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Pitfalls in the MCD diagnostic work-up

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DRESDEN concept

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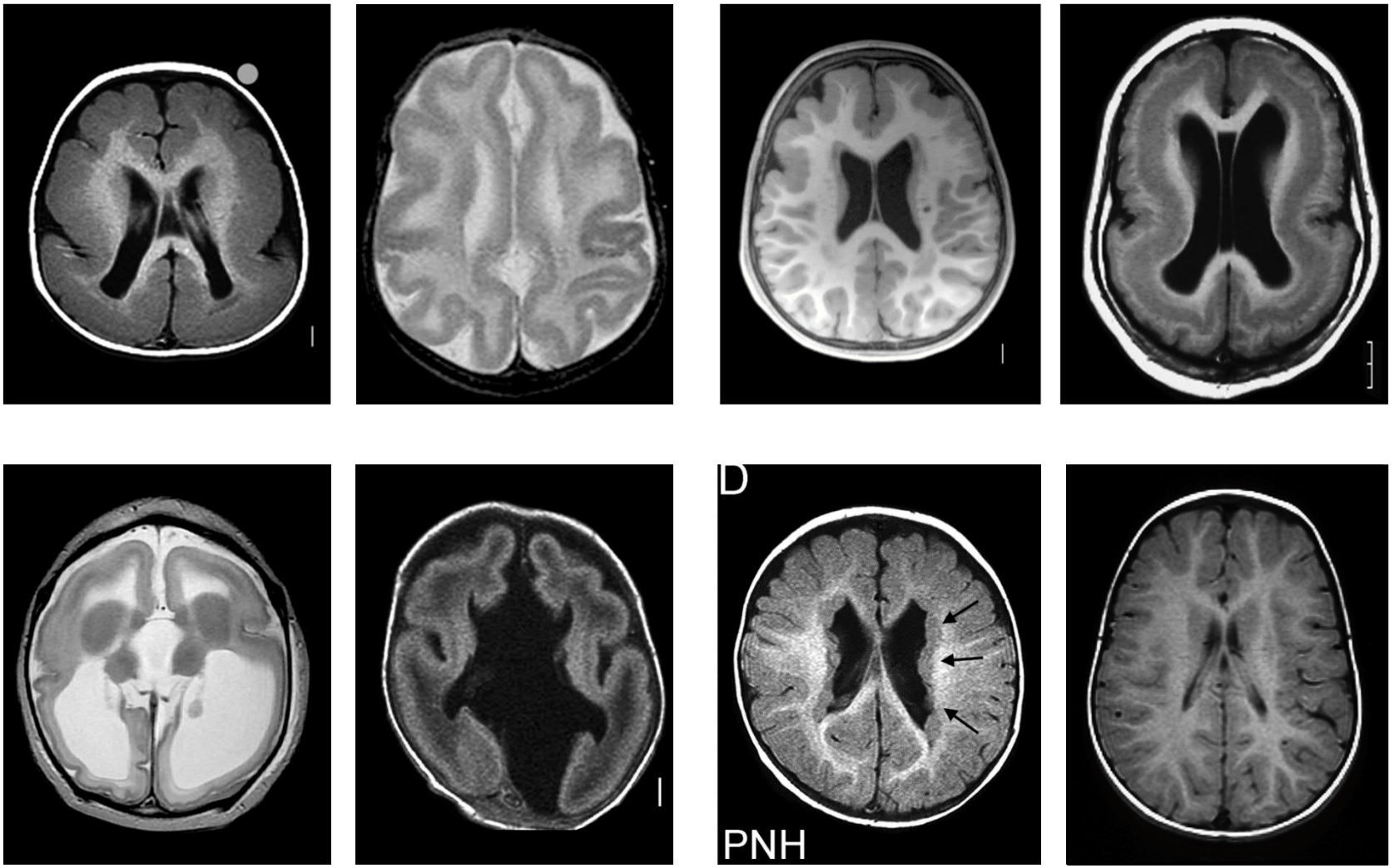
MCD are recognizable, if you are familiar with them (requires training)



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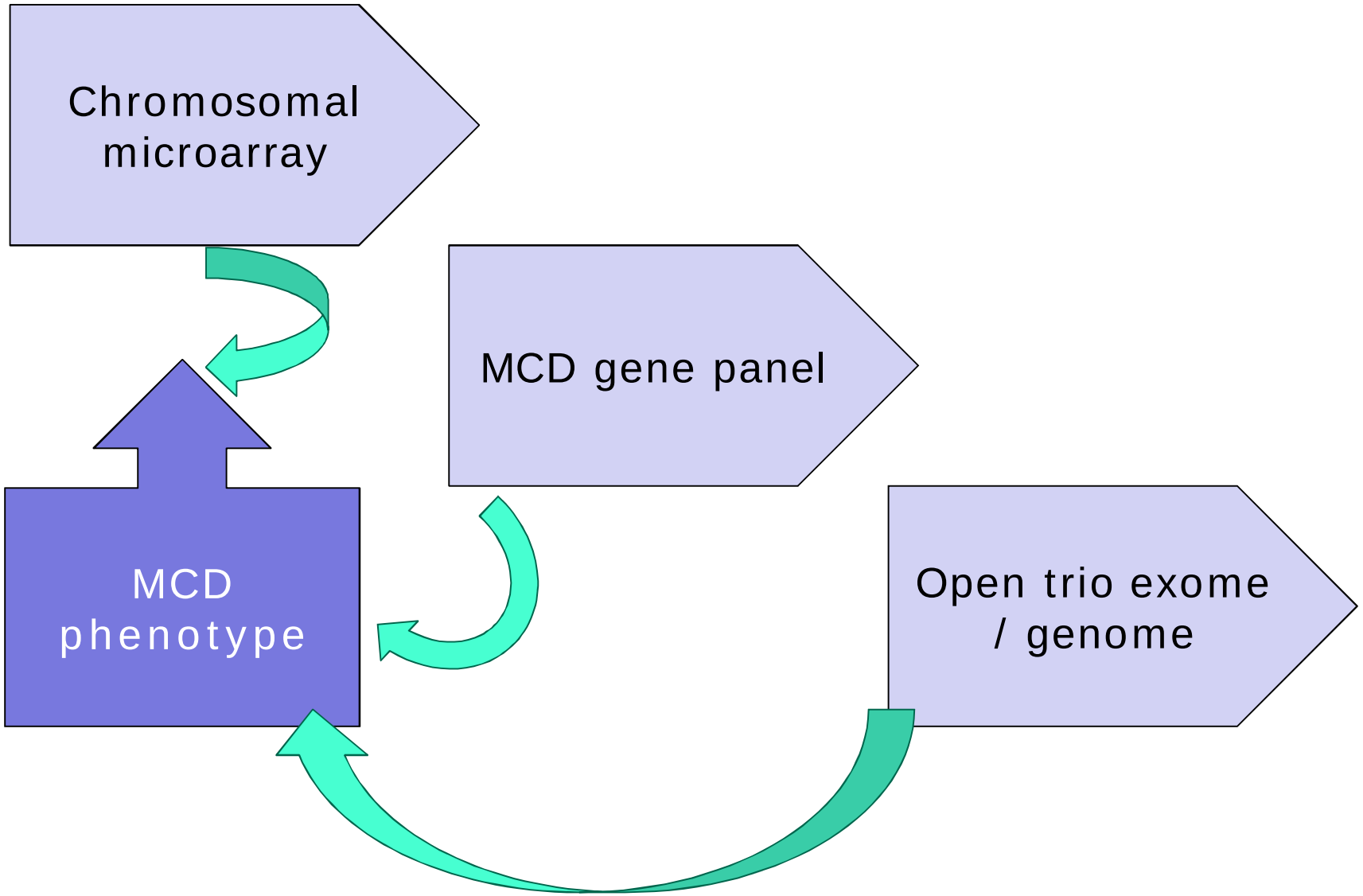
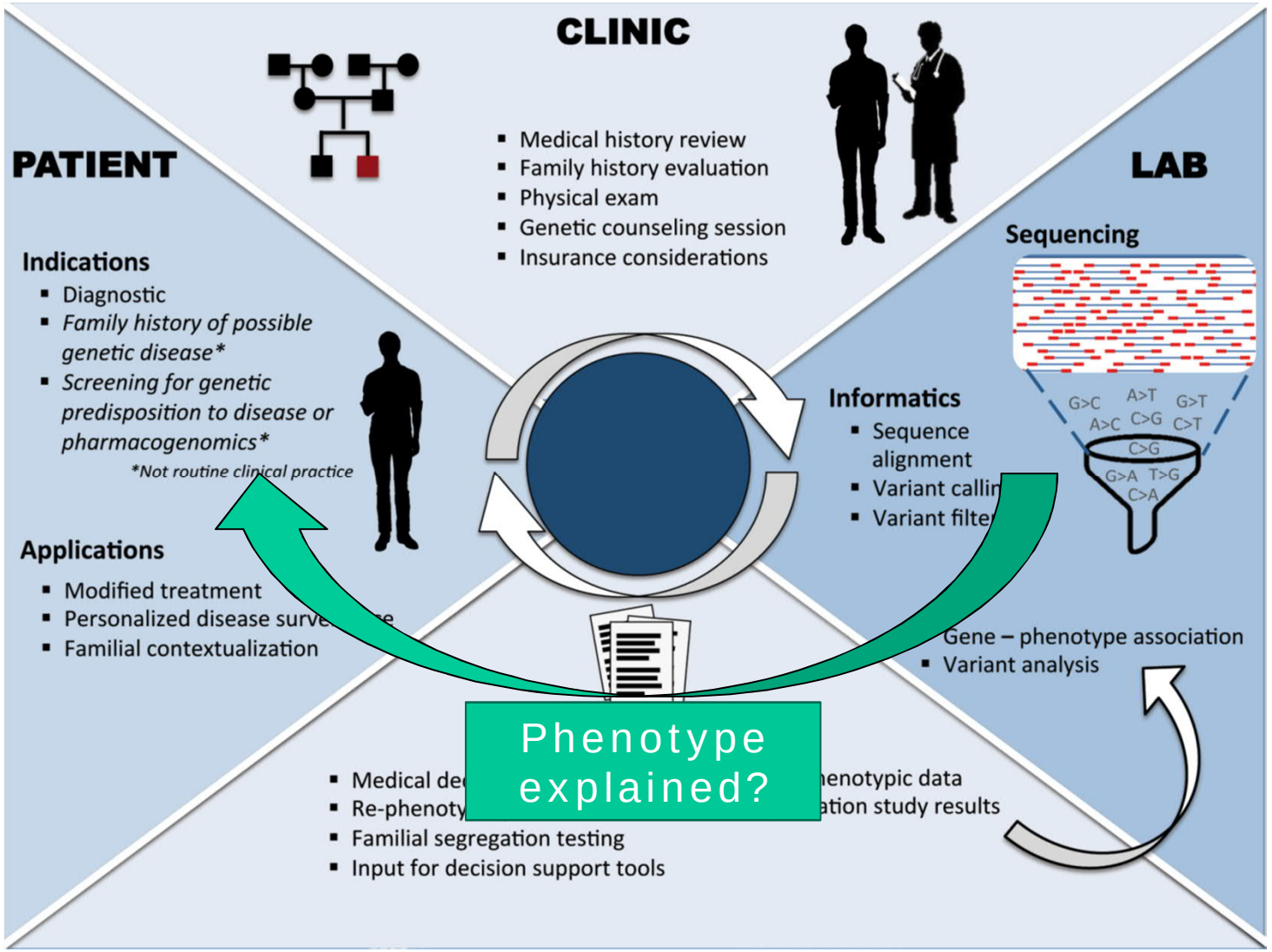
General diagnosic approach implies genome-wide testing

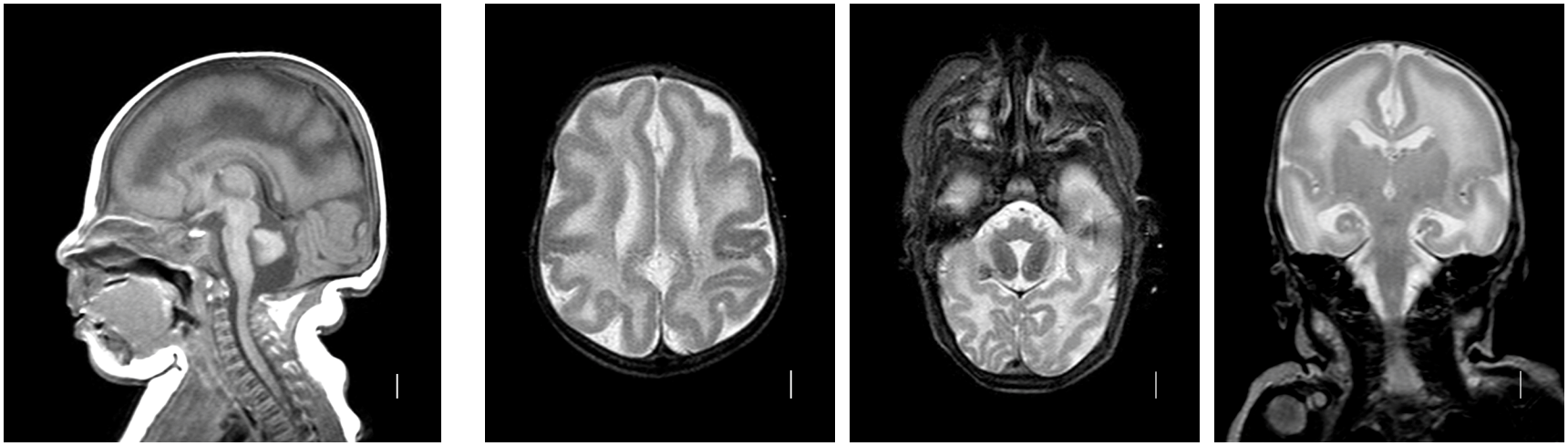


Diagnostic work-up requires a feedback loop between laboratory results and clinic



From Krier at al. 2016. PMID: 27757064

Patient 1. Neonatal seizures, lissencephaly; diagnostic targeted test

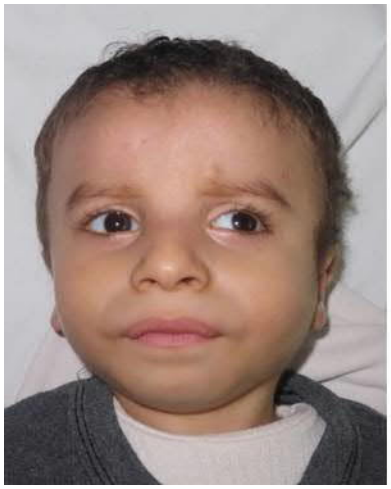


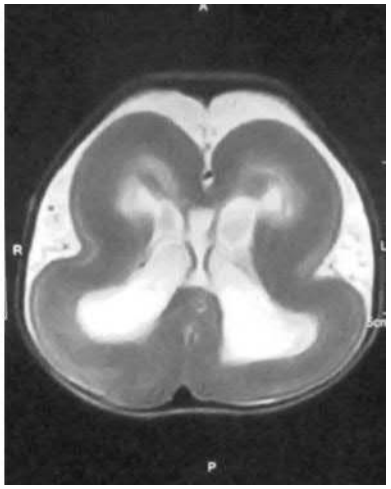
- Normal chromosomal microarray
- Whole exome sequencing
  - VOUS x2 – but BOTH were inherited from her father
    - RELN: c.1386C>T (p.C462C) potential splice defect
    - RELN: c.5200C>G (p.L1734V)

RELN deletion/duplication analysis: maternal deletion exon 4

VOUS – variant of unknown significance

Patient 2. Microlissencephaly, additional diagnostic test



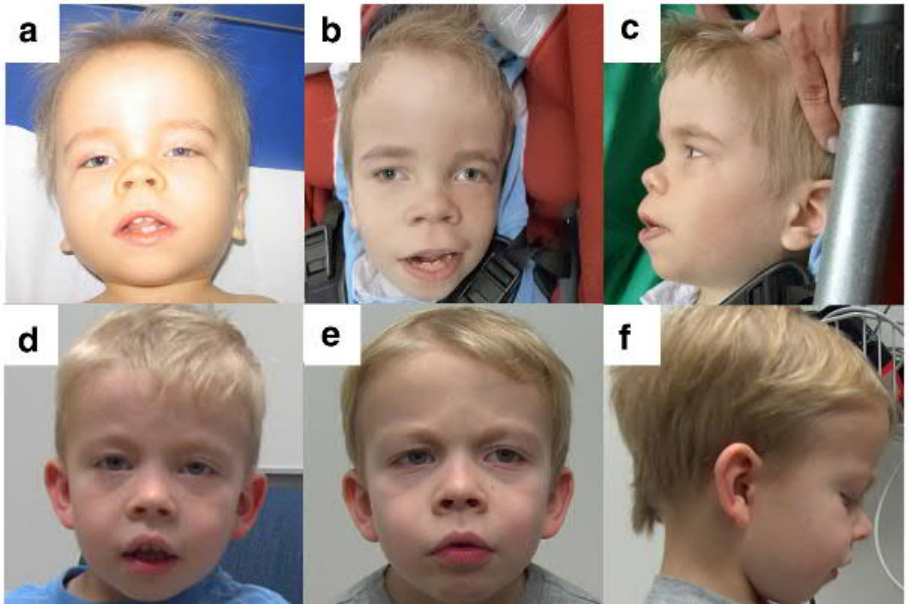


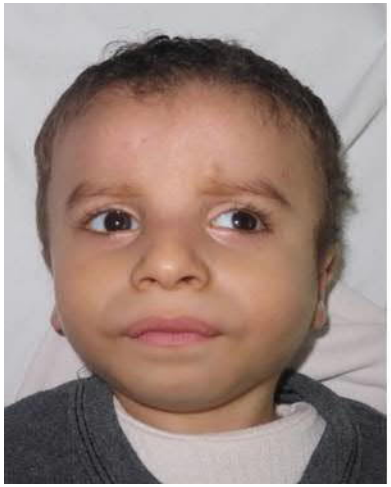
Trio exome sequencing
NM_152641.2(ARID2):
c.4523G>A:p.Gly1508Asp
de novo (PS2, PM2, PP3
– likely pathogenic)

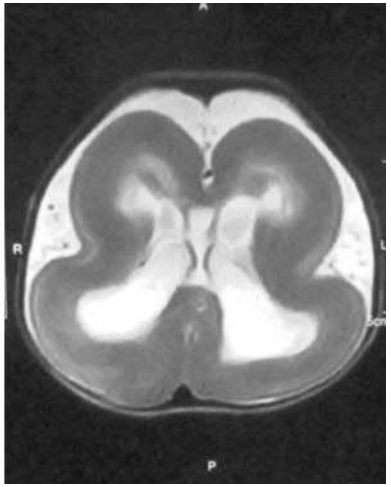
FA: parents 1st degree cousins;
global developmental delay,
seizures, OFC – 5 SD

Courtesy of Professor Maha Zaki, OFC – occipital frontal circumference; WES -

ARID2: Coffin-Siris syndrome or Coffin-Siris like phenotype





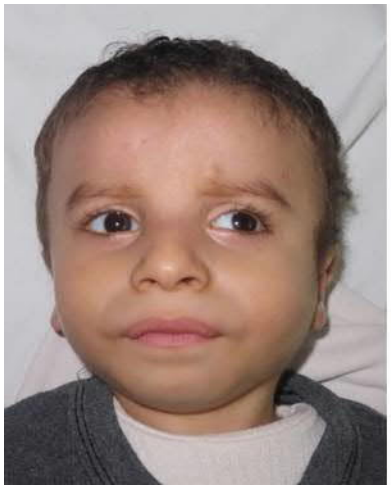


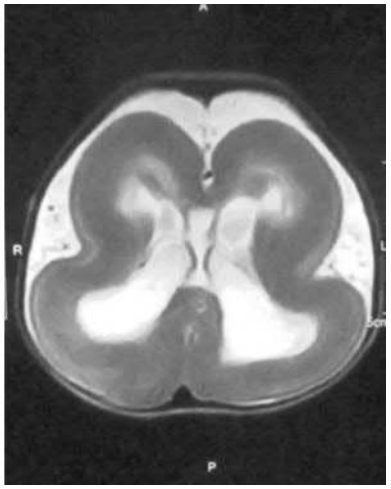
- SWI/SNP phenotype spectrum includes microcephaly, but no patients with ARID2 mutations presented with microcephaly

- SWI/SNP disorders were never associated with lissencephaly

PMID:28124119 (2017), PMID:29698805 (2019)

Patient 2. Boy with microlissencephaly, additional diagnostic test





- Trio exome sequencing NM_152641.2(ARID2): c.4523G>A:p.Gly1508Asp de novo (PS2, PM2, PP3 – likely pathogenic)
- CNV analysis reveals deletion of PAFAH1B1 (LIS1)

Family: parents 1st degree cousins;

global developmental delay, seizures, OFC – 5 SD

Courtesy of Professor Maha Zaki

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Patient 3. Pontocerebellar hypoplasia with COL4A1

- FA unremarkable
- Born full term with
  Length 46 cm (-2 SD)
  Weight 2360 g (-2.3 SD)
  OFC 31.5 cm (-2.5 SD)
- Global developmental delay
- Severe microcephaly
  OFC at 20m - 6 SD
- Brain MRI:
  pontocerebellar hypoplasia

- Normal CMA
- Various panels: normal
- Open trio exome sequencing:
  NM_001845.5(COL4A1):
  c.3950G>A p.Gly1317Asp de novo (likely pathog.)

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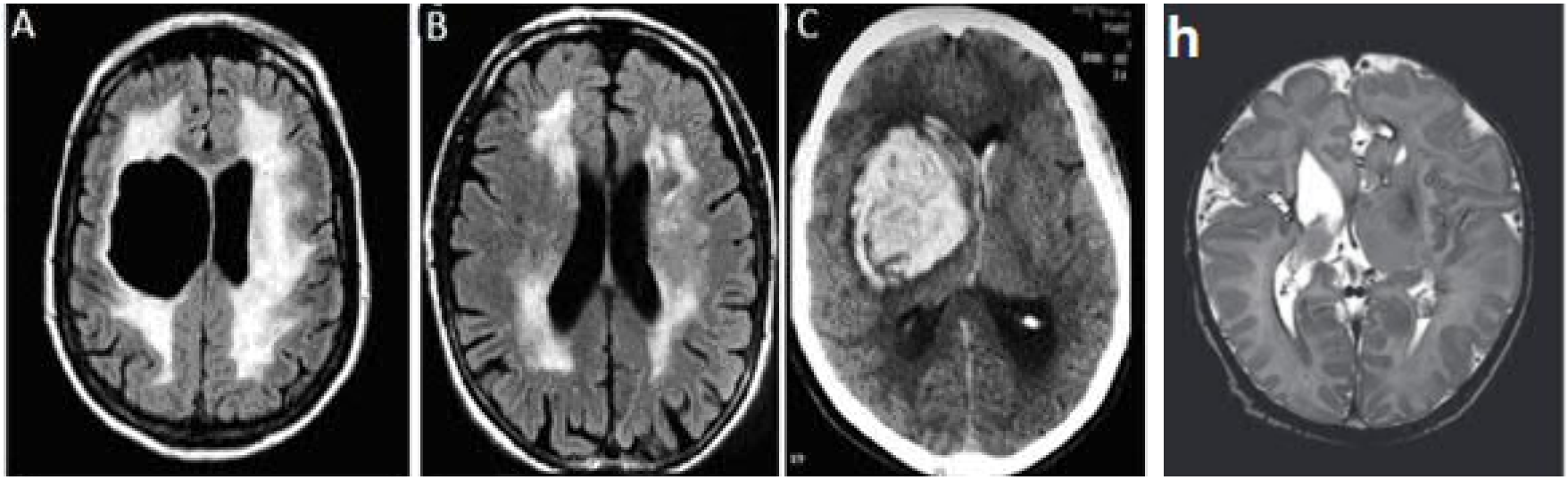
PCH is not a COL4A1-related disorder



Porencephaly | Leukencephalopathy | Hemorrage | Schizencephaly

Small-vessel brain disease of varying severity

Plaisier E, Ronco P. COL4A1-Related Disorders. 2009 Jun 25 [Updated 2016 Jul 7]. In GeneReviews®; Meuwissen et al. PMID: 25719457

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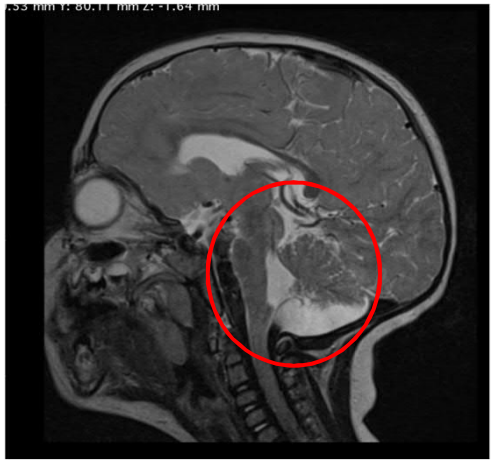
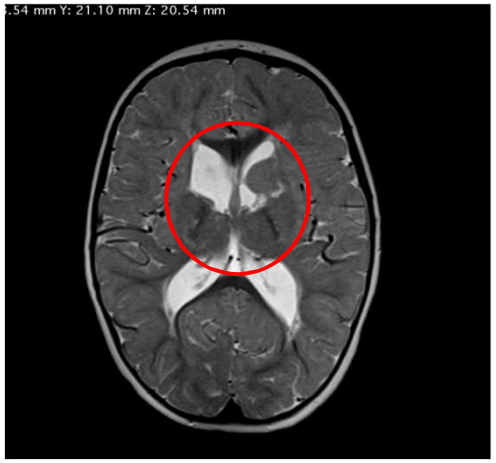
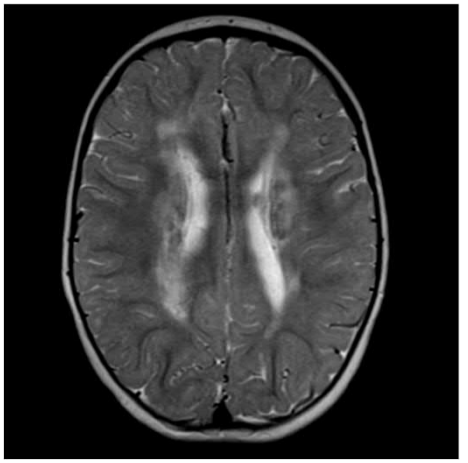
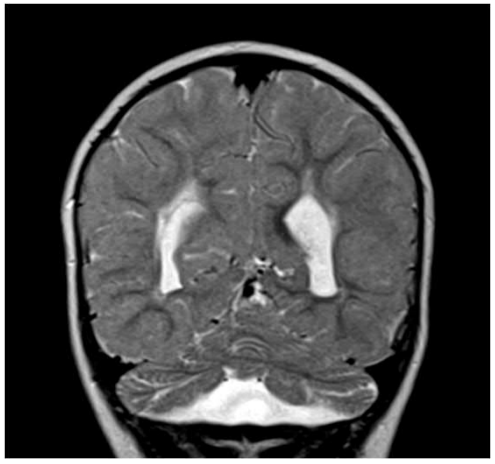
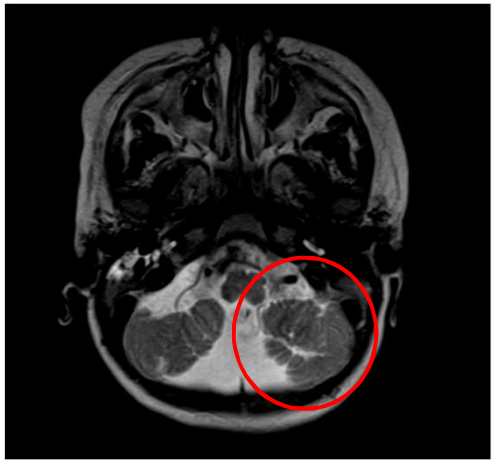
Patient 3 does not have PCH, but COL4A1 related disorder





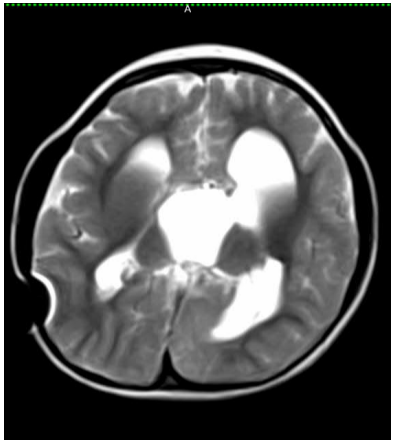






Small-vessel brain disease

Patient 4. IUGR, hydrocephalus, profound ID and seizures; de novo variant in TUBB3, low quality scans



4 years

- IUGR: BL 47 cm (-2.37 SD), OFC 32 cm (-2.46 SD)
- Hydrocephalus operated at 8m, complicated with post-operative hemorrhage and venriculitis and multiple revisions
- 4y - no developmental milestones, complex focal seizures
- OCF 40.5 cm (-7.46 SD), L 93.5 cm (-2.5 SD), ptosis
- NM_001197181.1(TUBB3): c.317C>T, p.(Thr106Met) likely pathogenic

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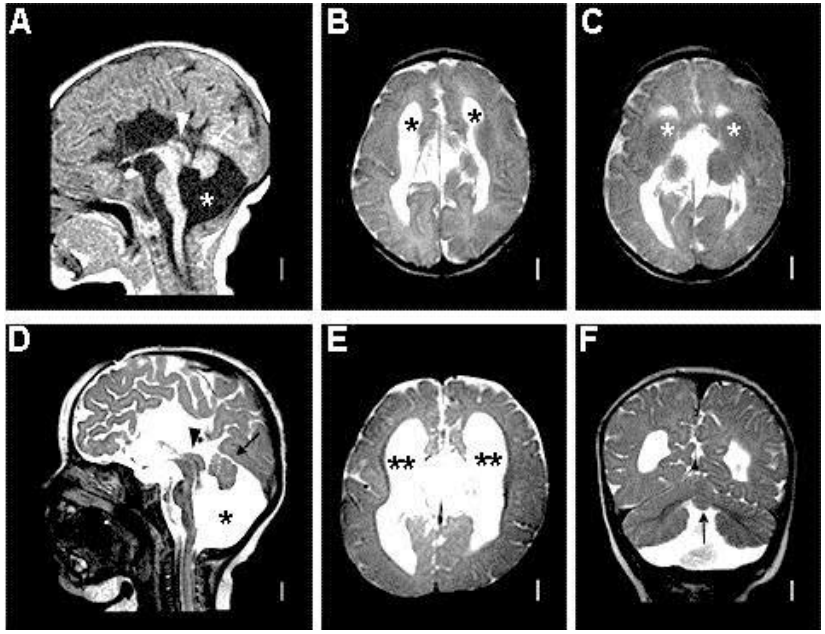
Hydrocephalus is not a core feature of the tubulinopathies

Key features of tubulinopathies:

- Dysmorphism / unusual orientation of basal ganglia
- Partial / complete agenesis of the corpus callosum
- Cerebellar dysplasia / hypoplasia
- Thick tectum

6 days



5 months

Early MRI images are typical for tubulinopathy spectrum

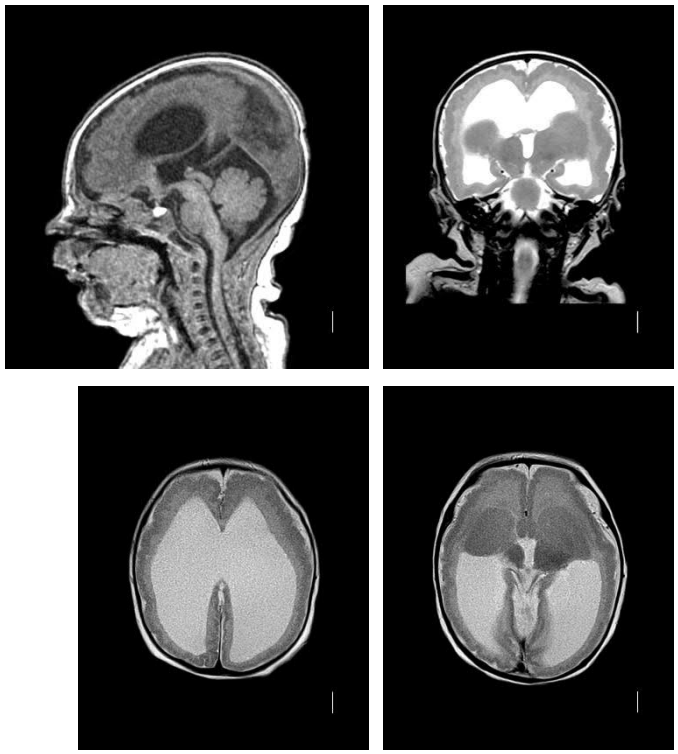
Patient 5. IUGR and abnormal gyration due to trisomy 18

- FA: parents are 1st degree cousins from Afganistan, two siblings died shortly after birth from unknown cause

- Gestational diabetes
- 39+5 GW: W 2 kg (-3.3 SD), L 44 cm (- 3.4 SD), OFC 33 cm (- 1.4 SD), Apgar 1/6/8
- Heart defect (VSD, PDA), AV block
- Cholestasis
- Head US - abnormal gyration

Chromosomal microarray: trisomy 18

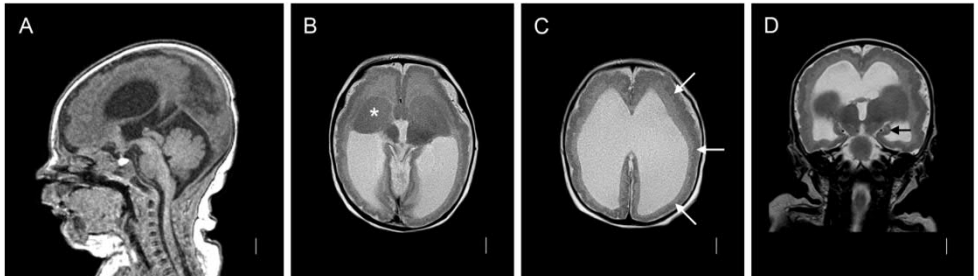
Patient 6. Hydrocephalus, respiratory distress, feeding difficulties, neonate seizures; GRIN2B



- Tonic seizures at 3 weeks, developed into generalized tonic-clonic seizures refractory to the therapy
- G-tube feeding
- No milestones reached by 3y
- Normal length and OFC
- Brain MRI with very thin corpus callosum, thick tectum, diffuse bilateral PMG, enlarged lateral ventricles, dysplastic basal ganglia
- NM_000834.3(GRIN2B):c.1916C>T p.(Ala639Val), likely pathogenic, was not considered to be causative for MCD, suggested mutation in tubulin or MAP encoding genes

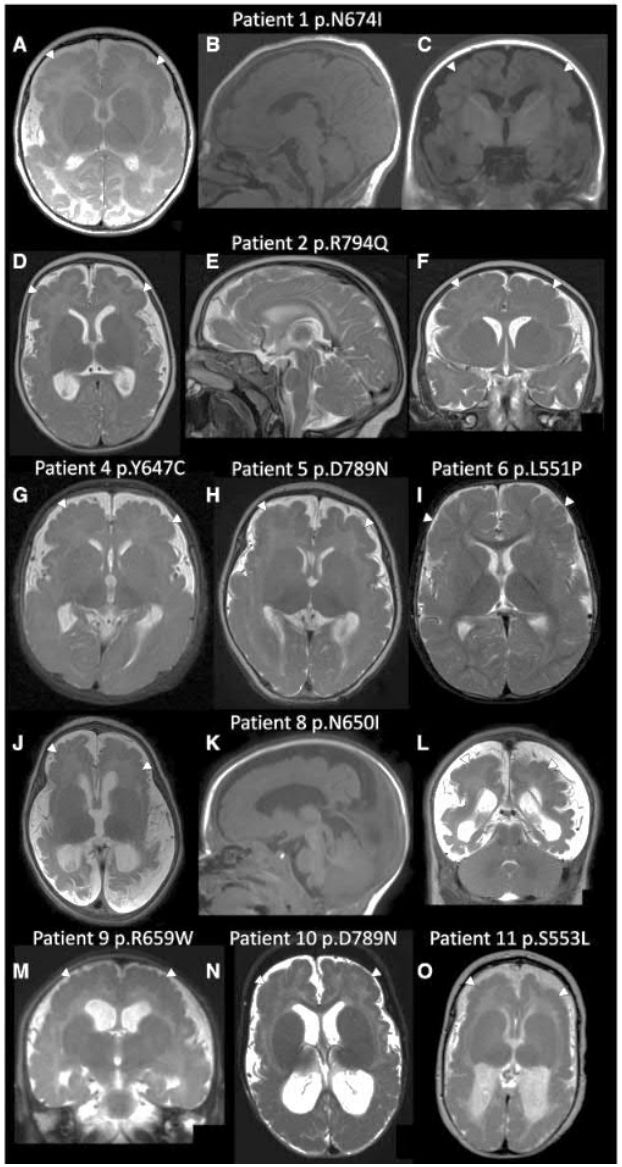
Patient 6. Expansion of GRIN2B-associated phenotype

P.1



Platzer at al 2017. PMID:28377535

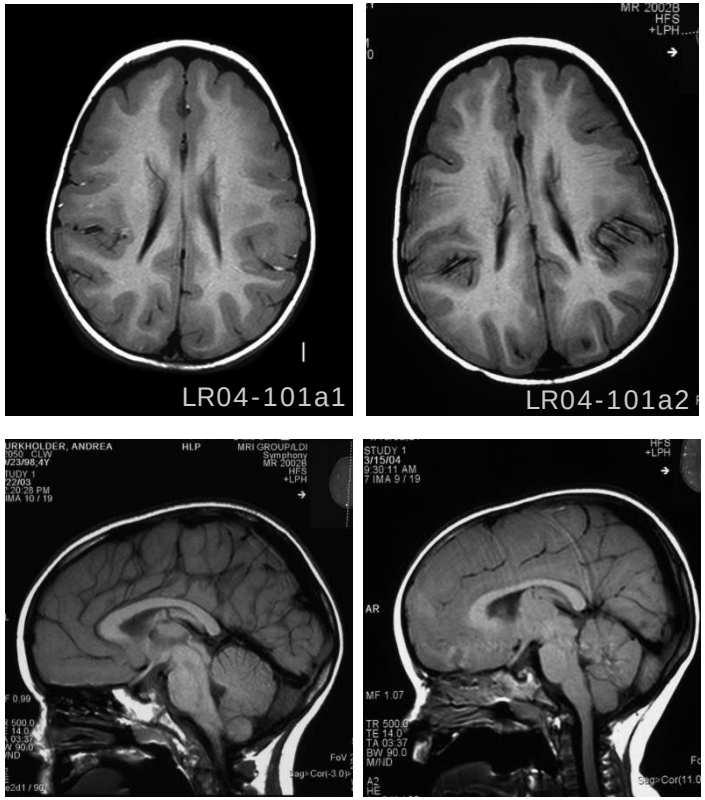
Other NMDA receptor encephalopathies are also associated with MCD: GRIN1



- Extensive bilateral PMG, severe ID, microcephaly, therapy-resistant epilepsy
- GRIN1 mutations reported in patients with non-synd. ID and epileptic encephalopathy and movement disorders
- The reason why some GRIN1 (and GRIN2B) patients get MCD is uncertain

Fry et al. 2018. PMID: 29365063

Patient 7. Frontal pachygyria (< 10 mm), seizures and macrocephaly; similar affected sibling

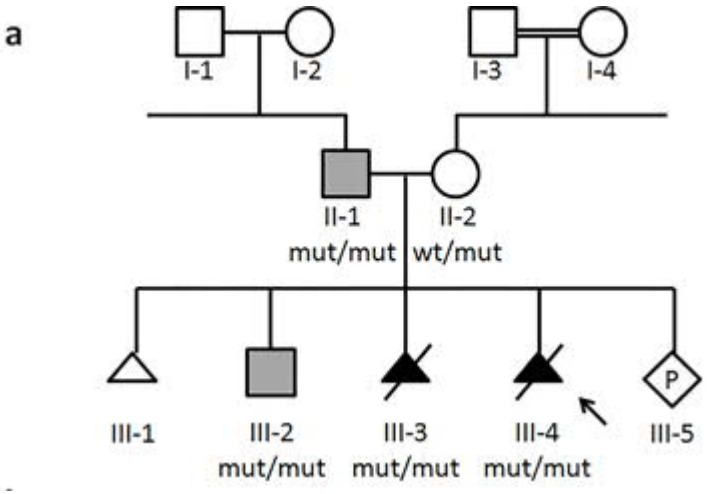


- NM_003805(CRADD):c.382G>C, p.Gly128Arg homozygous in both affected
- Mutation previously reported in a large Mennonite family with mild non-synd. ID (no MRI)
- Re-evaluation of the previously published family: identical MCD pattern

CRADD mutations detected in 4/13 families with frontal pachygyria (cortical thickness < 10mm)

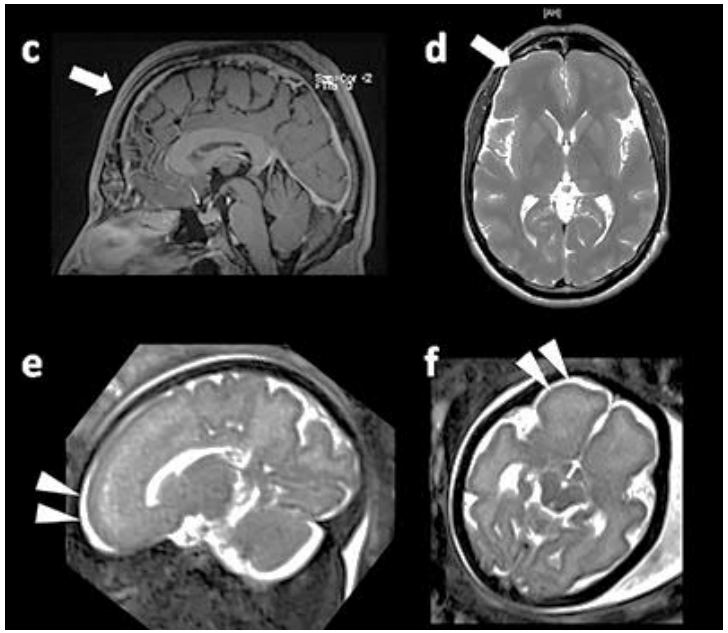
Puffenberger at al. 2012. PMID: 22279524; Di Donato et. al. 2016. PMID: 27773430

Pathogenic variants can be inherited from unaffected / undiagnosed parents



NM_003805.4(CRADD):
c.52_59delGCAGAGGT /
p.Ala18Ilefs*47

Father



37. GW
Frontal AGY

Harel et al. 2017. PMID:28686357

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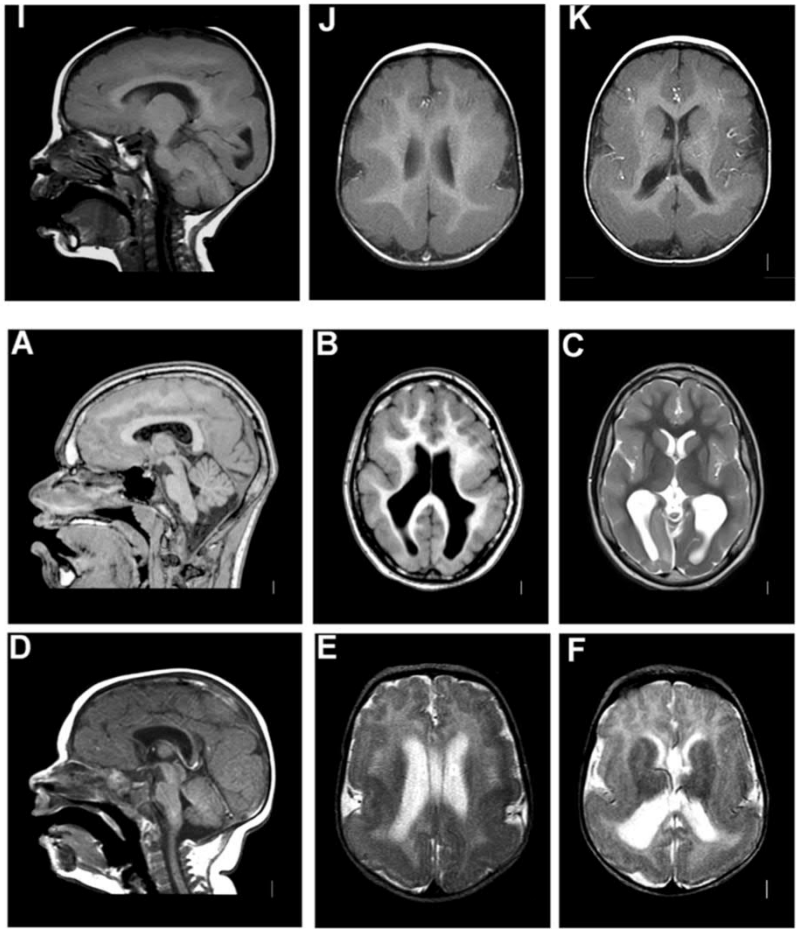
“Phenotypic spectrum can be different from the literature

Key features of tubulinopathies:

- Variable MCD
- Dysmorphism / unusual orientation of basal ganglia
- Partial / complete agenesis of the corpus callosum
- Cerebellar dysplasia / hypoplasia
- Thick tectum

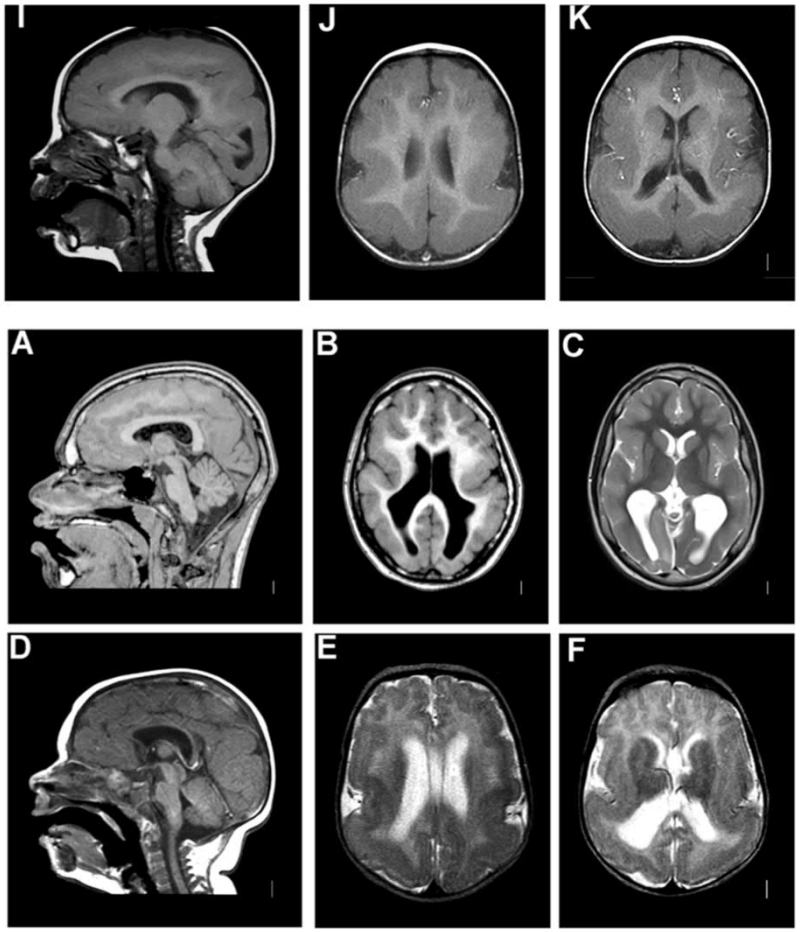


TUBG1:p.Ser259Leu

TUBG1 phenotype is similar to LIS1, DYNC1H1, or KIF5

Key features of TUBG1-associated disease:

- 8 patients reported
- Posterior predominant pachygyria
- No malformations of corpus callosum, brain stem, cerebellum or basal ganglia



TUBG1:p.Ser259Leu

Brock et al. 2018. PMID: 29706637

Clinical interpretation of variants in novel disease genes requires a patient cohort

Available platforms (Matchmaker Exchange):

- GeneMatcher, DECIPHER, PhenomeCentral, MyGene2, Matchbox, AGHA Patient Archive, IRUD…

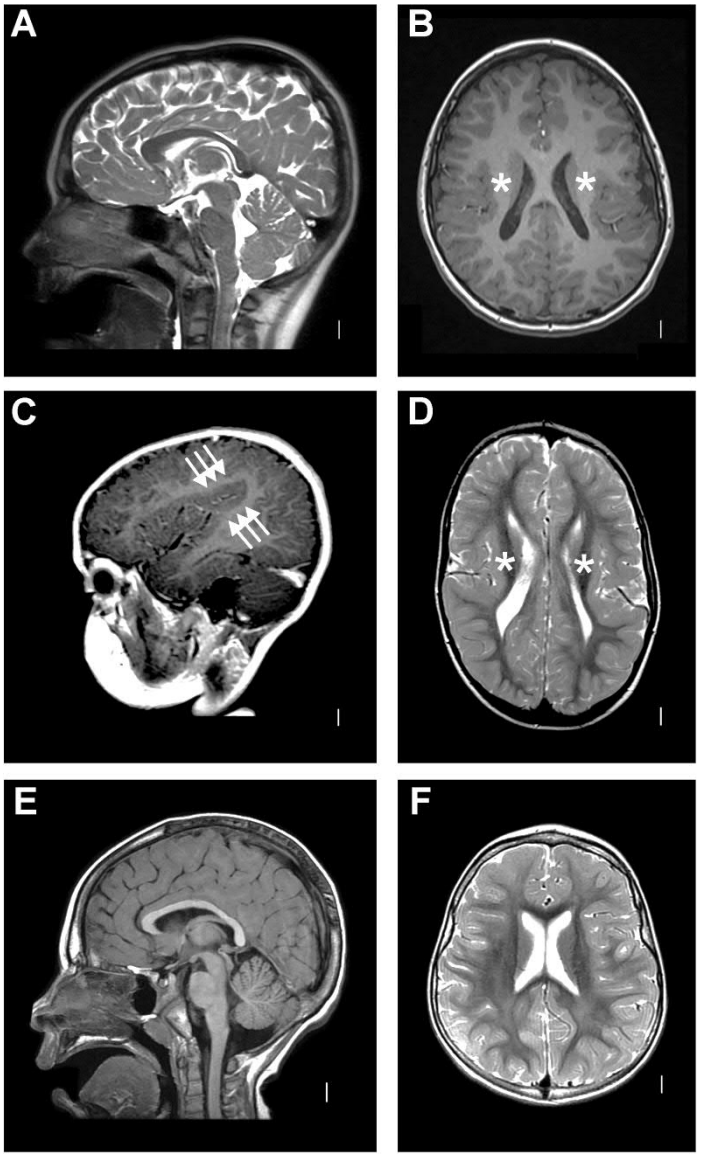
"Data don't make any sense,
we will have to resort to statistics."

GeneMatcher:

> 150 citations in Pubmed

Philippakis et al. 2015. PMID: 26295439 ; Sobreira et al. 2015. PMID: 26220891

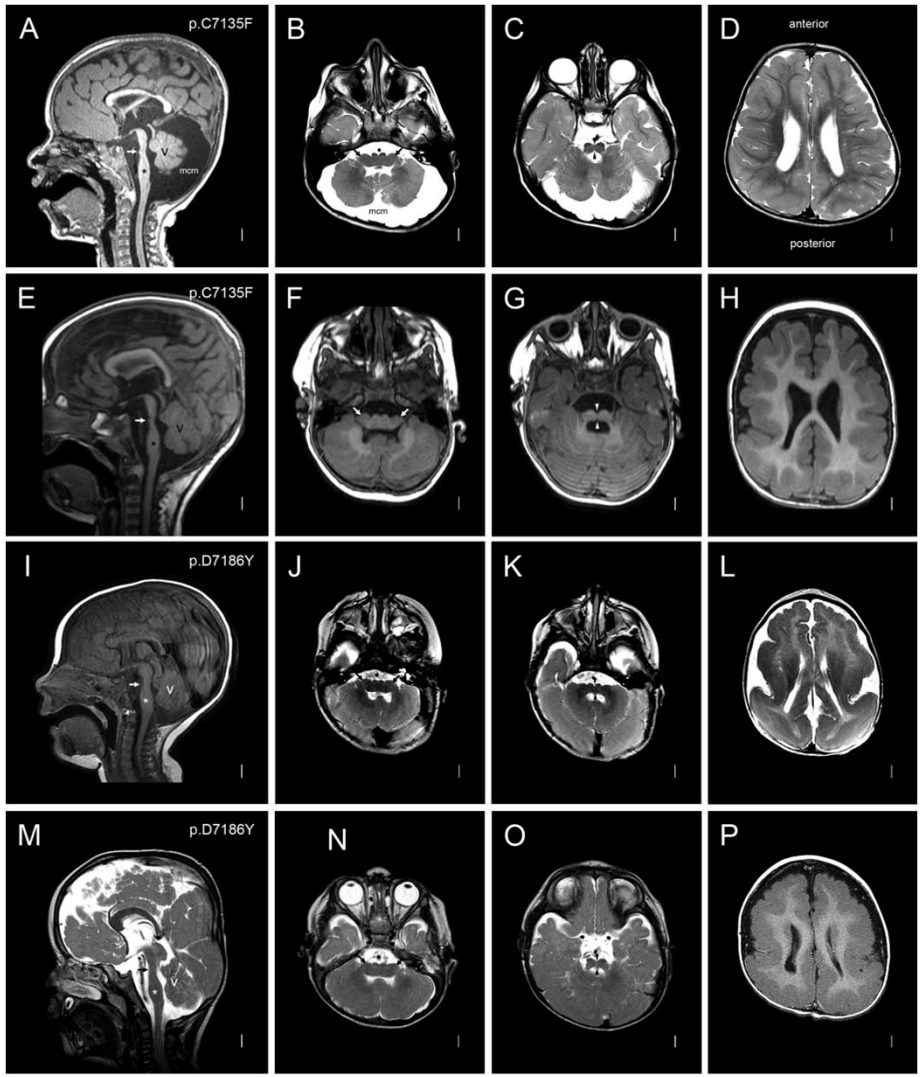
Successful GeneMatching: MAPK8IP3



- 13 individuals with de novo heterozygous variants (10 missense and 3 truncating)
- NDD phenotype with variable brain malformations: cerebral and cerebellar atrophy, hypoplastic corpus callosum and perisylvian PMG
- PMG in two patients with recurrent p.Leu438Pro
- None of other mutations presented with MCD

Platzer et al. 2019. PMID:30612693: NDD neurodevelopmental disorder

Majority of gene discoveries in MCD cohorts are still done through direct phenotyping



- 3 unrelated patients with lissencephaly and a distinct brain stem malfromation with deficient midline crossing
- 5 additional individuals with the same pattern
- MCD consistent with diffuse pachygyria with posterior gradient and cortex thickness < 10mm
- “Bowtie” brainstem on mid-sagittal images looking like inverted totem bird wings on axial images
- 7 missense mutations and 1 deletion in MACF1

Dobyns et al., Mancini. 2018. PMID: 30471716

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MACF1 was also proposed to as an MCD candidate gene by a forward genetic screen

nature COMMUNICATIONS

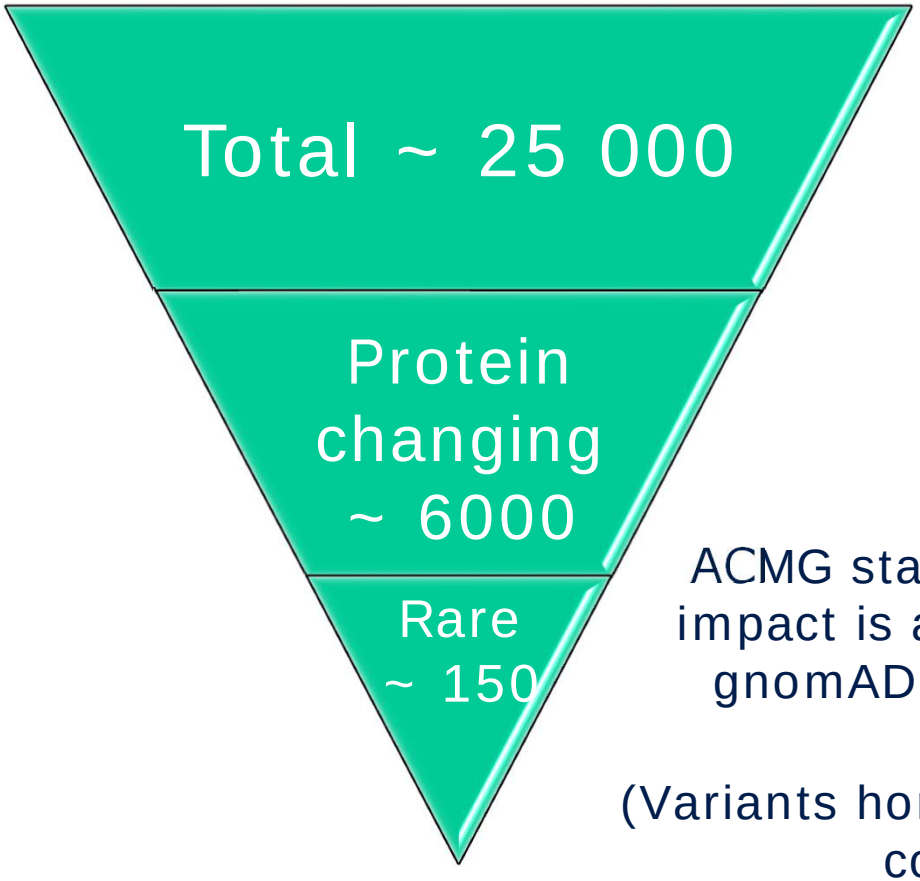
ARTICLE

DOI: 10.1038/s41467-018-04880-8 OPEN

Identification of genes associated with cortical malformation using a transposon-mediated somatic mutagenesis screen in mice

I-Ling Lu[1], Chien Chen[2,3], Chien-Yi Tung[4,5], Hsin-Hung Chen[3,6], Jia-Ping Pan[4], Chia-Hsiang Chang[1,7], Jia-Shing Cheng[1], Yi-An Chen[1], Chun-Hung Wang[1], Chia-Wei Huang[1], Yi-Ning Kang[1], Hsin-Yun Chang[1], Lei-Li Li[1], Kai-Ping Chang[3,8], Yang-Hsin Shih[3,6], Chi-Hung Lin[4,5,9], Shang-Yeong Kwan[2,3] & Jin-Wu Tsai[1,10,11]

Population allele frequency is a main criterium for variant prioritization, but also a pitfall

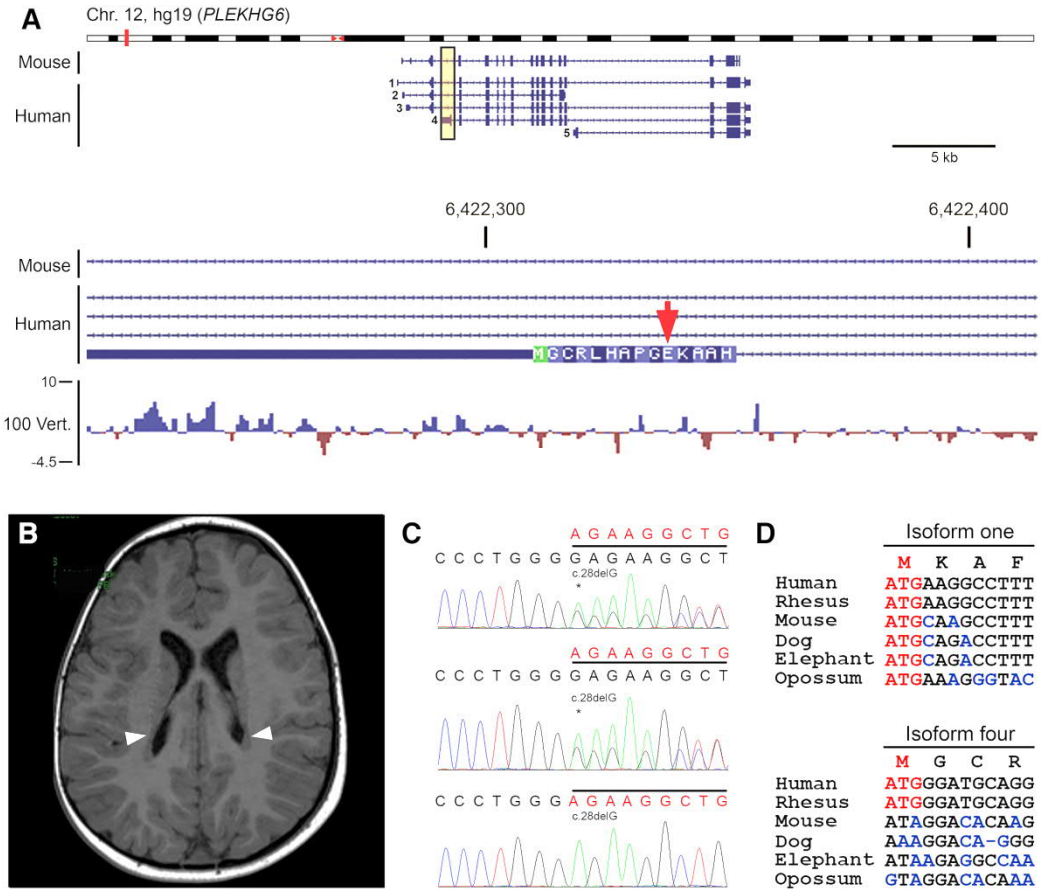


ACMG stand alone evidence of benign impact is a allele frequency of > 5% in gnomAD, 1000 Genoms project etc.

(Variants homozygous in gnomAD are often considered to benign)

ACMG – American colleage of medical genetics

PLEKHG6 disease causing variant would not pass most clinical filters as listed x1 homozygous in gnomAD



- Child with mild-moderate ID and periventricular nodular heterotopia (occipital horns and trigone)
- NM_001144857.1 (PLEKHG6):c.28delG p.(Glu10Argfs*31) hom.
- Selected as a variant located within validated human transcripts that have no ortholog in mice

O'Neill et al. Robertson. 2018 PMID: 30517861

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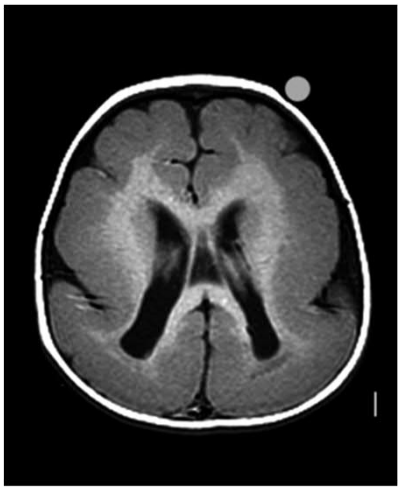
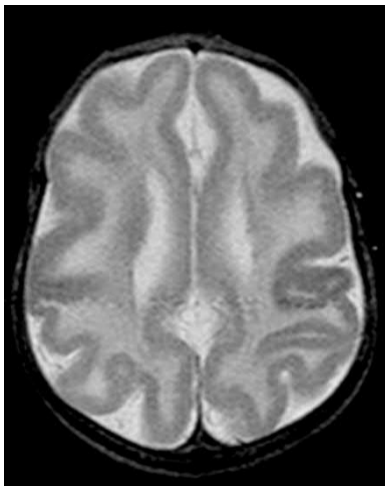
Every diagnostic step has its pitfalls

- Disorder is not recognized clinically (insufficient data, insufficient expertise)
- Complex phenotype due to two (or more) monogenic disorders in one patient
- Genetic test does not cover the whole mutation spectrum
- Phenotypic spectrum can be broader when currently known
- Variants can be inherited from seemly unaffected parents
- Population databases can include affected individuals, variants might not be fully penetrant

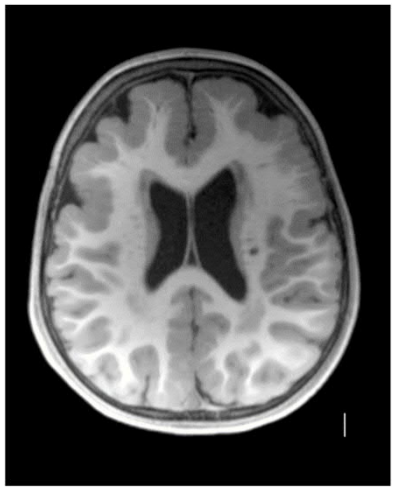
MCD are recognizable



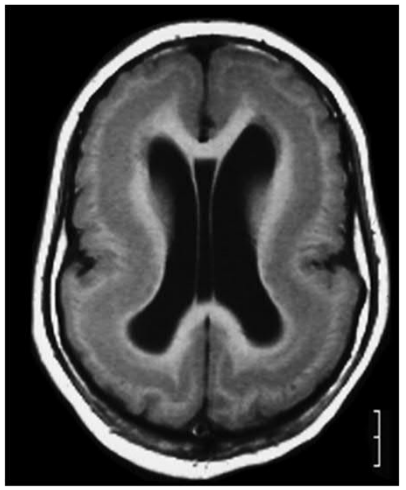
PAFAH1B1 (LIS1)



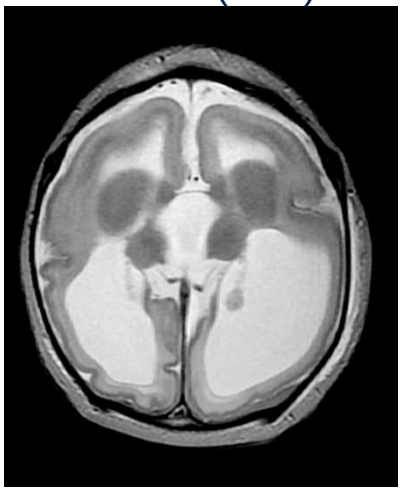
RELN



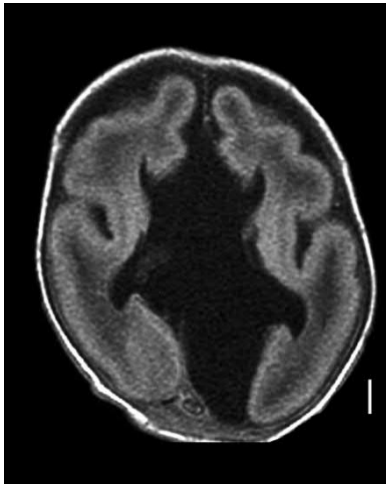
GPR56



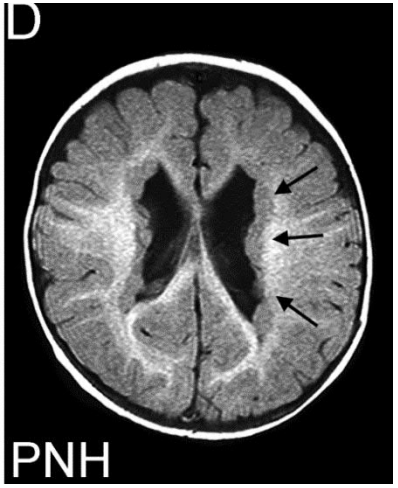
DCX



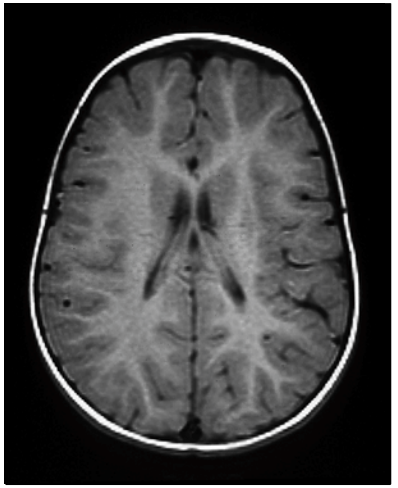
TUBA1A



ARX



FLNA



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