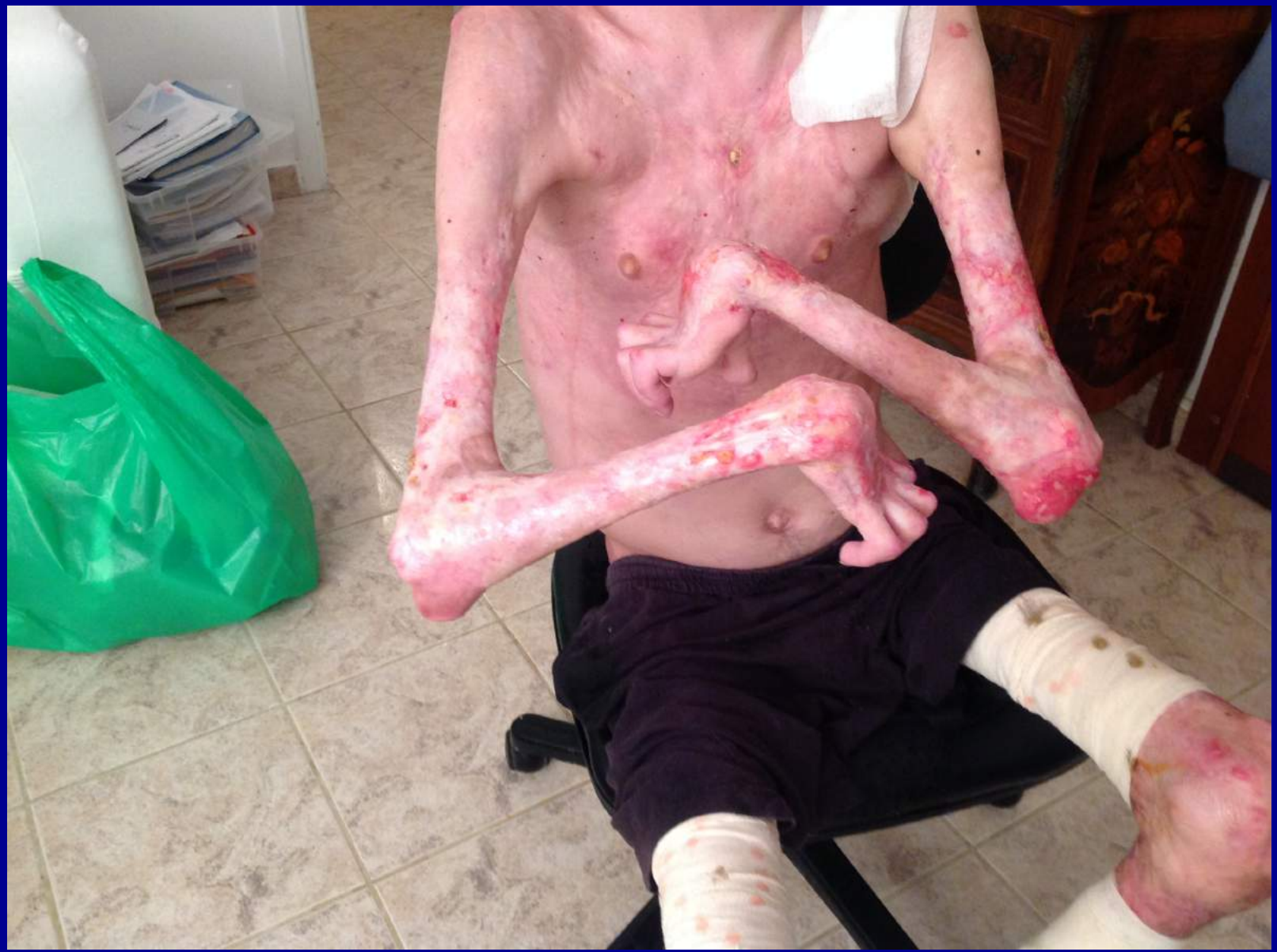
Sodium Thiosulfate – July 2014 (1 month)



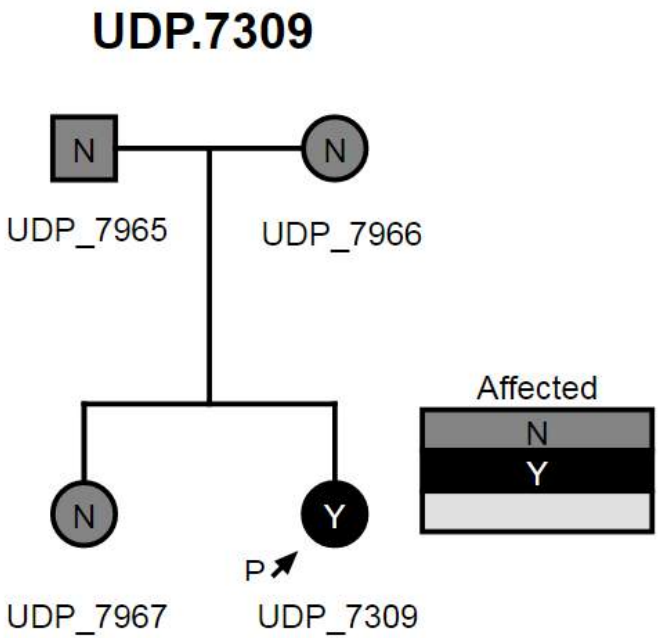
Sodium Thiosulfate – August 2014 (2 months)



Sodium Thiosulfate – October 2014 (4 months)



UDP 7309

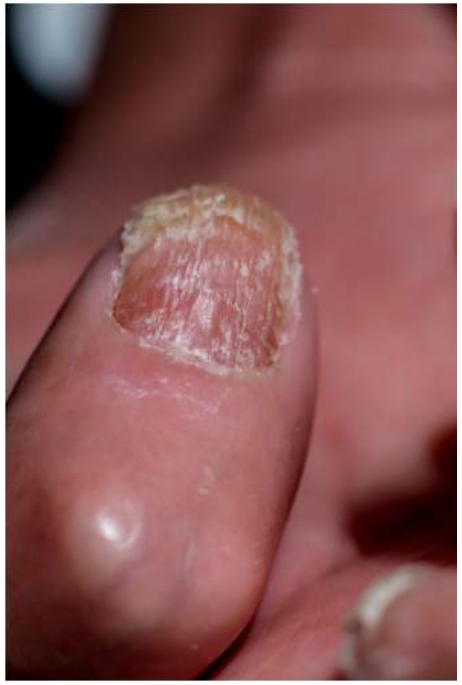


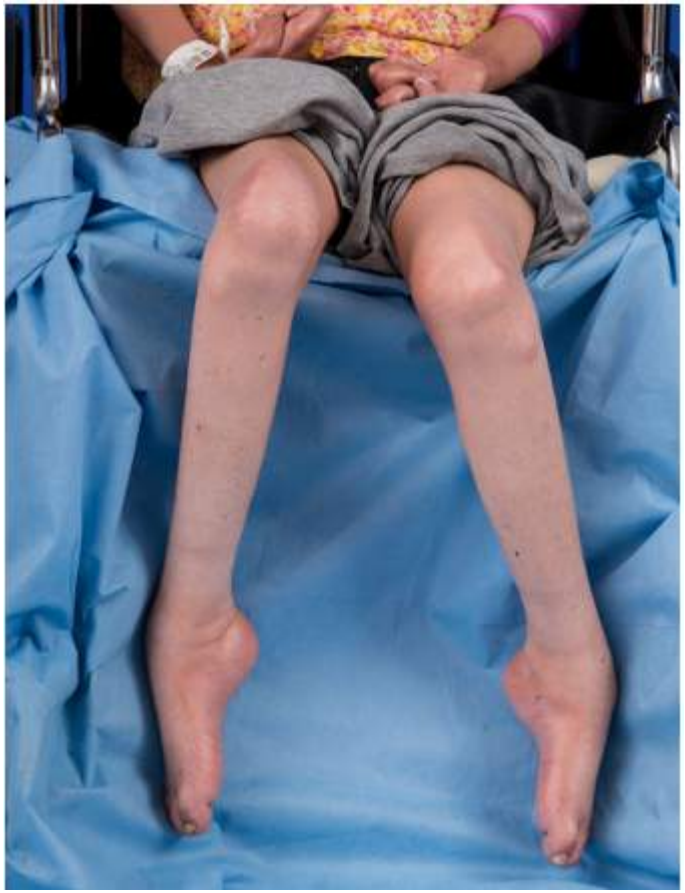
38 year-old woman with:

- **Ichthyosis**
- **Dysmorphic face**
- **Brittle hair**
- **Contractures**
- **Peripheral neuropathy**
- **Developmental delay**
- **Short stature**
- **In-utero grown retardation**
- **Neurosensory hearing loss**

Camilo Toro, MD

UDP 7309





Camilo Toro, MD

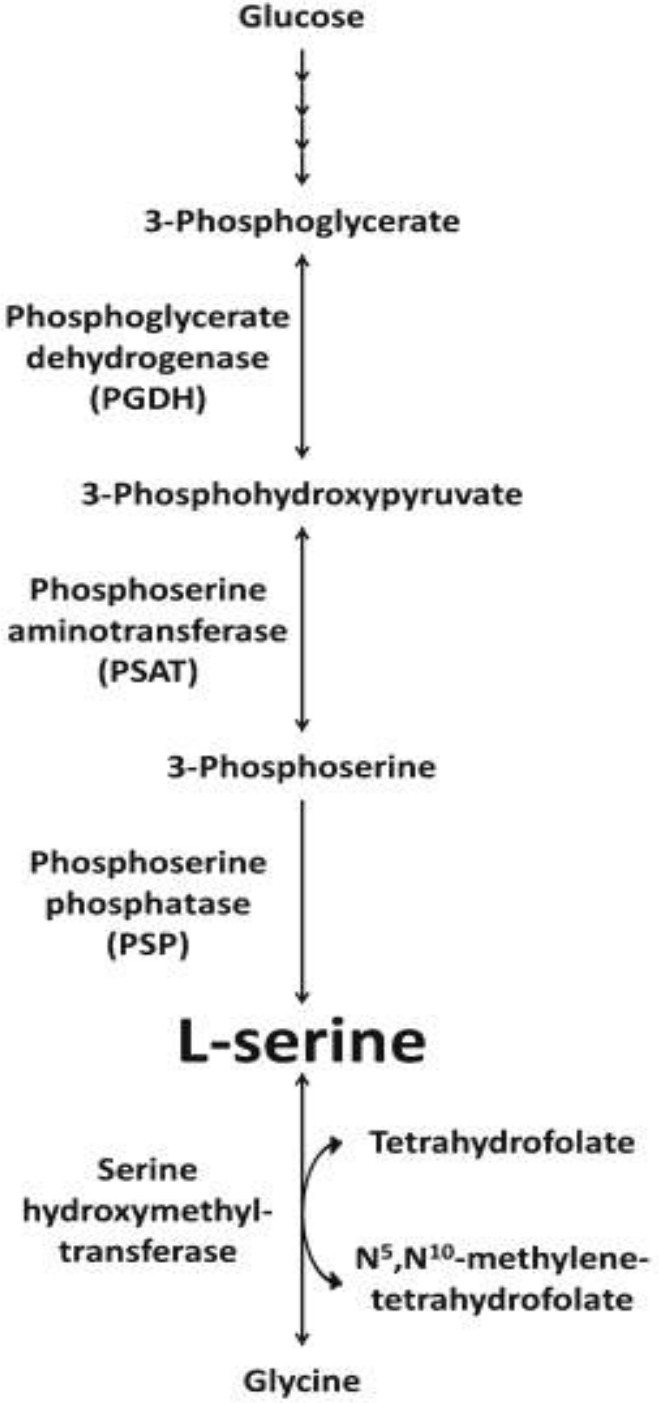
UDP 7309: Disease-associated variants

GENE	PHENOTYPE
KLHL40	Nemaline myopathy
TTN	Myopathy, MD
SMO	Basal cell CA
GUSB	MPS VII
WDR81	Ataxia
PSAT1	Neu-Laxova syndrome 2

PSAT1
Phosphoserine aminotransferase 1

- NEU-LAXOVA SYNDROME (NLS2)

- PHOSPHOSERINE AMINOTRANSFERASE DEFICIENCY



- **CSF and plasma amino acid analyses showed extremely low L-serine levels, confirming that the mutations are functionally deleterious and verifying the diagnosis.**

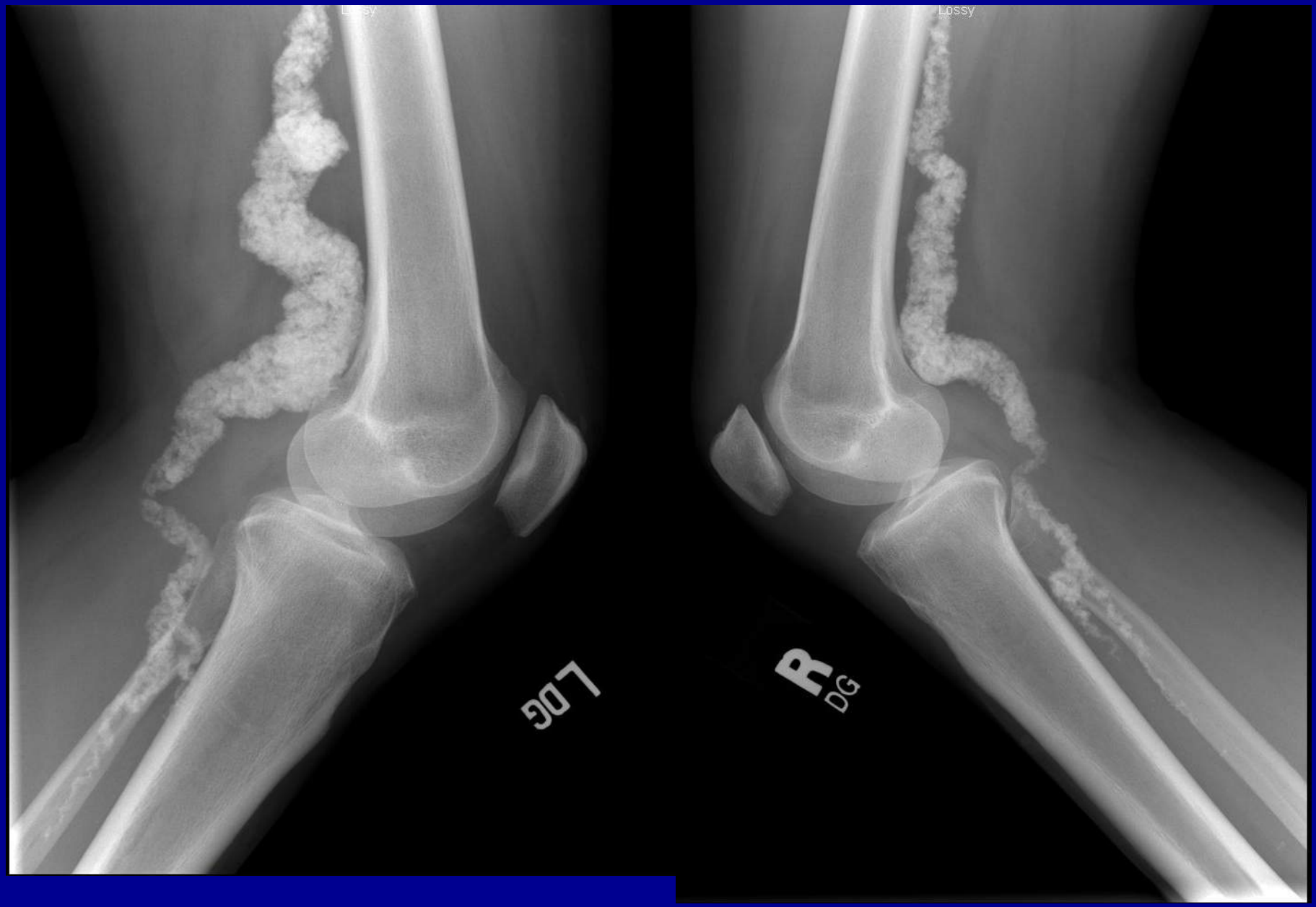
- **The patient was treated with L-serine in substrate amounts, i.e., 4 g/day.**

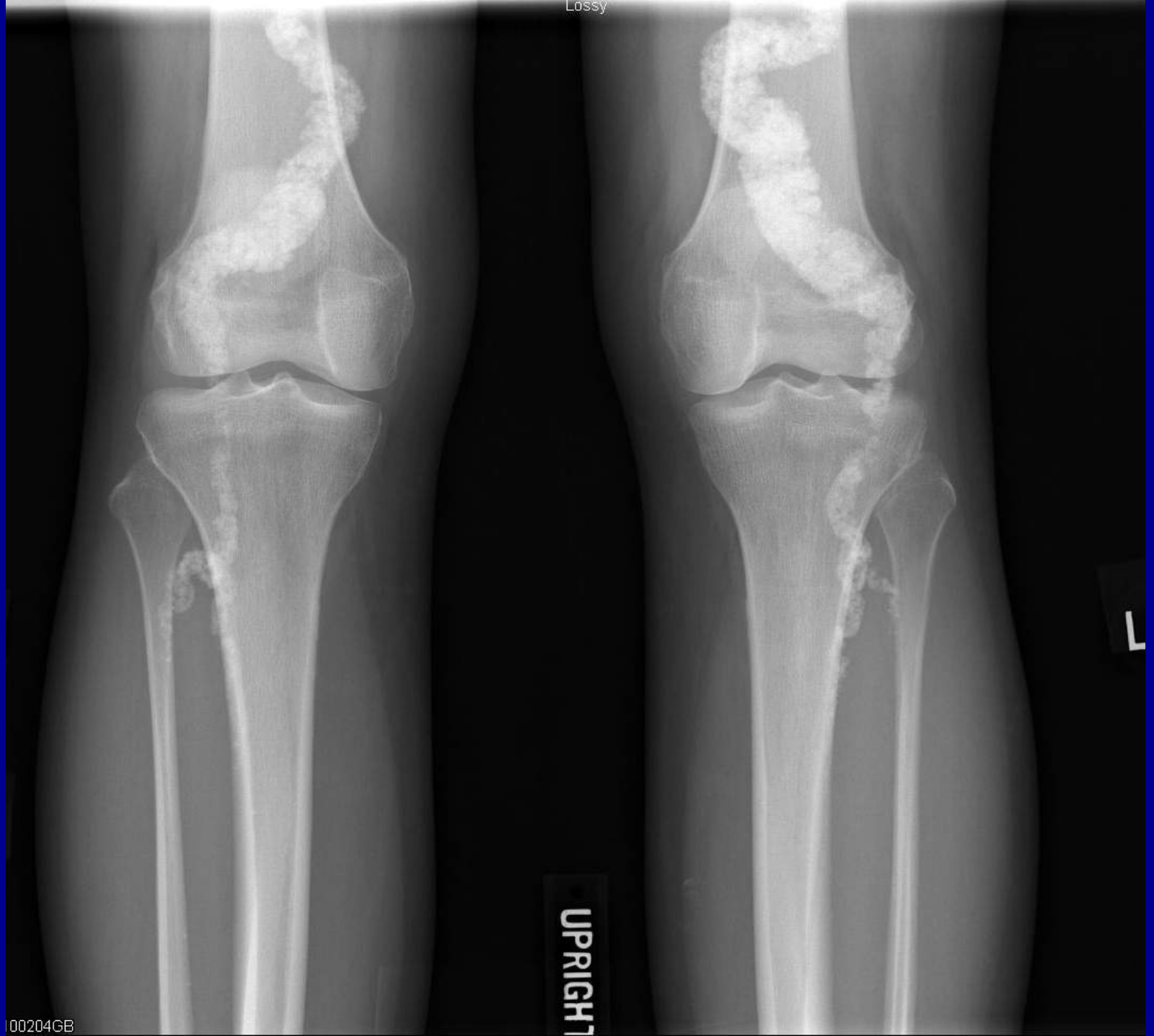
- **The ichthyosis resolved.**

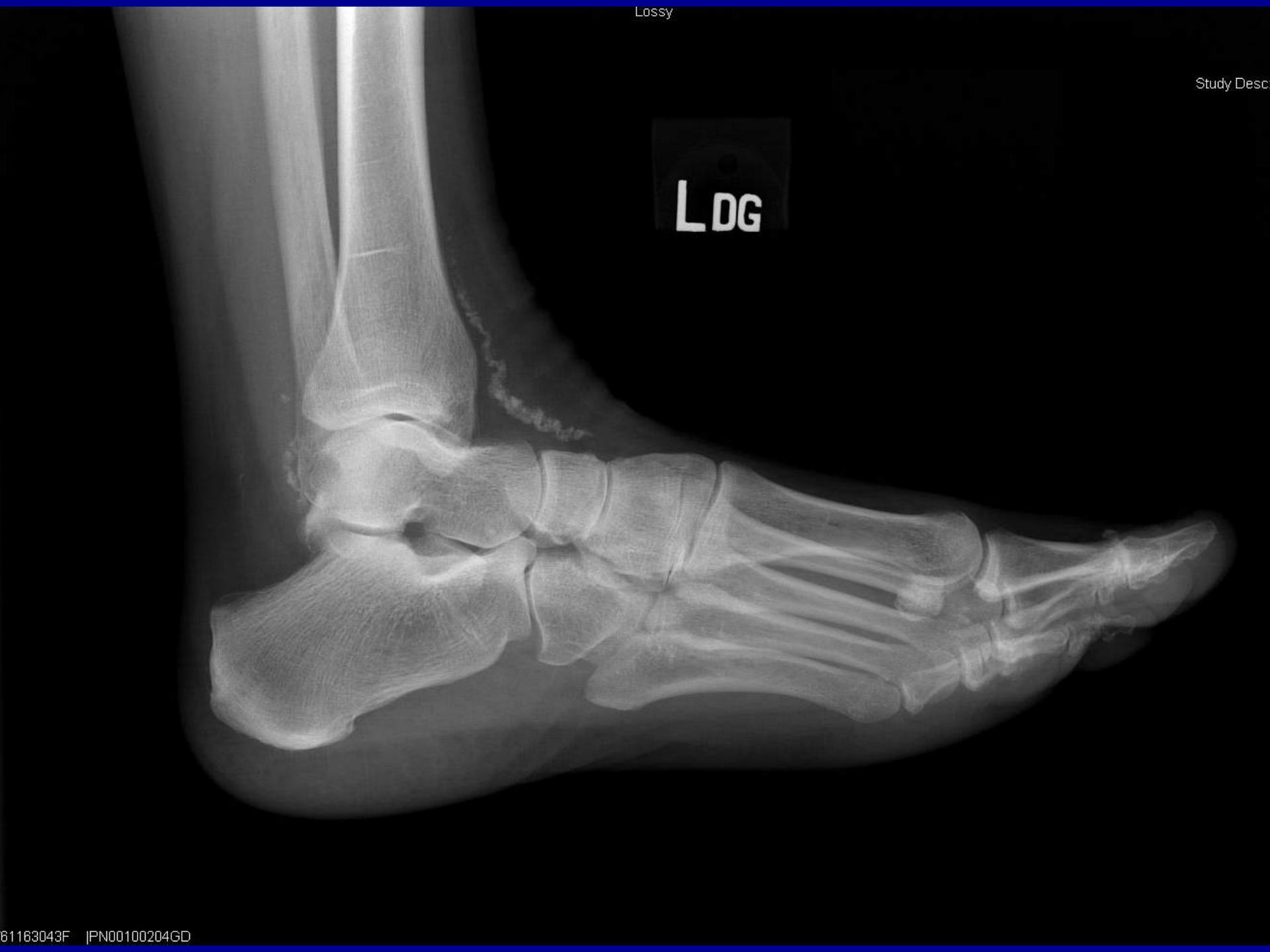
5 Adult Siblings with these Clinical Symptoms and Signs:

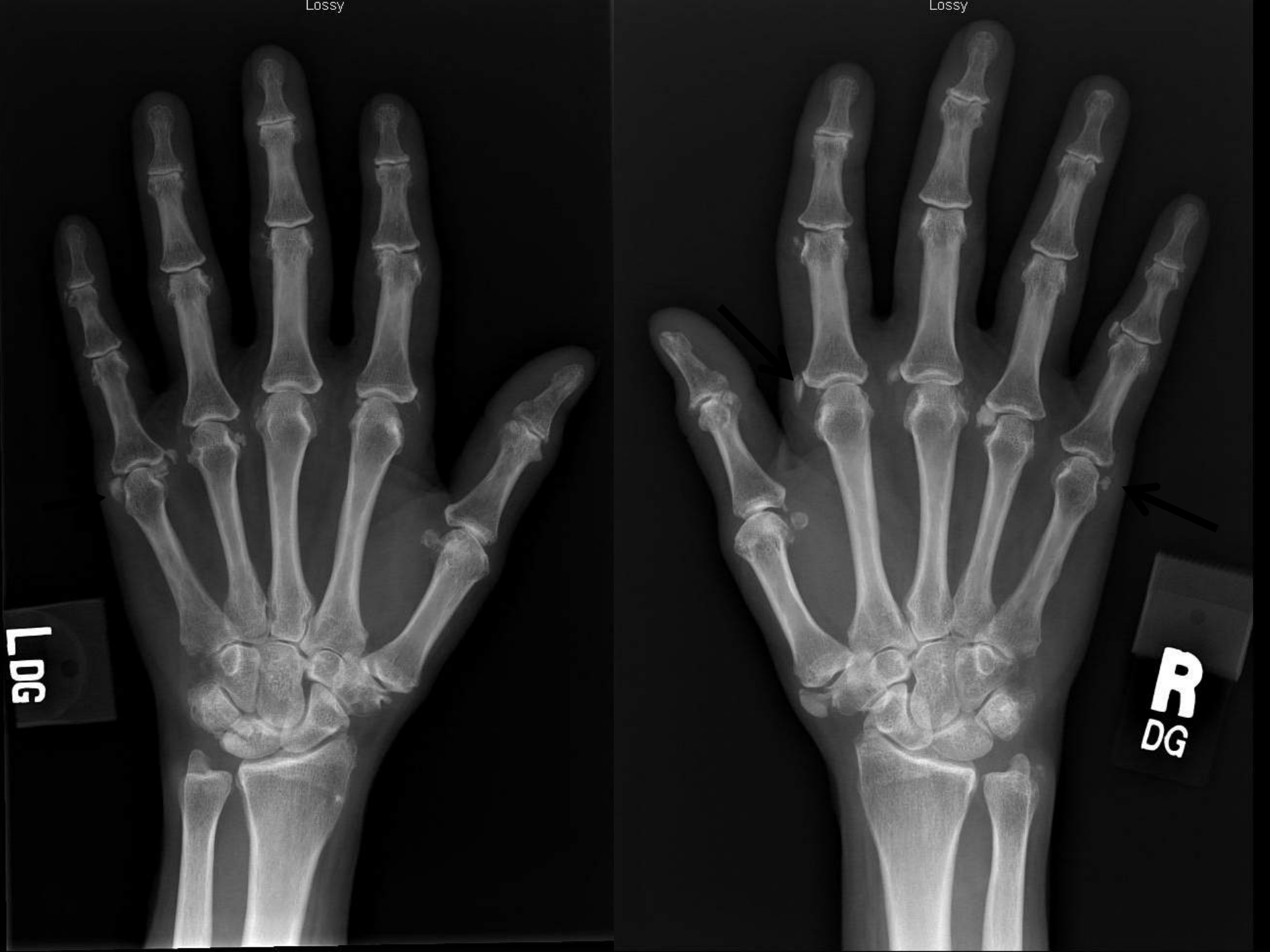
- Intermittent claudication of calves, thighs, buttocks
- Chronic ischemic pain of the feet
- Joint pain in the hands
- Arterial calcification of lower extremities
- Spared coronary arteries

Femoral-Popliteal Artery Calcification

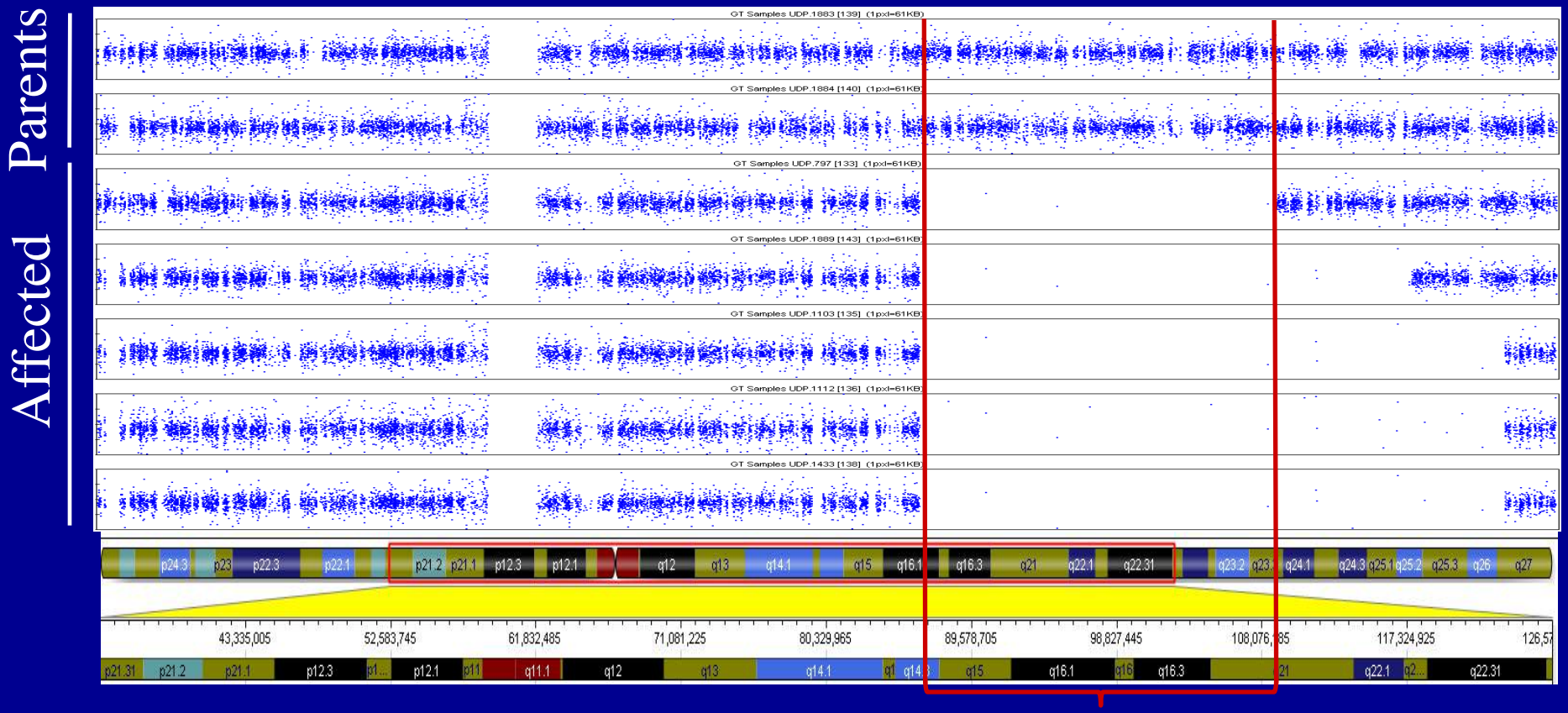



Lossy
UPRIGHT
L
00204GB


Lossy
Study Desc
L DG
61163043F |PN00100204GD


Lossy
Lossy
LDG
R
DG

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



Region of Identical Homozygosity

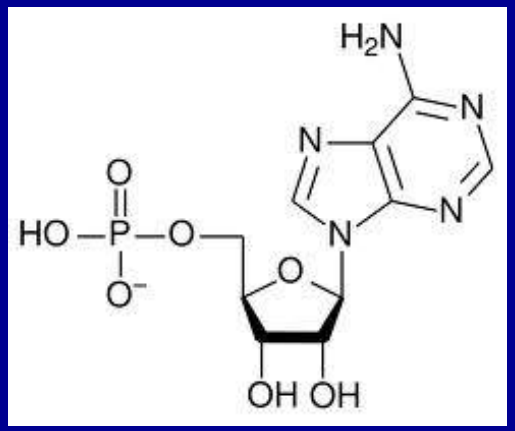
Dr. Tom Markello

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

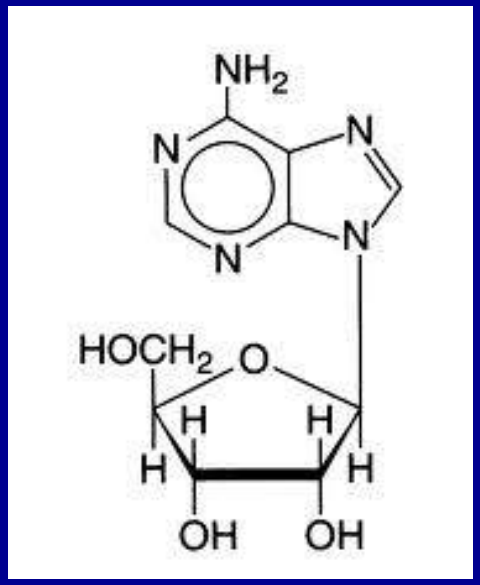
T. Markello, C. St. Hilaire

NT5E Encodes CD73



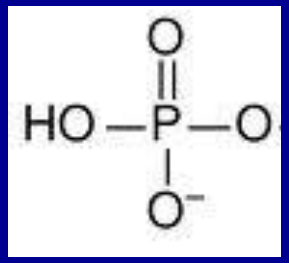
AMP

CD73



Adenosine

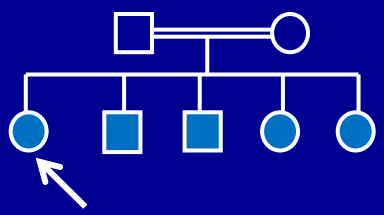
+



Pi

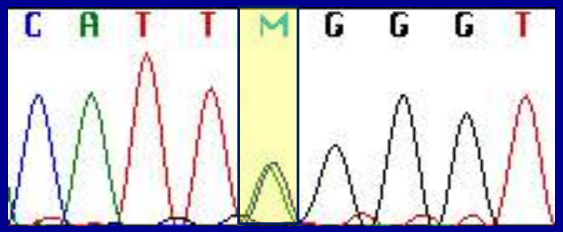
NT5E Sequencing Analysis

Family 1 (UDP)

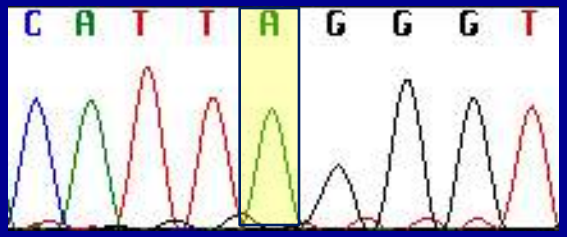


c.662C>A, S>X

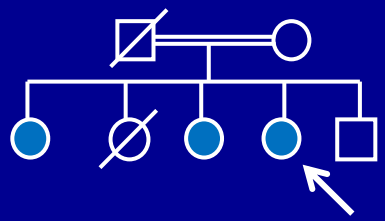
Parent



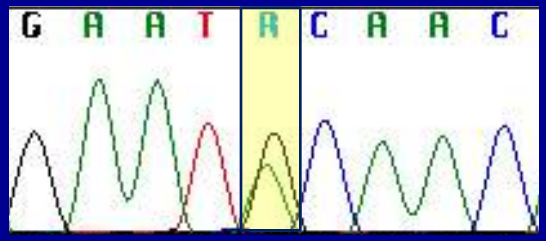
Affected

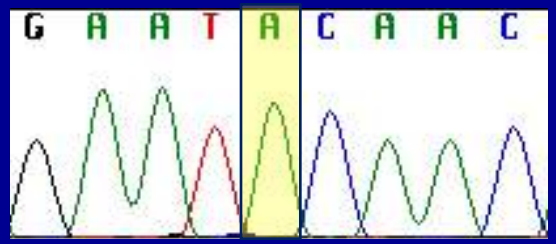


Family 2 (Kleta)

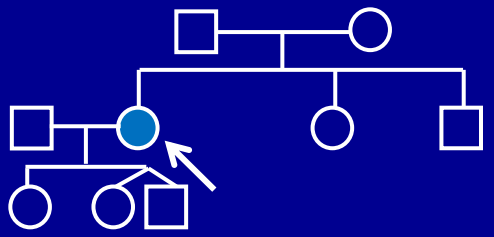


c.1073G>A, C>Y



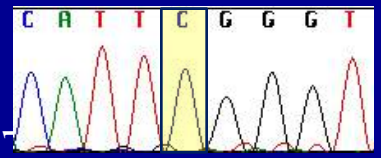


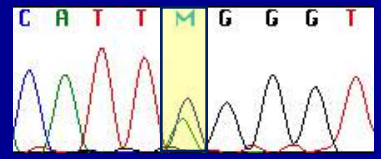
Family 3 (Nussbaum)



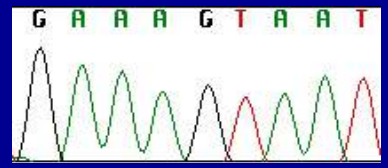
c.1069dupA/c.662C>A

Affected Contro

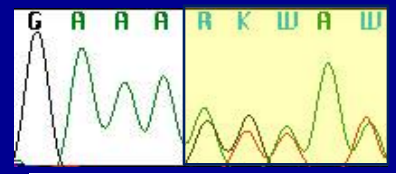




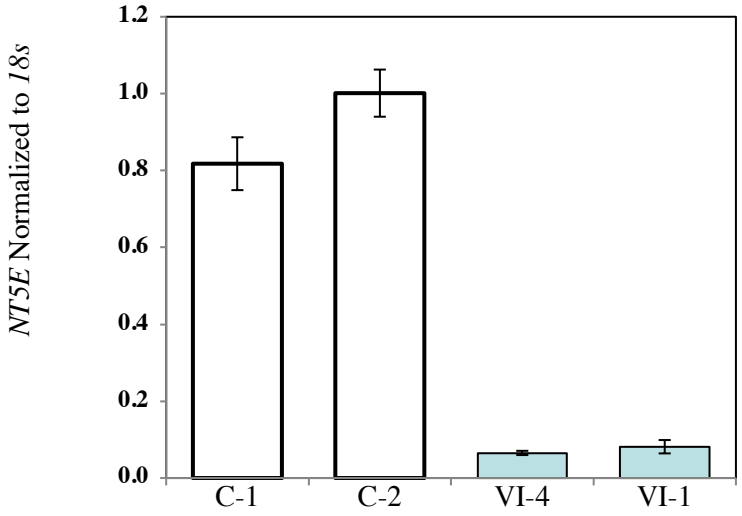
Control

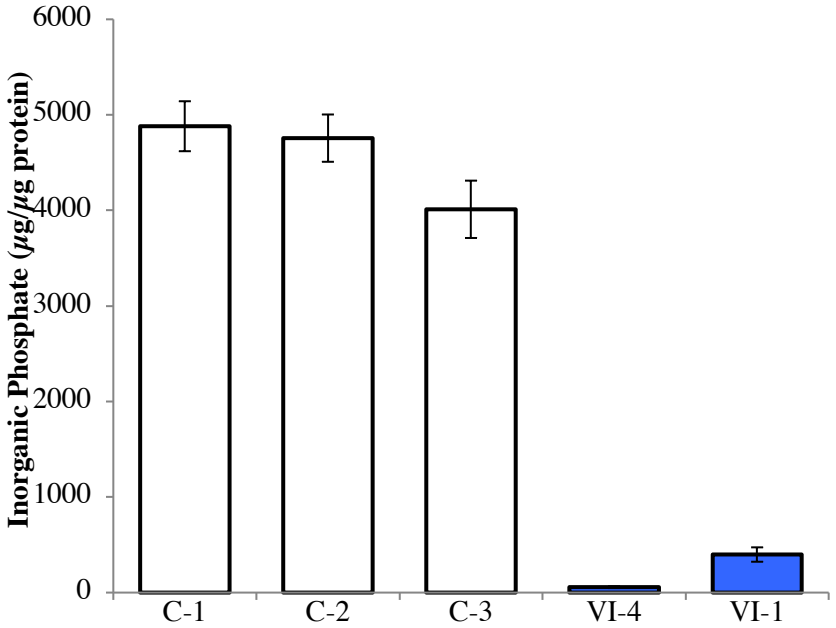


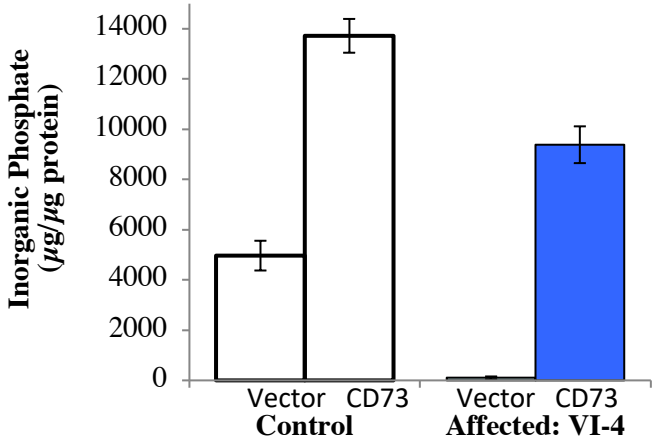
Affected



S. Ziegler

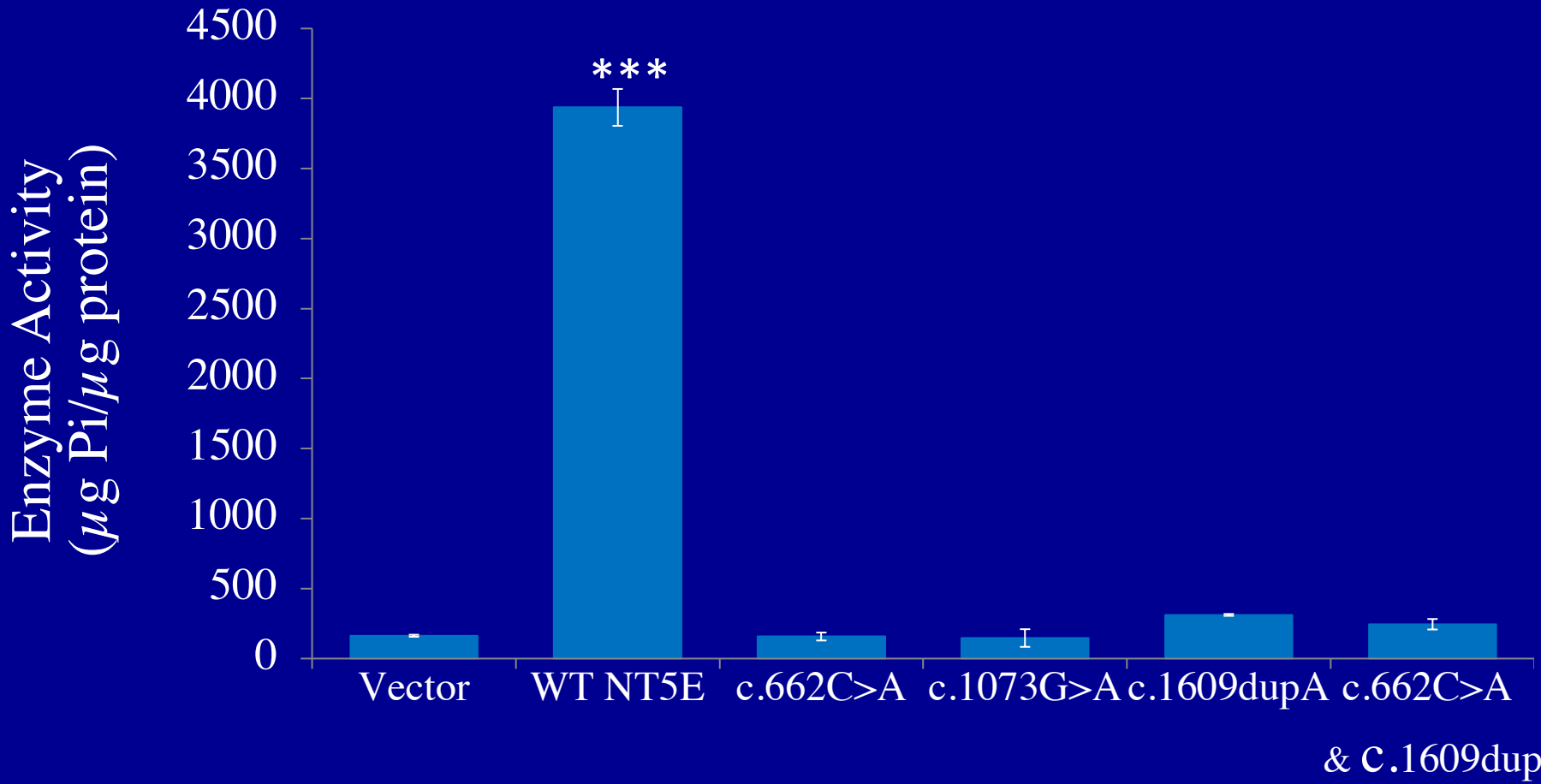






Drs. C. St. Hilaire, M. Boehm

Enzyme Activity in Mutagenized Constructs

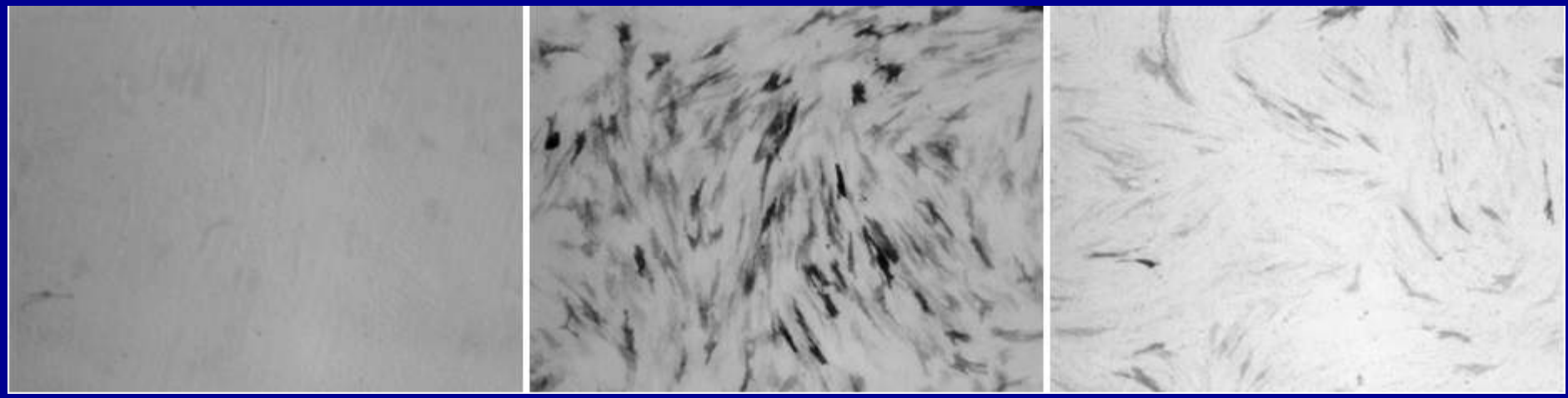


Vectors containing patient *NT5E* mutations transfected into HEK293 cells

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

Increased Fibroblast Staining for Alkaline Phosphatase

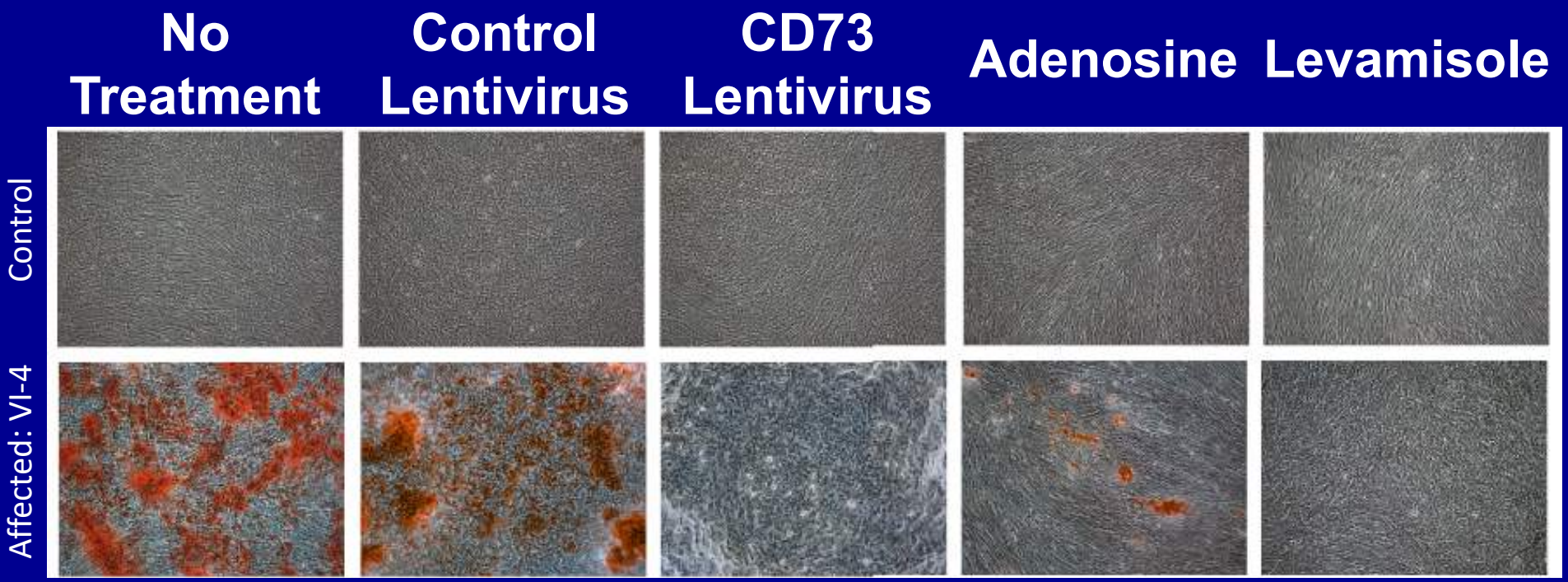
Control | Affected | Affected + Adenosine



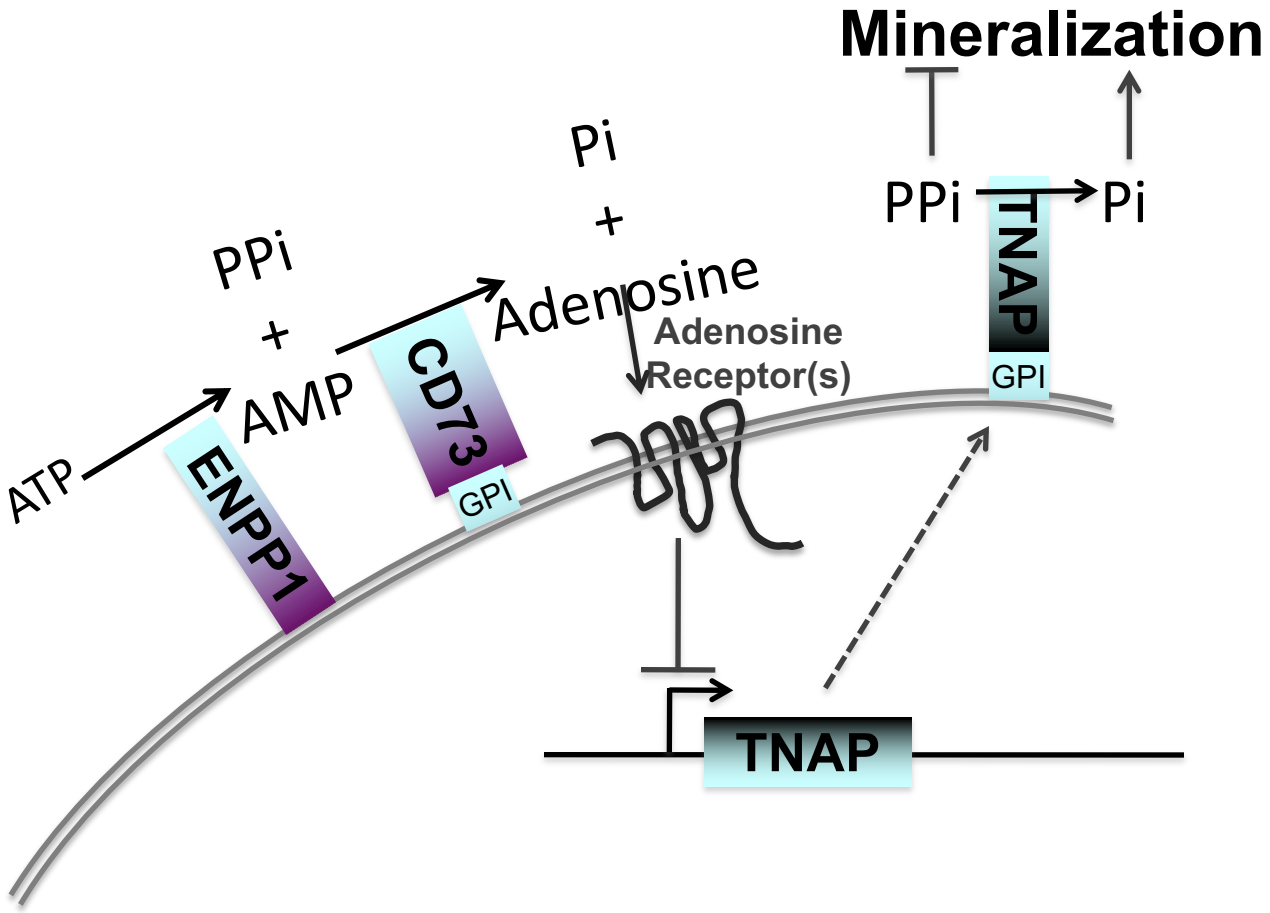
Adenosine treatment of cells reduces alkaline phosphatase staining.

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