a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

GENETIC BASIS OF PYRIDOXINE-RESPONSIVE NEONATAL EPILEPSY IN CONSANGUINEOUS FAMILIES

Presented by:
Hilal Al Shekaili

CHILD & FAMILY RESEARCH INSTITUTE

Graduate Student | Friedman Lab
Child and Family Research Institute
Medical Genetics | University of British Columbia

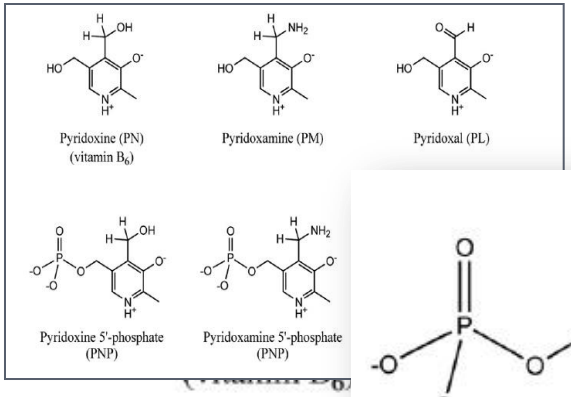
OUTLINE

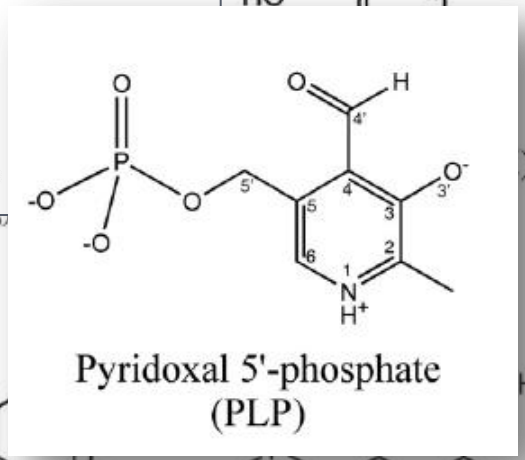
- Absorption & Metabolism of pyridoxine
- Knowledge gaps on pyridoxine metabolism & transportation
- Pyridoxine-dependent epilepsy
- Description of the studied family
- Molecular work
- Results
- Future steps

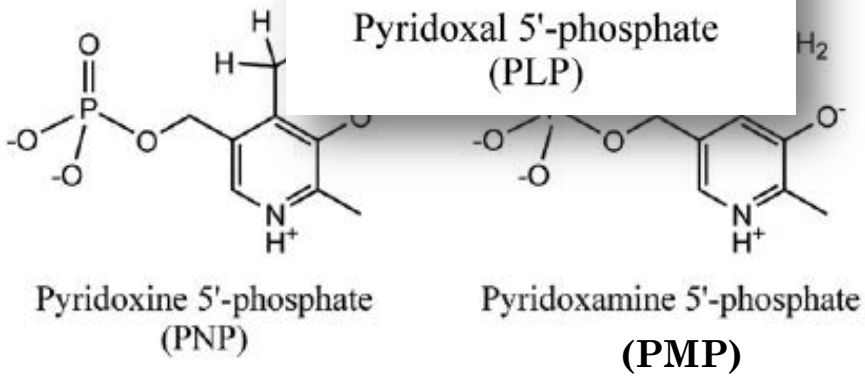
Metabolism of pyridoxine

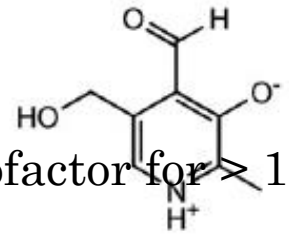
- Vitamin B6 is a generic term:

  = six interconvertible pyridine compounds (vitamers)

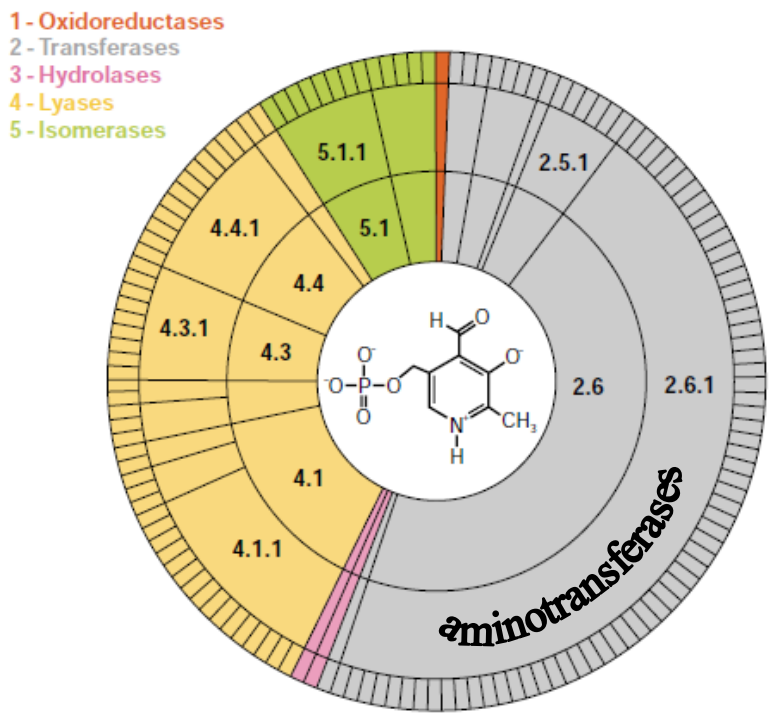








- cofactor for > 140 enzymes

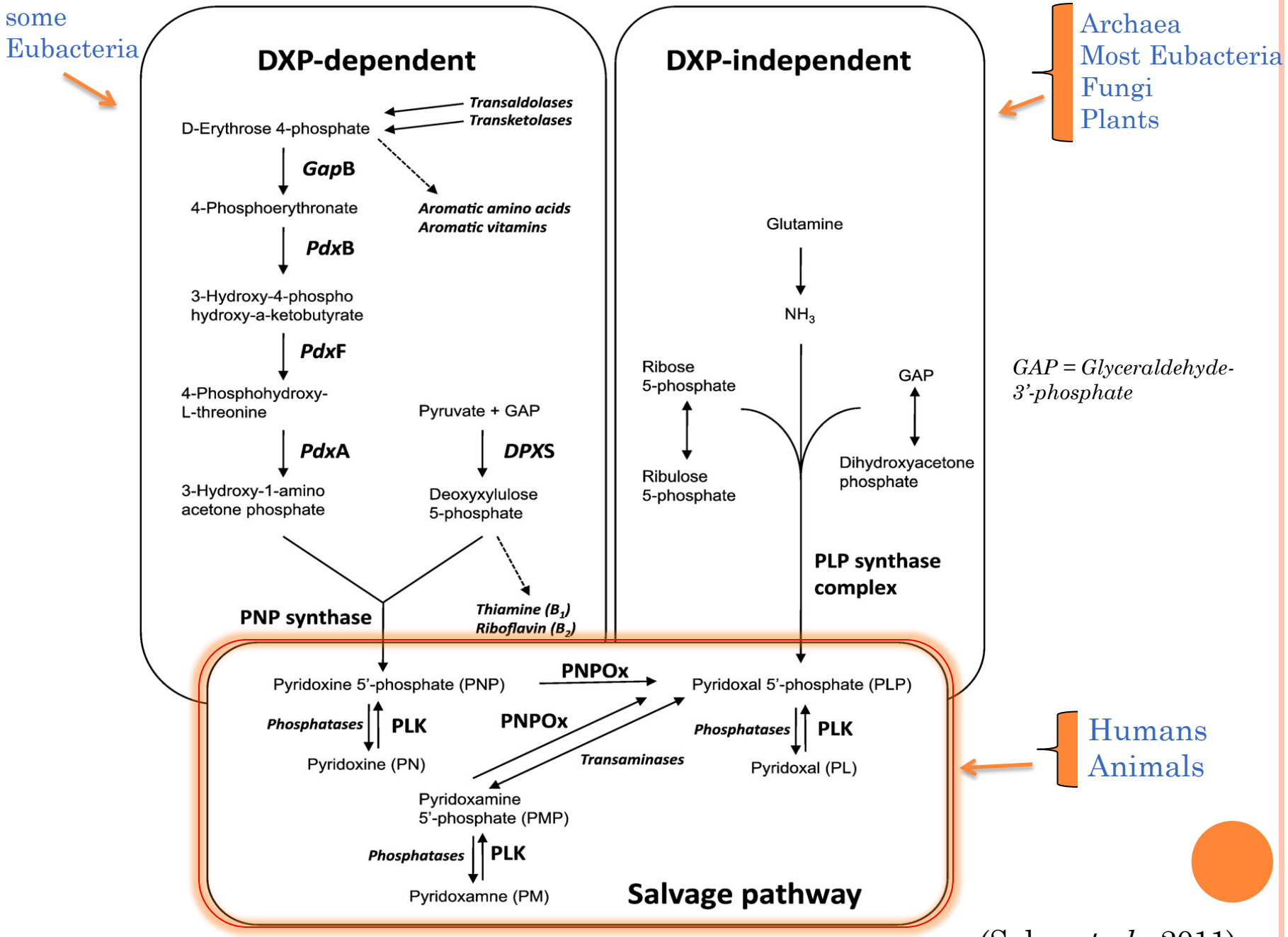


(Salvo *et al.*, 2011; Percudani and Peracchi, 2003)

Metabolism of pyridoxine

- All living beings require vitamin B6 for their existence
- only microorganisms and plants are able to synthesize it *de novo*
- All other organisms acquire vitamin B6 from food and interconvert its different forms (Salvage pathway).

(Salvo *et al.*, 2011; Mooney *et al.*, 2009)



(Salvo *et al.*, 2011)

Metabolism of pyridoxine

- Humans obtain vitamin B6 from dietary and bacterial sources (normal microflora in the large intestine)

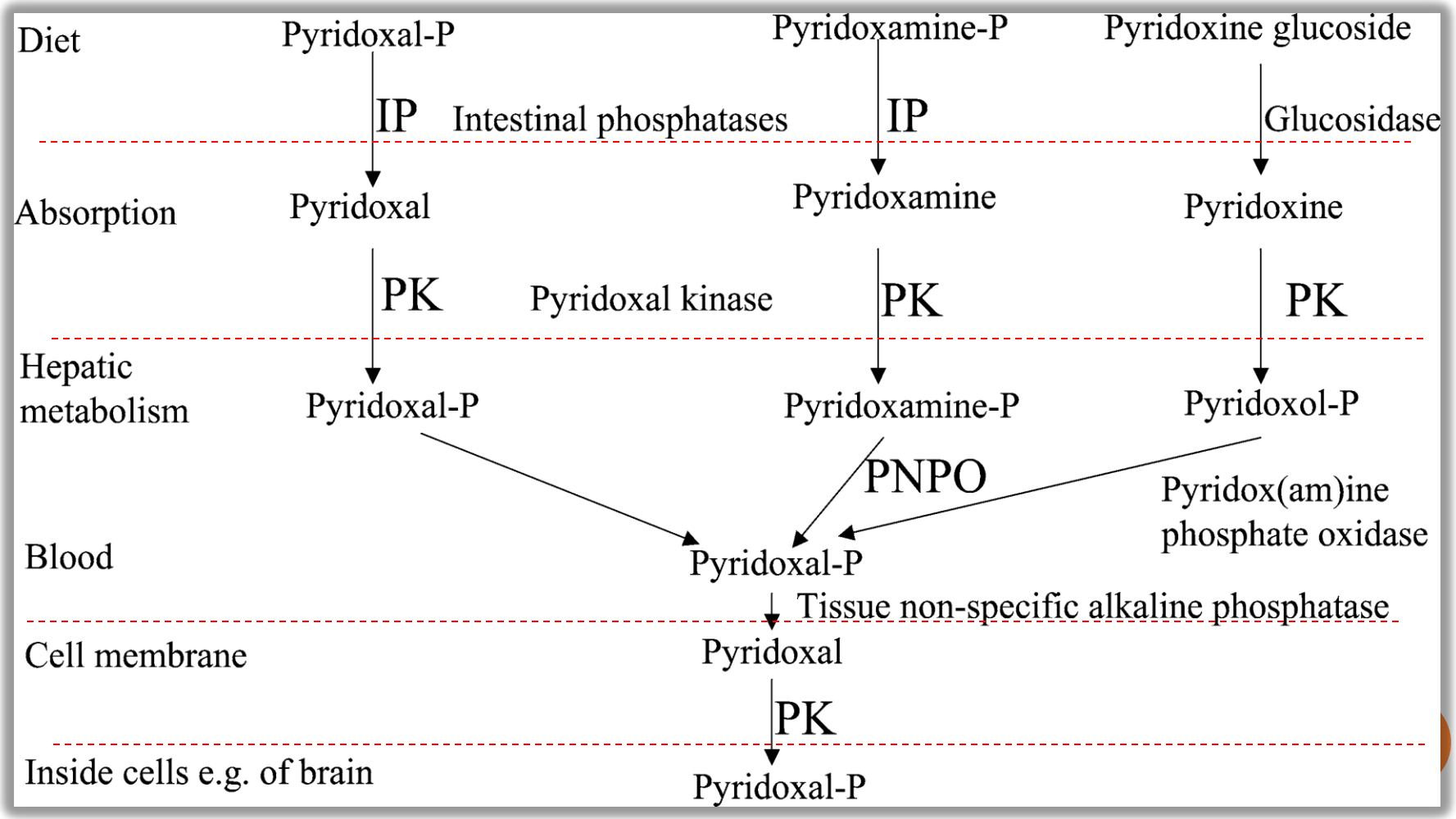
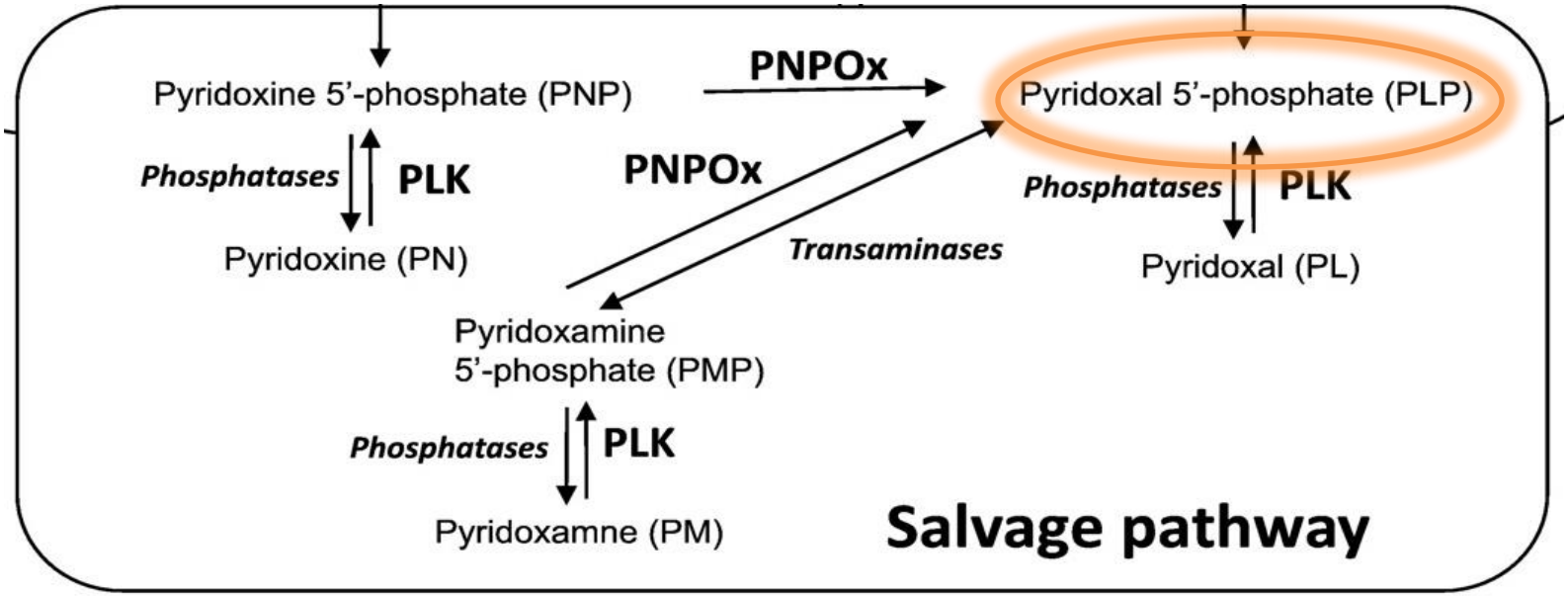


Fig.: A proposed route of vitB6 absorption (Said, 2011; Clayton, 2006)

Gaps in our knowledge of pyridoxine metabolism and transportation

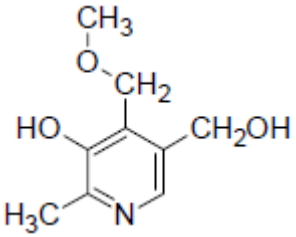
- Regulation of intracellular levels of PLP/ Salvage pathway?



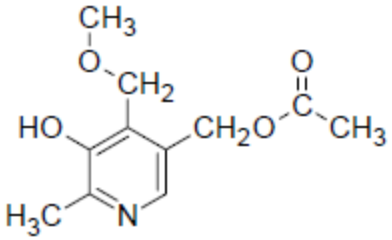
(Mooney *et al.*, 2009; Salvo *et al.*, 2011)

Gaps in our knowledge of pyridoxine metabolism and transportation

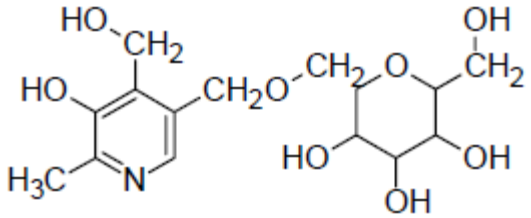
- How PLP is supplied to B6-dependent enzymes?
- A variety of different PN, PM, and PL derivatives have been described for which the precise function is not understood



4'-O-Methylpyridoxine



5'-O-Acetyl-4'-O-methylpyridoxine



5'-0-(β-D-Glucopyranosyl) pyridoxine

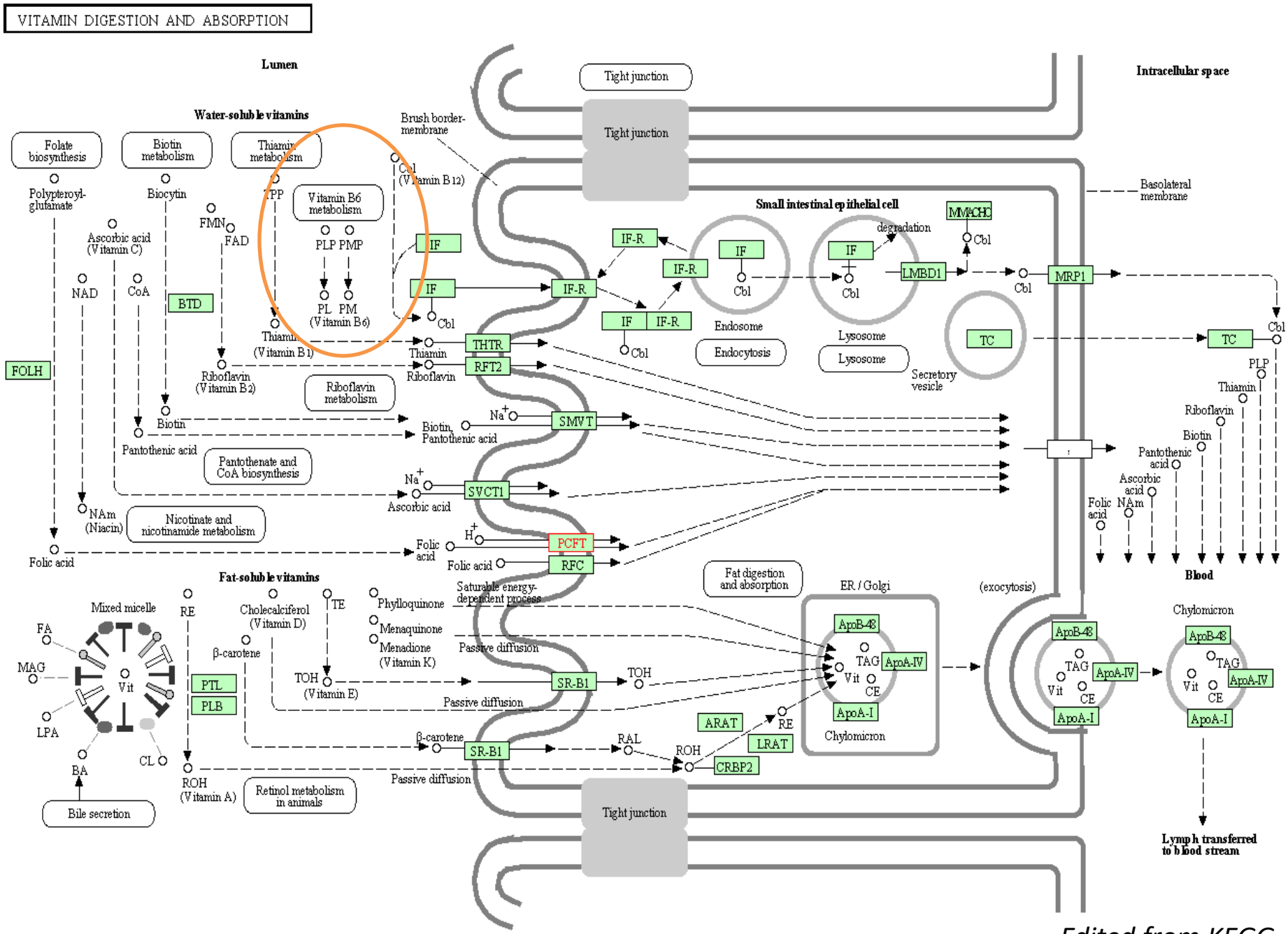
- About 20% of the putative PLP-dependent enzymes encoded by the human genome have unknown catalytic activity

(Mooney *et al.*, 2009; Percudani and Peracchi, 2003)

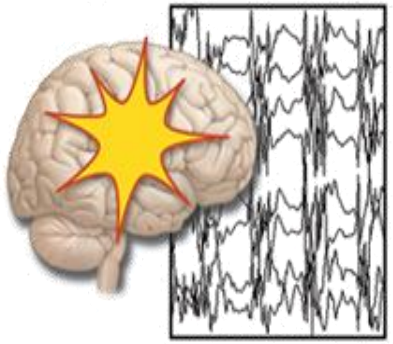
Gaps in our knowledge of pyridoxine metabolism and transportation

- The only vitamin B6 transporters identified so far are the yeast transporters, Tpn1p and Bsu1, and PUP1 in plant species *Arabidopsis* (first to be identified in plants).
  - Plant mutant phenotypes → rescued by exogenous PN. This implied that there is an uptake system in plants.
- In humans, no vitB6 transporter has been identified to date
- Multiple experimental evidence indicated the existence of an efficient and specific carrier-mediated mechanism of vitamin B6 uptake by human intestinal, colonic, as well as renal cells.

(Szydlowski *et al.*, 2013; Said *et al.*, 2002; Said *et al.*, 2003; Said *et al.*, 2008)


VITAMIN DIGESTION AND ABSORPTION
Lumen
Intracellular space
Water-soluble vitamins
Folate biosynthesis
Biotin metabolism
Thiamin metabolism
Vitamin B6 metabolism
Brush border-membrane
Tight junction
Tight junction
Basolateral membrane
Small intestinal epithelial cell
Polypteroyl-glutamate
Biocytin
TPP
FMN
FAD
PLP PMP
PL PM (Vitamin B6)
Cbl (Vitamin B12)
Ascorbic acid (Vitamin C)
NAD
CoA
BTD
Thiamin (Vitamin B1)
Riboflavin (Vitamin B2)
FOLH
Riboflavin metabolism
Biotin
Pantothenic acid
Pantothenate and CoA biosynthesis
NAm (Niacin)
Nicotinate and nicotinamide metabolism
Folic acid
IF
IF-R
Cbl
THTR
RFT2
Thiamin
Riboflavin
SMVT
Biotin, Pantothenic acid
SVCT1
Ascorbic acid
PCFT
RFC
Folic acid
Endosome
Endocytosis
Lysosome
Secretory vesicle
degradation
MMACHC
LMBD1
MRP1
TC
Fat-soluble vitamins
Mixed micelle
FA
MAG
LPA
BA
CL
Vit
PTL
PLB
Bile secretion
RE
ROH (Vitamin A)
Retinol metabolism in animals
Cholecalciferol (Vitamin D)
TE
TOH (Vitamin E)
β-carotene
Phylloquinone
Menaquinone
Menadione (Vitamin K)
Saturable energy-dependent process
Passive diffusion
SR-B1
ARAT
LRAT
CRBP2
RAL
ROH
Fat digestion and absorption
ER / Golgi
(exocytosis)
ApoB-48
ApoA-IV
ApoA-I
TAG
Vit
CE
Chylomicron
Blood
PLP
Thiamin
Riboflavin
Biotin
Pantothenic acid
Ascorbic acid
Folic acid
NAm
Lymph transferred to blood stream
04977 4/7/11
(c) Kanehisa Laboratories
Edited from KEGG

Pyridoxine-dependent epilepsy (PDE)



- Rare, autosomal recessive disorder
- Estimated incidence of 1:20,000 to 1:600,000
- characterized by recurrent seizures in the prenatal, neonatal, or postnatal period, which are:
  - typically resistant to conventional anticonvulsant treatment.
  - show remarkable response to the administration of pyridoxine (vitamin B6)
  - recur soon after the pyridoxine is stopped.

(Sadilkova *et al.*, 2009; Clayton, 2006; Baumgartner-Sigl *et al.*, 2007; Striano *et al.*, 2009; Mills *et al.*, 2005; Walker *et al.*, 2000)

PYRIDOXINE DEPENDENCY: REPORT OF A CASE OF INTRACTABLE CONVULSIONS IN AN INFANT CONTROLLED BY PYRIDOXINE

By A

A Gene for Pyridoxine-Dependent Epilepsy Maps to Chromosome 5q31

Valérie Cormier-Daire,[1,*] Nathalie Dagoneau,[1,*] Rima Nabbout,[2] Lydie Burglen,[1] Clotilde Penet,[1] Christine Soufflet,[2] Isabelle Desguerre,[2] Arnold Munnich,[1] and Olivier Dulac[2]

- Genetic cause discovered in 2006 by Mills *et al*.

nature medicine

Mutations in antiquitin in individuals with pyridoxine-dependent seizures

Philippa B Mills[1], Eduard Struys[2], Cornelis Jakobs[2], Barbara Plecko[3], Peter Baxter[4], Matthias Baumgartner[5], Michèl A A P Willemsen[6], Heymut Omran[7], Uta Tacke[7], Birgit Uhlenberg[8], Bernhard Weschke[8] & Peter T Clayton[1]

Pathophysiology of PDE

- In most affected infants, PDE is caused by mutations in the antiquitin gene (*ALDH7A1*)

(Mills *et al.*, 2006; Gospe, 2012)

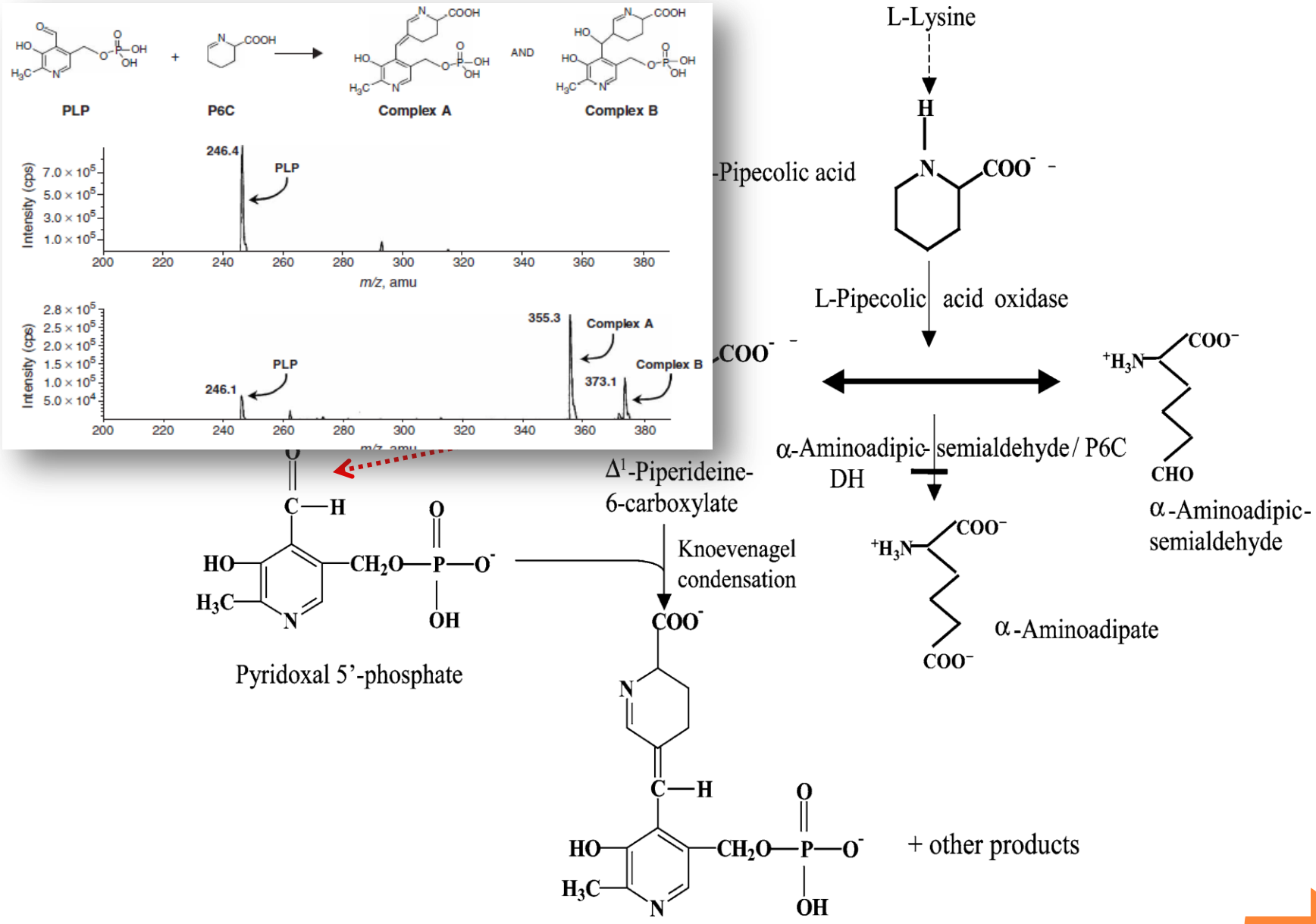
Pathophysiology of PDE

mutations in the antiquitin gene (*ALDH7A1*)

inactivation of α-aminoadipic semialdehyde dehydrogenase

an enzyme that functions within the cerebral lysine catabolism pathway

(Sadilkova *et al.*, 2009)



metabolism of L-lysine via the pipecolic acid pathway

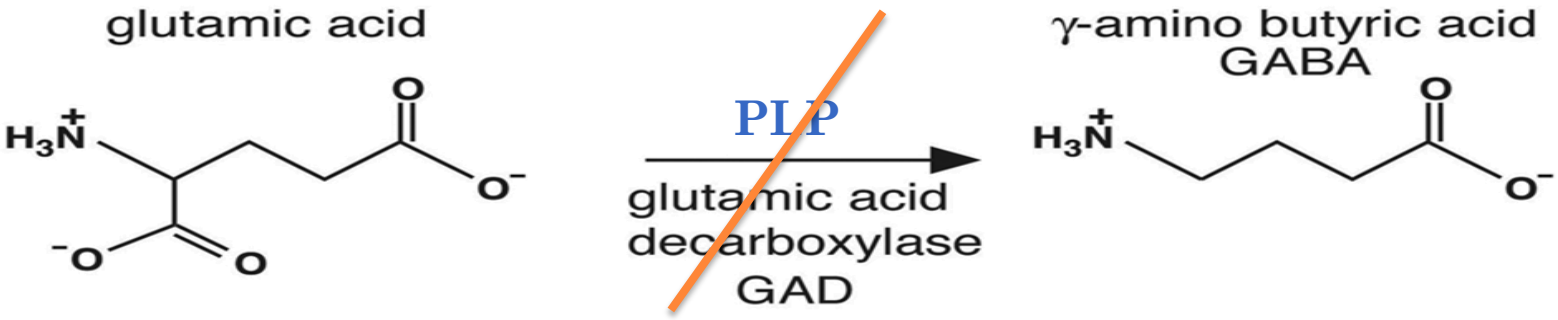
(Clayton, 2006; Mills *et al.*, 2006)

PDE bimarkers

- Elevated α-AASA in plasma or urine
- Elevated pipecolic acid in plasma

(Stockler *et al.*, 2011; Mills *et al.*, 2010)

Hypothesized cause of seizures



↑ excitatory neurotransmitter

↓ inhibitory neurotransmitter

(Murty *et al.*, 2013)

- Currently *ALDH7A1* is the only gene for which mutations are known to underlie PDE.

- However, locus heterogeneity has been reported in some families and other genes seem to be involved.

- Nearly 5% of children with a typical clinical picture of PDE harbor no detectable mutation of *ALDH7A1*.

- Identifying causative genes in such families:

  - Improved treatment for these patients.

  - Fill knowledge gaps about pyridoxine metabolism and transportation in the human body.

(Bennett *et al.*, 2005; Gospe, 2012)

Aim of this project

To characterize the genetic defect underlying PDE in a consanguineous Omani Arab family with two affected children who have a PDE-like clinical picture but negative ATQ biomarkers.

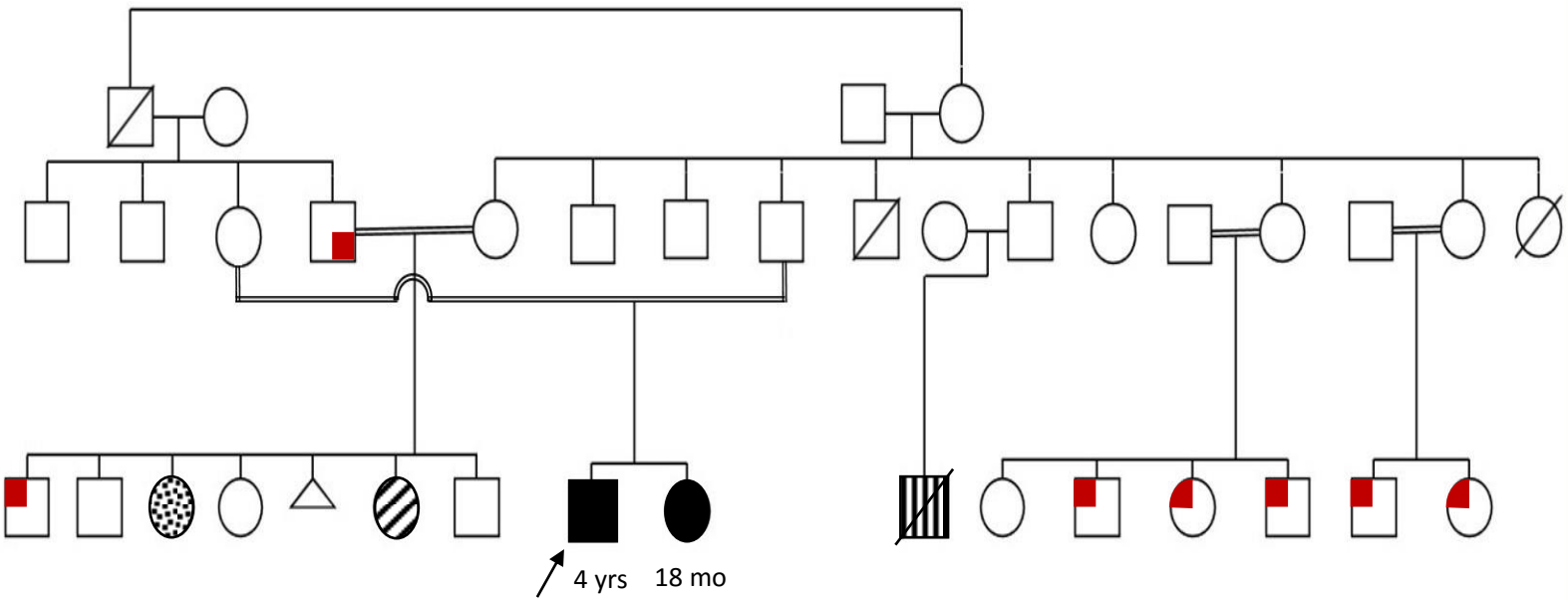
Oman

- Total population: 2,773,479 (2010 census)
- Omanis: 1,957,336
- previous study based on a large sample of the population in Oman has shown the rate of consanguinity in the general Omani population is around **55%**

- " Because of the strictly endogamous nature of the tribal groups in Oman, all marriages would be expected to be consanguineous to some degree, albeit at a level beyond that of second cousins"

(Al Khabori and Patton, 2008; Rajab and Patton, 2000)

Family pedigree



Prelingual
Hearing loss

Microcephaly
Spastic quadriparesis
Global developmental delay
Pigmentary retinopathy
Intractable seizures partially responsive to pyridoxine

Delayed milestones
Hirschsprung's disease
Spastic quadriparesis
Intractable seizures

Partail seizures

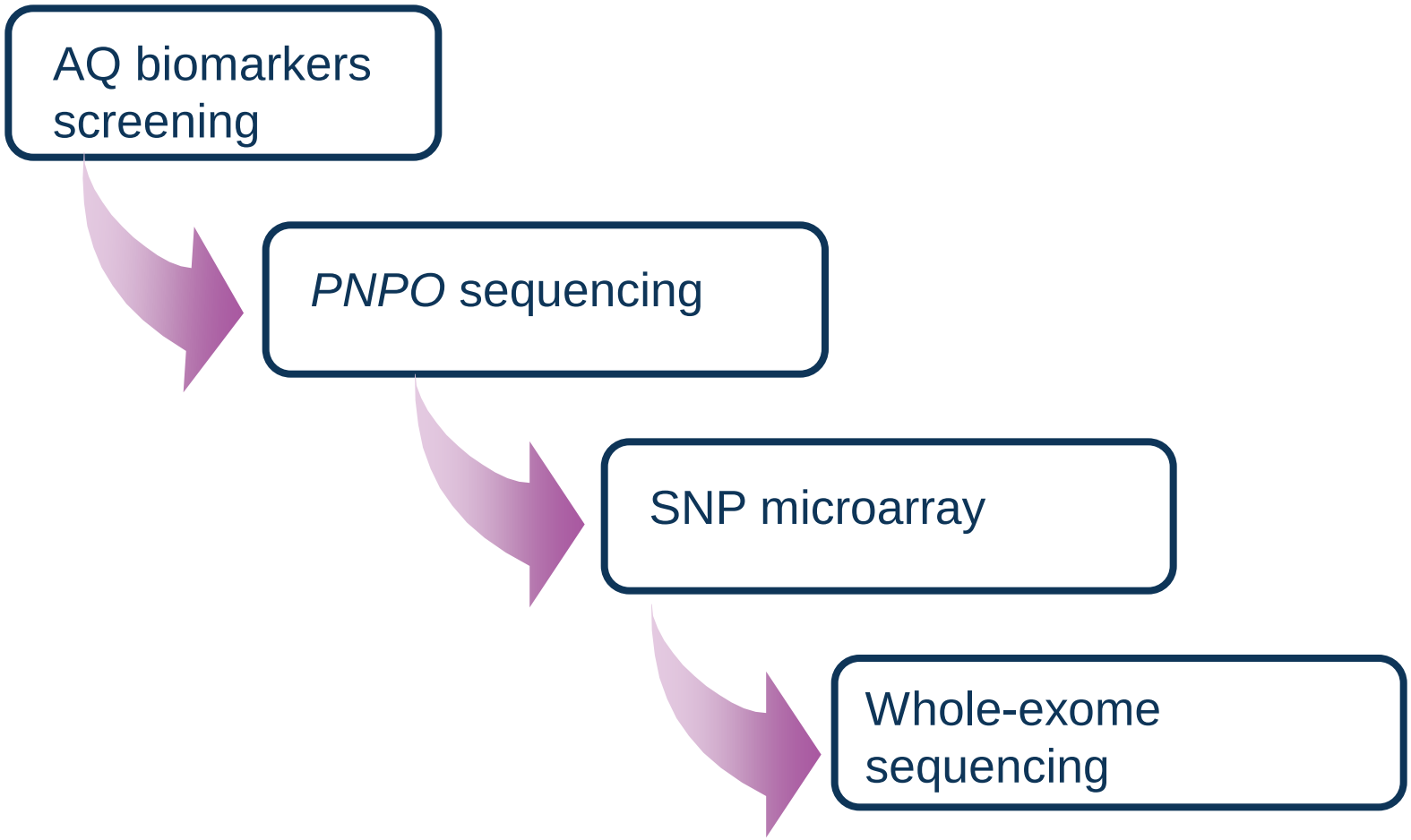
Pyridoxine – responsive epilepsy

Anencephaly

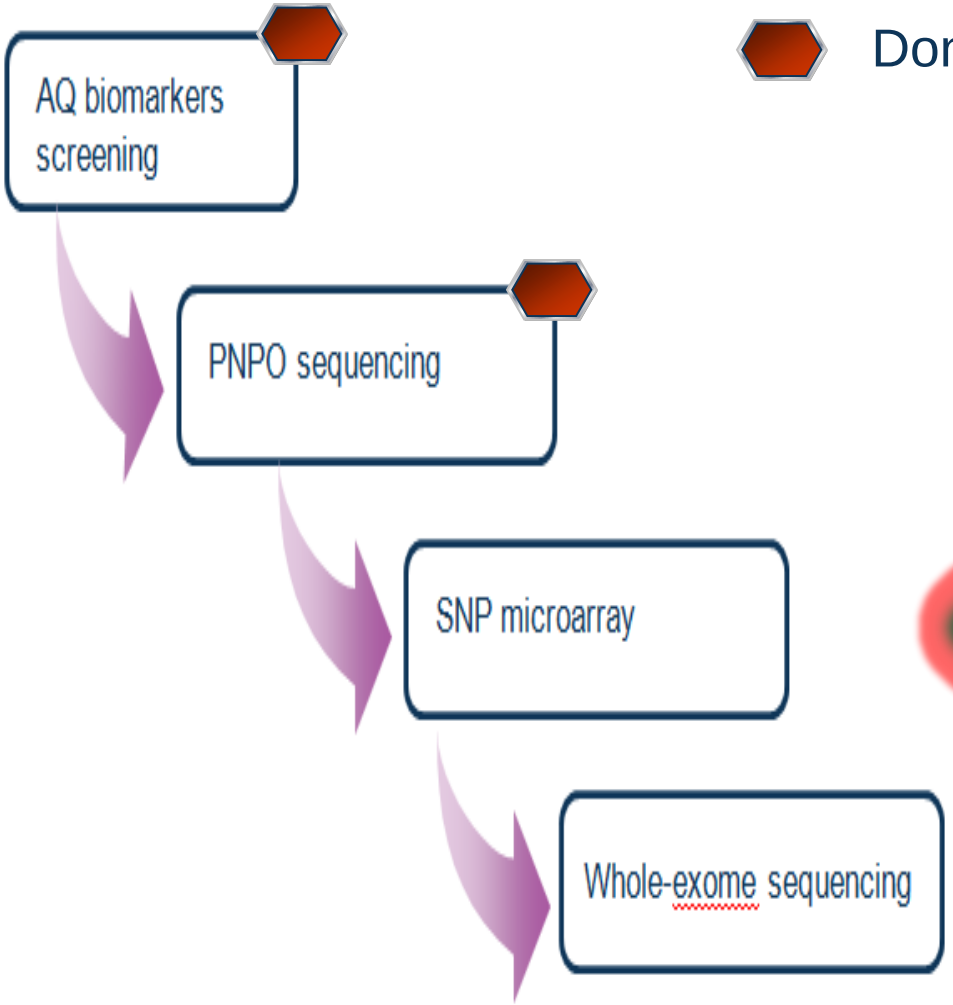
Description of the phenotype

- Seizures started 3-4 weeks after birth
- Breakthrough seizures with febrile illness
- Refractory to multiple anti-epileptics
- Dramatically abolished with vitamin B6 treatment
- EEG: Burst suppression
- Antiquitin biomarkers:
  - Negative urinary α-AASA
  - Normal plasma pipecolic acid

Molecular work



Molecular work



- Done by GDMC, both -ve
- Done at BCCH for index pateint, -ve (Plecko *et al.*, 2014)

Pyridoxine responsiveness in novel mutations of the *PNPO* gene

SNP microarray

- Whole-genome SNP genotyping was performed on the father and the two affected children
- Illumina HumanOmni5-Quad array chip
- Call rates for these samples were > 99.9% giving rise to a coverage of about 4.3 million SNPs per sample.
- SNP density: 1 SNP per 0.738 kb

Runs of Homozygosity (RoH) mapping

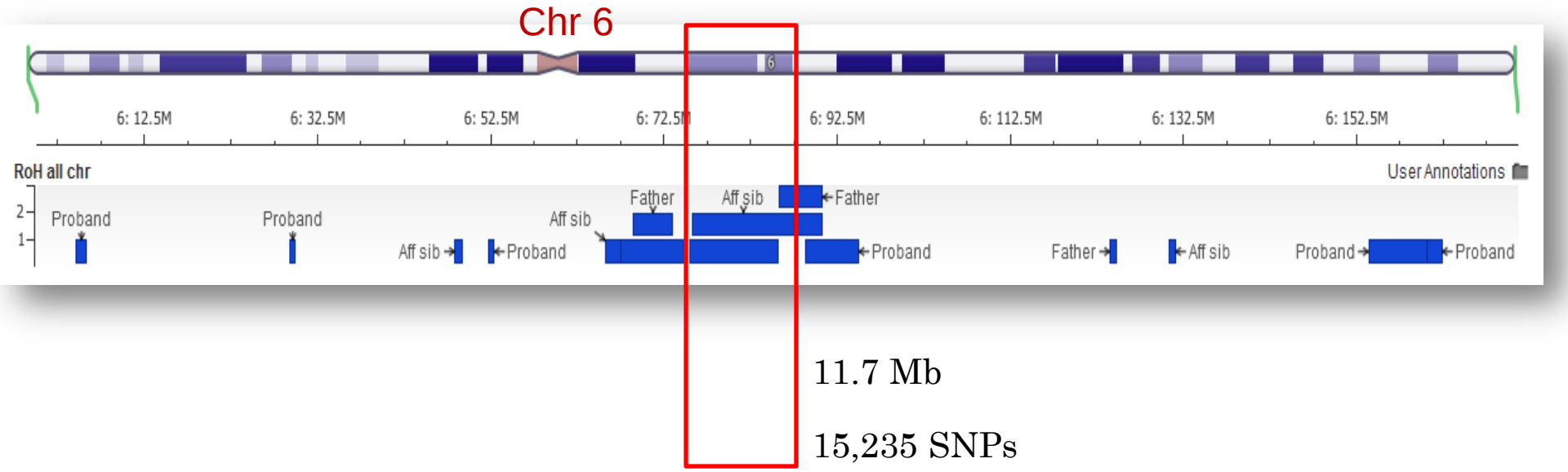
- was carried out using SNP & Variation Suite (SVS) software version 8.1.0 (Golden Helix), with the following conditions:

  1) min. RoH length: 500 kb
  2) min. no. of SNPs per RoH: 25
  3) allowing inclusion of up to one heterozygous call
  4) allowing inclusion of up to 5 missing genotypes
  5) max. gap between SNPs in an RoH: 100 kb.

- Based on these criteria, primary SVS run yielded total of **327** RoH's in the autosomes of the 3 individuals.

- Of these, only **46** regions were overlapping between the two affecteds

GOLDEN HELIX SNP & VARIATION SUITE



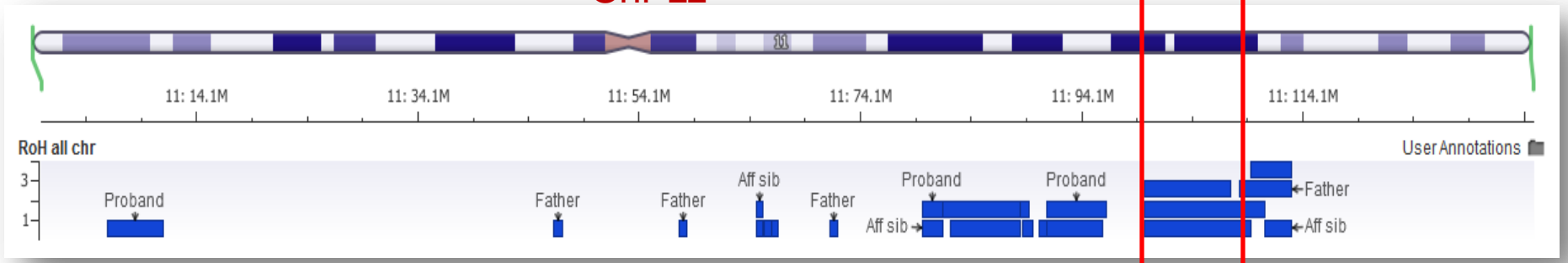
11.7 Mb

15,235 SNPs

Largest RoH (excluding X-chr)

GenomeBrowse™

Chr 11

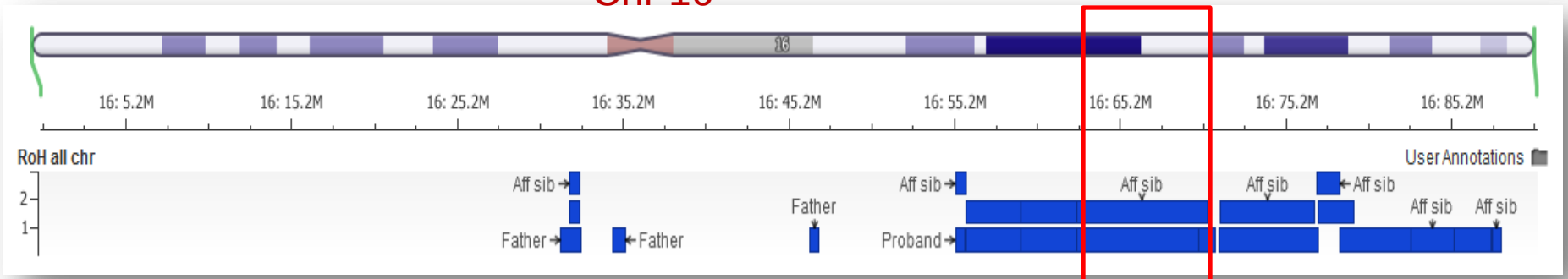


9.7 Mb

13,605 SNPs

Largest RoH overlap between affected sibs

Chr 16



7.86 Mb

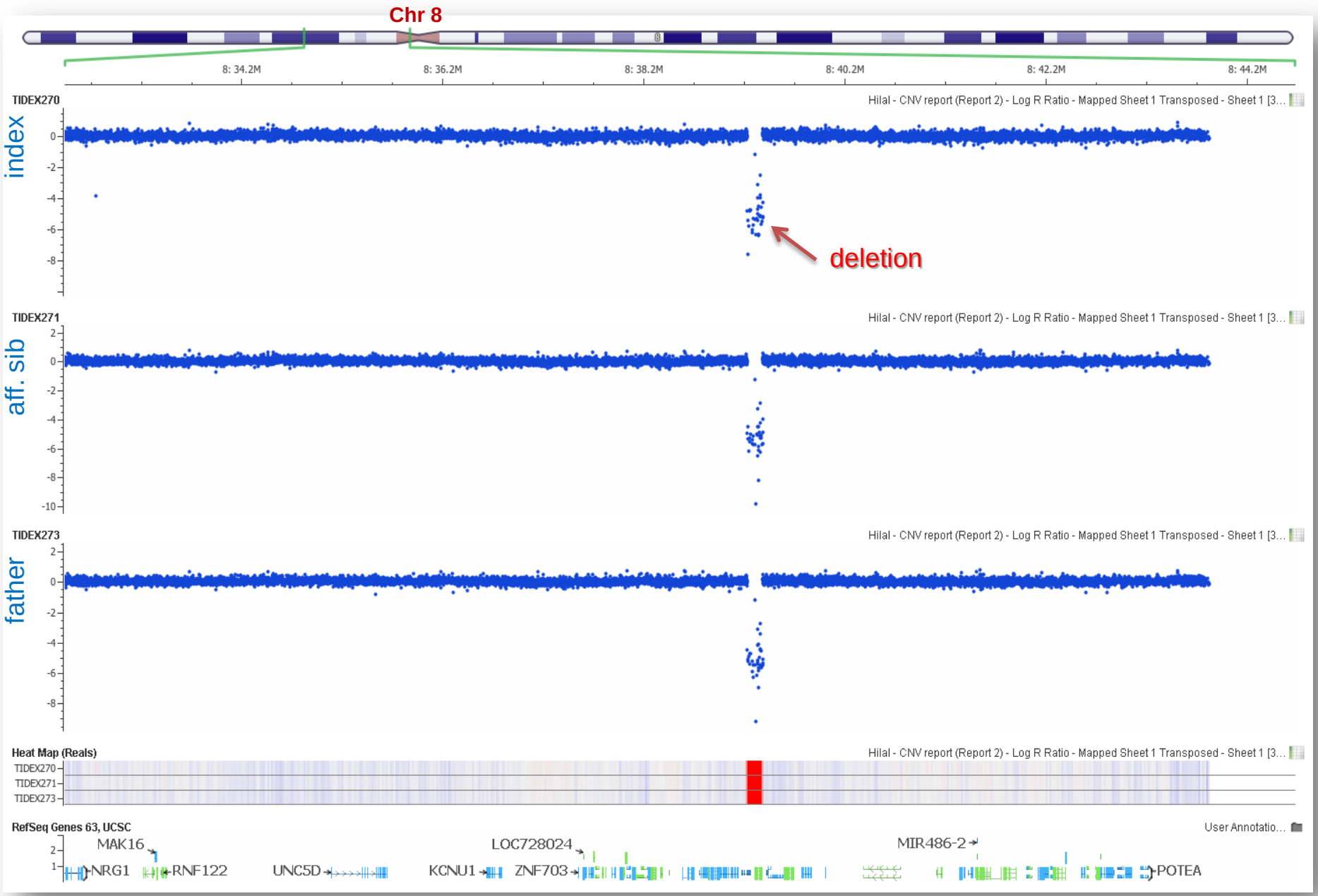
10,875 SNPs

GenomeBrowse™

CNV analysis

- SNP array dataset
- CNAM (Copy Number Analysis Method) tool in SVS
- No pathogenic CNV was found

GOLDEN HELIX SNP & VARIATION SUITE



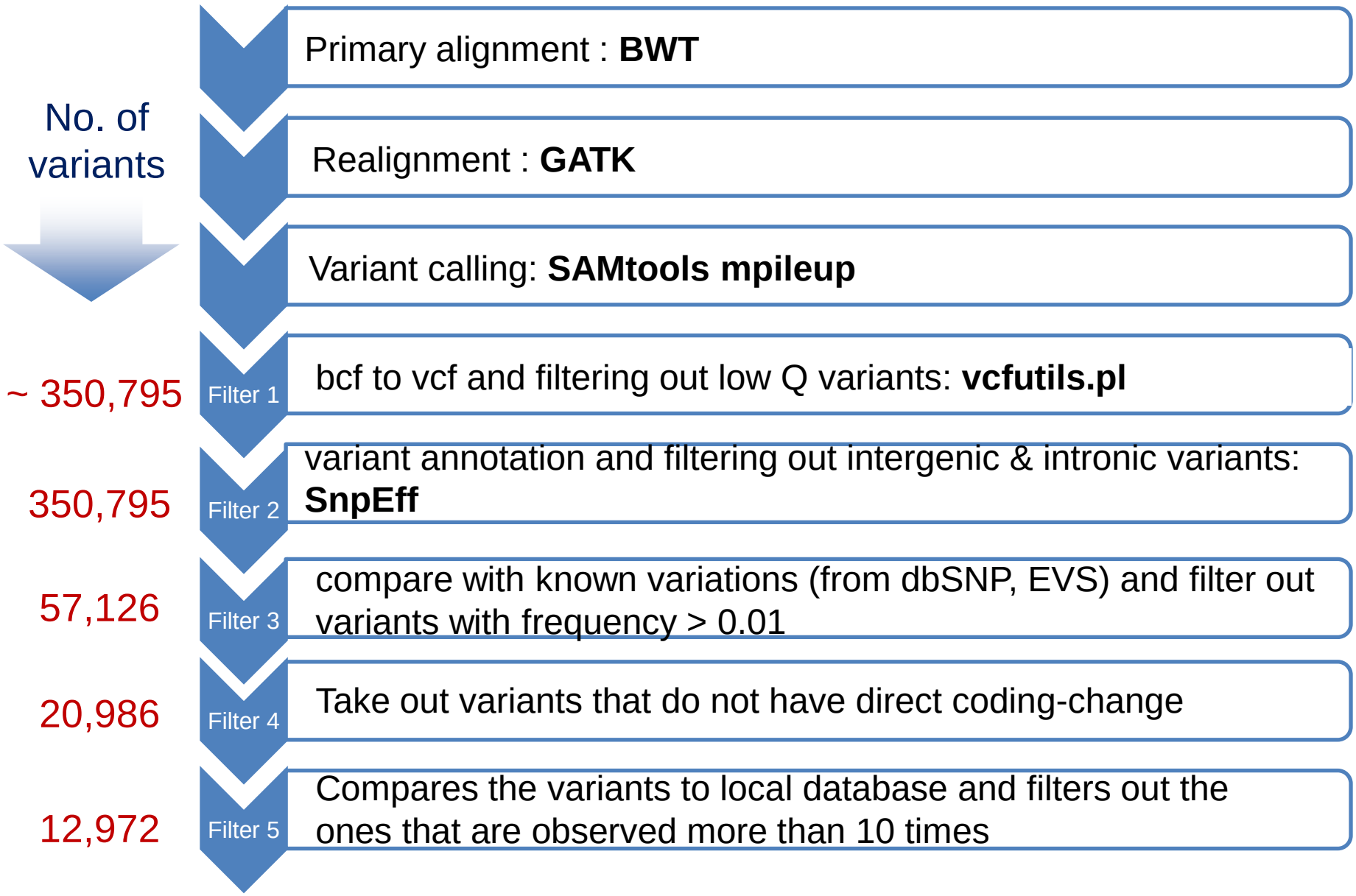
GenomeBrowse™

GenomeBrowse™

Whole-exome sequencing (WES)

- WES was performed on the mother and aff. sib from Perkin-Elmer using the following:
  - Exome capture using Agilent SureSelect V4 (51 Mb)
  - 100bp/paired end library construction
  - Illumina HiSeq 2000 for sequencing

WES analysis pipeline



WES analysis results

- **38** homozygous recessive variants

- The majority of these (32) were single-base substitutions causing missense changes, 4 were indels (3 of which caused frameshift mutation while one was an in-frame deletion), and 2 affected the splice donor/acceptor sites.

- Eleven of the 38 candidate genes had genomic coordinates that overlapped with mapped runs of homozygosity (RoH) in this family

WES analysis results

To prioritize these genes, we have set our candidate gene hypothesis to comprise:

- Lysine degradation pathway
- Proline metabolism
- vitamin B6 metabolism
- vitamin B6 transport

WES analysis results

- None of the 11 genes had a clear overlap with amino acid or vitamin B6 metabolic pathways.

- However, two genes are good candidates for PDE based on their functional relevance

Candidate gene 1

- Belongs to Solute Carrier super family
- ubiquitously expressed gene
- mediates the coupled movement of these ions across the plasma membrane.

Candidate gene 1

- 3 members of the solute carrier super family have been already described as vitamin transporters; these are:
  - SLC19A2 for thiamin (vitamin B1)
  - SLC19A1 and SLC46A1 for folate (vitamin B9)
- Mutations in SLC19A2 cause thiamine-responsive megaloblastic anemia syndrome (OMIM #249270)
- SLC46A1 mutations are associated with autosomal recessive hereditary folate malabsorption disease (OMIM #229050).

Candidate gene 2

- highly expressed in ovary and testis as well as within discrete brain areas.
- It encodes for a type II integral membrane protein
- cleaves a neuropeptide expressed both in the central nervous systems and in the periphery and is thought to function as a neurotransmitter.

Next steps:

- Recruiting more Omani families with similar phenotype.
- Collaboration with international teams working on PDE to find if any of our candidate genes is replicated in their cohorts
- Metabolomics assay of plasma & urine samples
- Functional/biochemical studies to validate candidate genes

Collaboration Offer

Recruitment of families:

- A clinical picture of early onset neonatal seizures that are refractory to conventional anticonvulsant treatment but responded well to PN treatment and have an inheritance pattern indicative of autosomal recessive disease.

- Exclusion of previously known causes of PDE/PREE based on the clinical phenotype and by:
  - **Biochemical testing:** of ATQ biomarkers (αAASA and pipecolic acid), amino acid profile and organic acid profile in patient's plasma, urine and/or cerebrospinal fluid (CSF).
  - **Genetic testing:** by Sanger sequencing of known genes (*ALDH7A1, PNPO,* and *ALPL*)

Collaboration Offer

- **Sharing of discovered candidate genes**

- **Contact:**

Hilal
Email: halshekaili@cfri.ca

PI: Dr. Clara van Karnebeek (chair, International PDE Consortium)
Email: clara@cmmt.ubc.ca

Acknowledgement

- My supervisor: Prof. J. Friedman
- All people at Friedman Lab
- **TIDEX team:**
  Clara van Karnebeek
  Colin Ross
  Maja Tarailo-Graovac
  Xiaohua Han
  Linhua Zhang
  Michelle Higginson
- **CMMT sequencing facility:**
  Joanne Denny
- Khalid Al-Thihli (Oman)
- Marion Coulter-Makie
- **Golden Helix team:**
  Rudy Parker
  Jami Bartole
  Cheryl Rogers
  Mary Makris