Microcephaly (MIC)

An update for the COST Neuro-MIG meeting

Seattle Children's
HOSPITAL • RESEARCH • FOUNDATION

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Satellite symposium to the EPNS 2019 in Athens

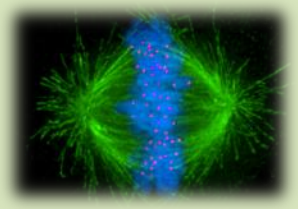
cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

Neuro-MIG

Outline

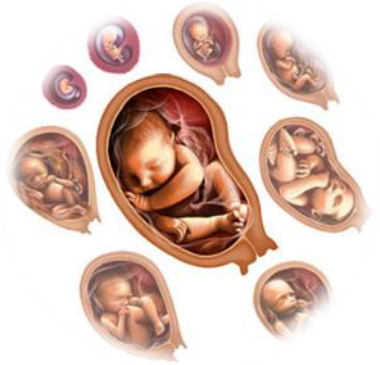
Overview of MIC classifications and phenotypes

Update on new MIC genes and syndromes



Future directions: diagnostics and mechanistic studies

MIC: General overview



- A descriptive term: A developmental defect that disrupts prenatal/early postnatal brain growth
  - Skull growth is dynamically determined by brain growth

- Neurologic sign rather than a disorder
  - More than 900 syndromes with MIC (OMIM, LDD)
  - Common neurologic sign
    - 1% of referrals to child neurologists are specifically for the evaluation of MIC
    - 15% of children referred to child neurologists for evaluation of developmental disabilities have MIC

- Approach to evaluation of affected individuals (in the literature and in clinical practice) is not uniform

Mirzaa, Ashwal and Dobyns, 2011

Clinical Diagnostic Classifications of MIC

- **Primary MIC (Microcephalia vera or "MCPH"):**
  - Time of onset: MIC is present since the 7th month of gestation
  - Aetiological: MIC is without an identifiable syndromic, environmental, or cytogenetic diagnosis; height, weight, brain MRI are normal in the majority of individuals
- **Secondary/syndromic MIC:**

  MIC is one of many brain and somatic abnormalities/features found on evaluation

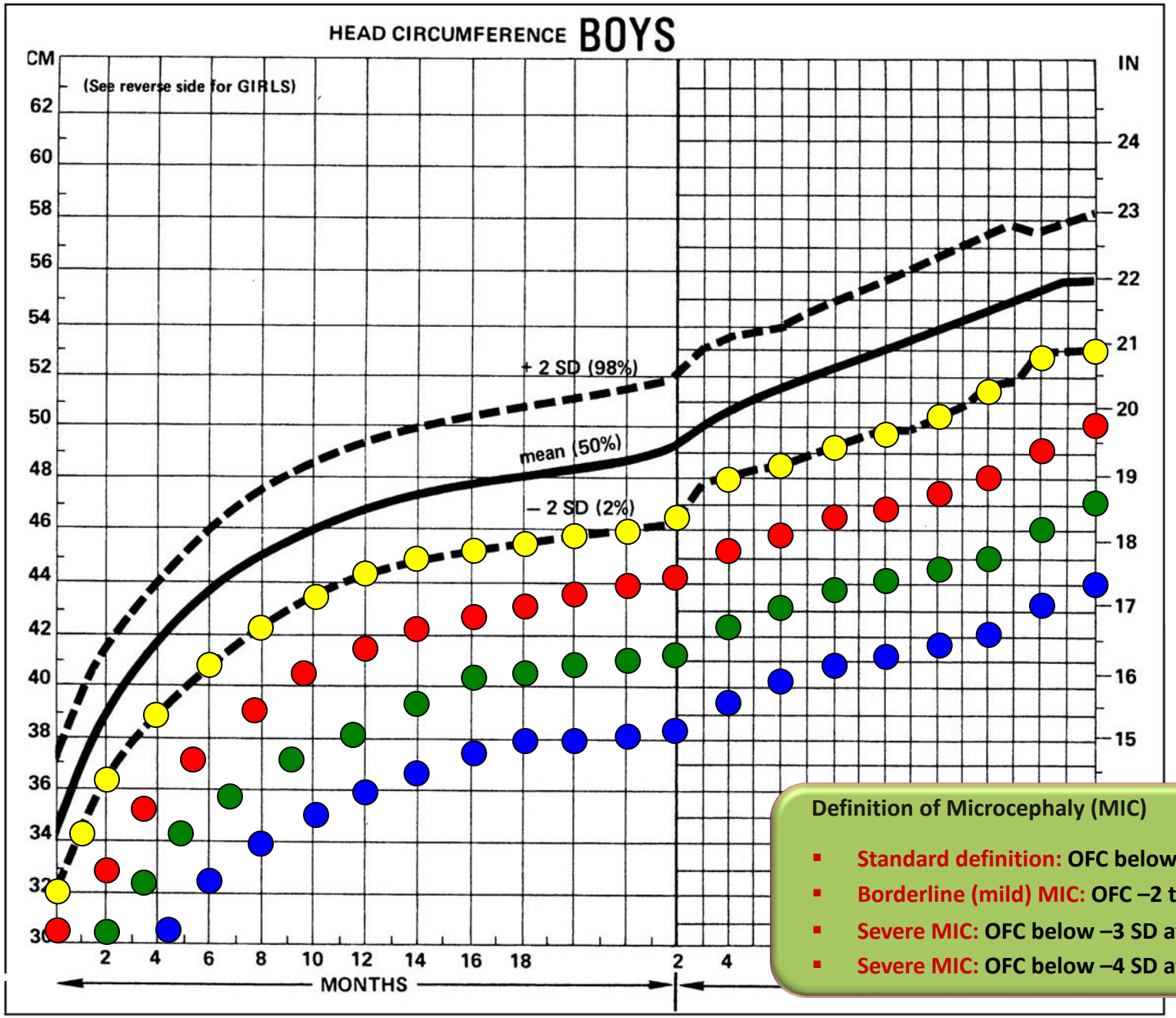
- **Congenital MIC:**

  MIC that is present at birth (birth OFC < 3 SD) - progressive

- **Postnatal MIC:**

  Normal/small-normal head size at birth, MIC develops and progresses during infancy/childhood

Severity of MIC impacts neurological outcome



+2 SD

0 SD

–2 SD

–4 SD

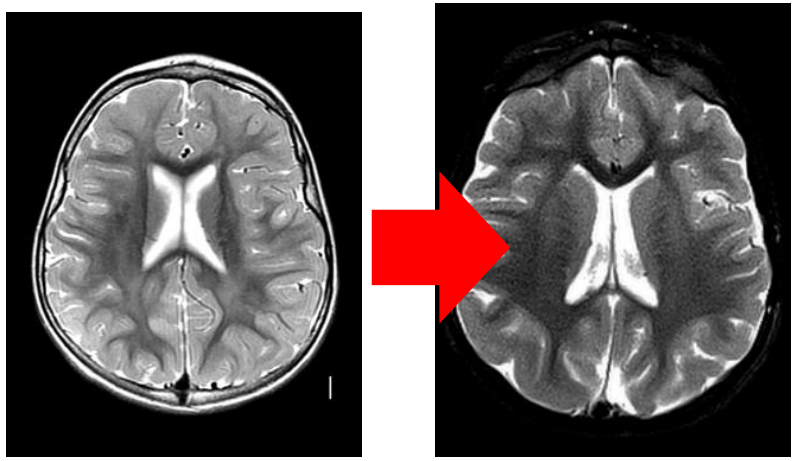
–6 SD

–8 SD

Definition of Microcephaly (MIC)

- **Standard definition:** OFC below –2 SD
- **Borderline (mild) MIC:** OFC –2 to –3 SD
- **Severe MIC:** OFC below –3 SD at birth
- **Severe MIC:** OFC below –4 SD at older ages

Correlations between MIC and neurological outcomes in children



OFC > – 2 SD

OFC < – 2 SD

ID/DD

- 50% increased risk for being developmentally delayed in children with MIC vs children without MIC
  - Correlation between MIC severity and developmental outcome:
    - Severe DD/ID in 10.5% of children with mild MIC (< 2 SD)
    - Severe DD/ID in 51.2% of children with severe MIC (< 3 SD)
- Even in children with normal school placement, 1.9% have MIC, and in many subtle learning disabilities are present

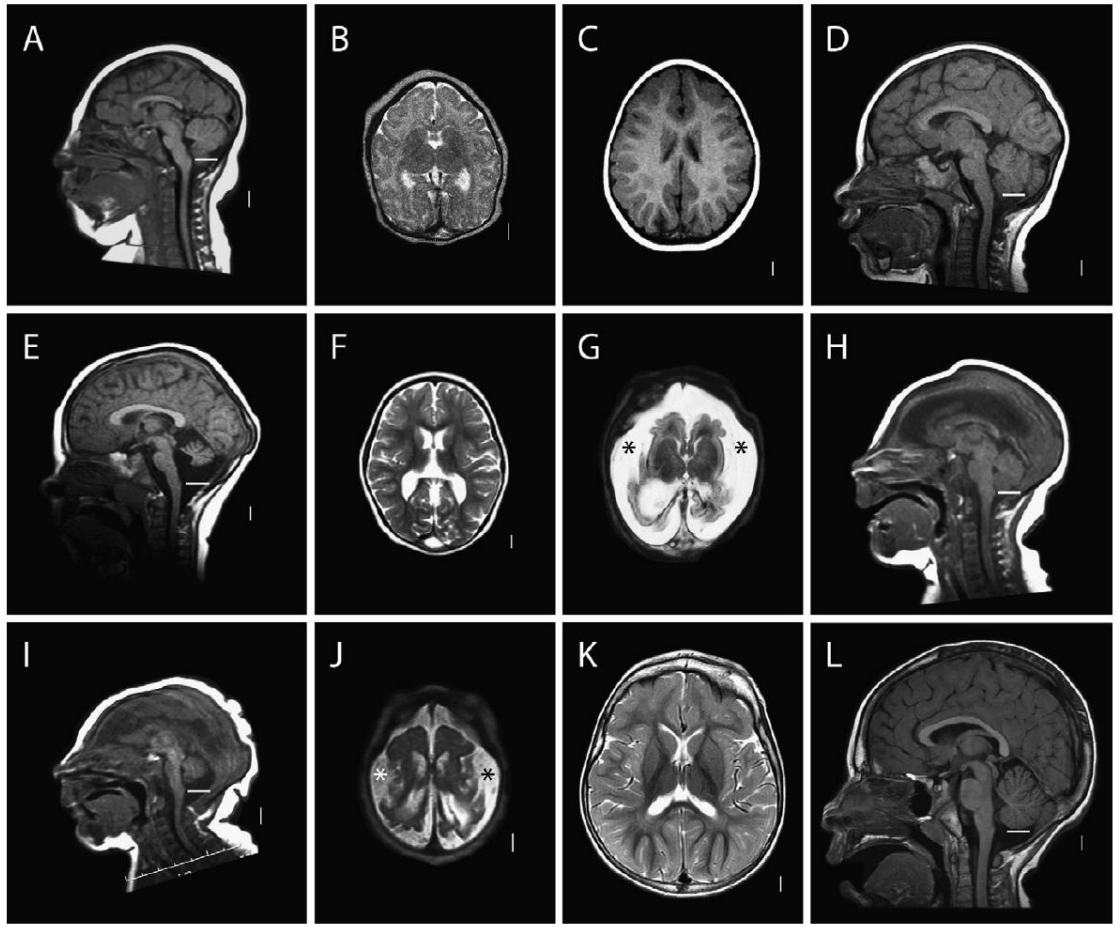
Epilepsy

- Prominent feature of some but not all MIC syndromes
- More common in children with postnatal MIC (50%) than congenital MIC (37.5%)

Mirzaa, Ashwal and Dobyns, 2011

MIC by neuroimaging features

1. **MIC with simplified gyral pattern only (primary MIC)**
2. MSG and enlarged extra-axial space (XAX)
3. MIC with disproportionate pontocerebellar hypoplasia (PCH)
4. MIC with disproportionate PCH and enlarged XAX
5. Microlissencephaly (MLIS) with true agyria-pachygyria
   - Barth MLIS syndrome
   - MOPD type 1
   - Norman-Roberts MLIS syndrome
6. MIC with diffuse periventricular nodular heterotopia
7. MIC with diffuse polymicrogyria (MDP)
8. MIC with diffuse cortical dysplasia
9. MIC with other brain malformations (holoprosencephaly)

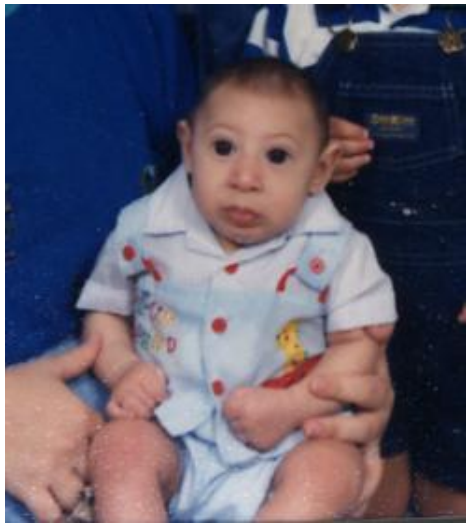


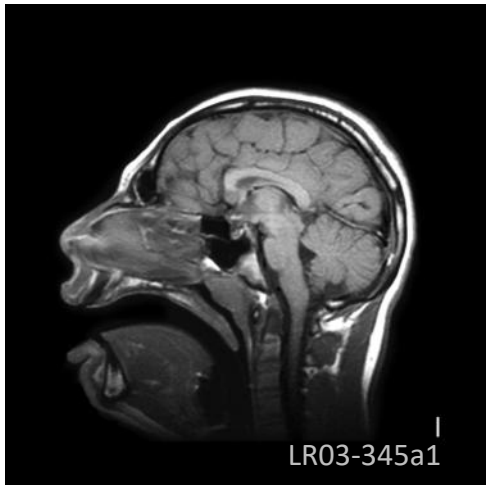
Basel-Vanagaite et al., 2010

ASPM related microcephaly

The most common ”primary MIC”

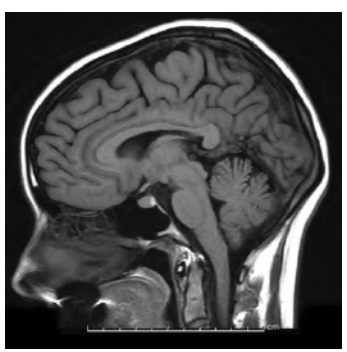
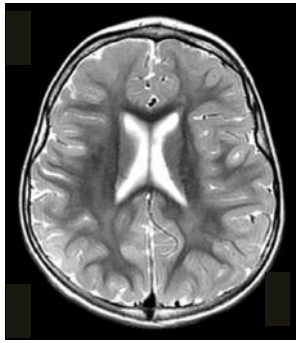
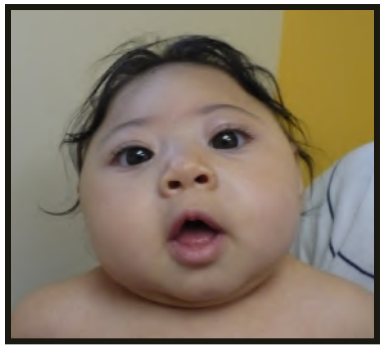
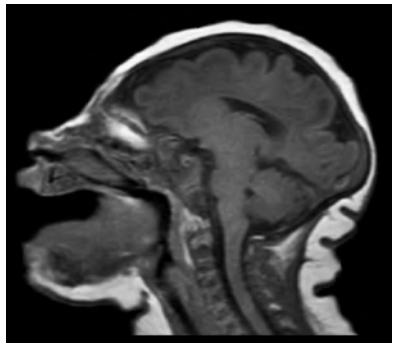
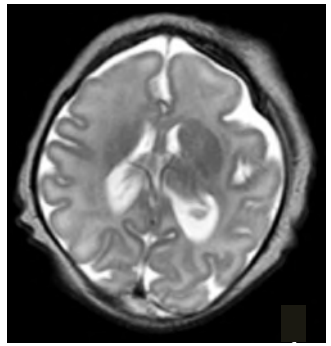
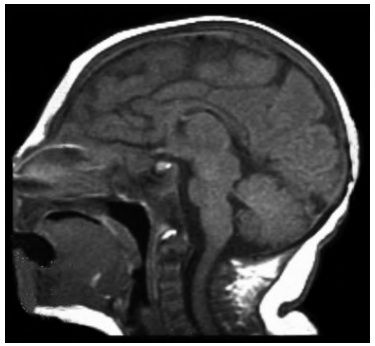
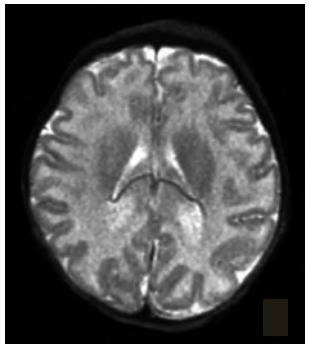
- **MIC with simplified gyral pattern, relatively normal growth (stature), and high function**
- Congenital MIC:
  - Birth OFC < 3 SD below the mean
  - Later OFC 4 - 8 SD below the mean
- Mild growth deficiency, stature –2 to –3 SD (syndrome, nutritional)
  - Feeding difficulties (initially), poor feeding
- Mild-moderate developmental delay
  - Many walk between 1-2 years
  - Develop limited language skills
  - Several self-help skills
  - Survival into adulthood is typical
- Normal tone or mild spasticity, moderate or severe spasticity less common
- Seizures uncommon and typically easily controlled (*ASPM* > other genes)



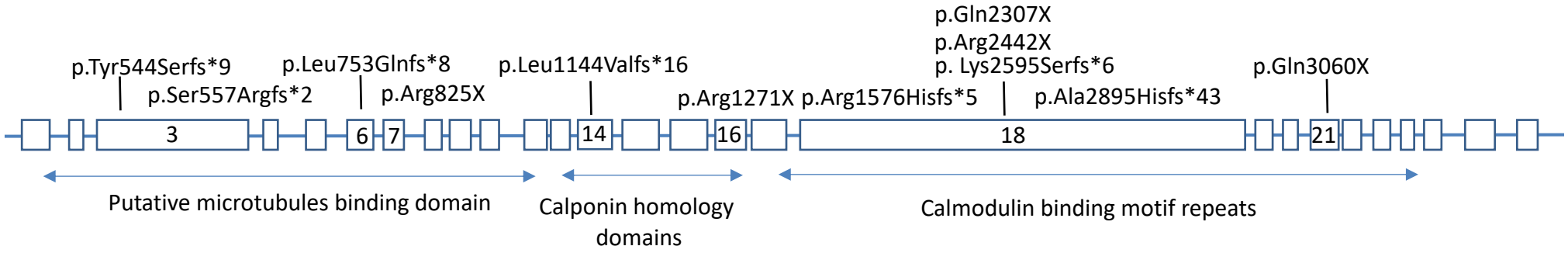


Scheck et al., unpublished

ASPM related microcephaly

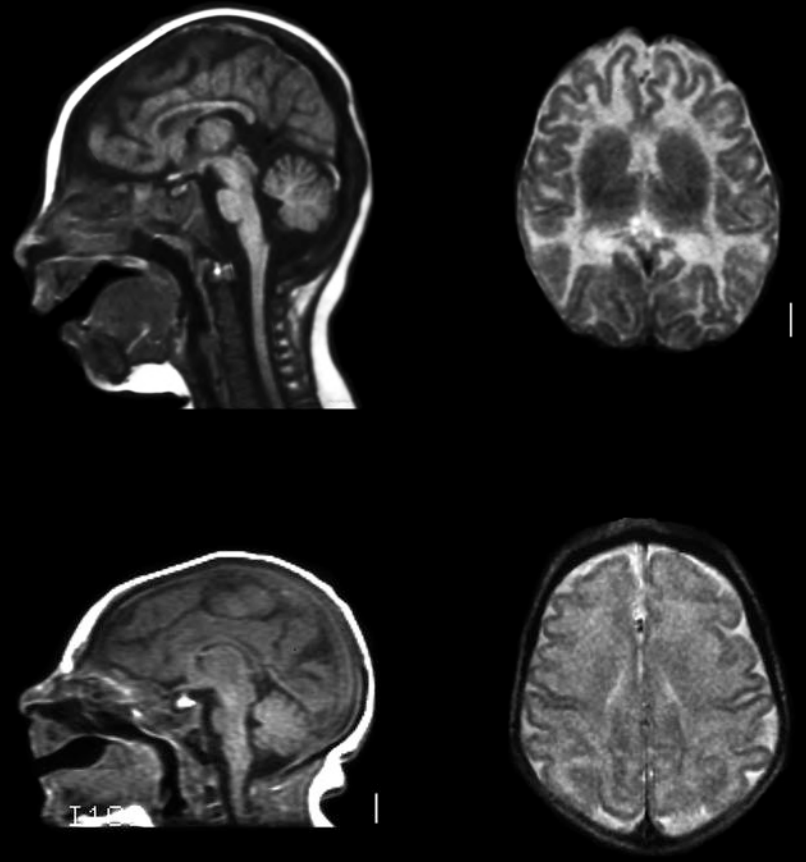
Normal **LR10-267 at 4 weeks** **LR04-029 at 5 weeks**



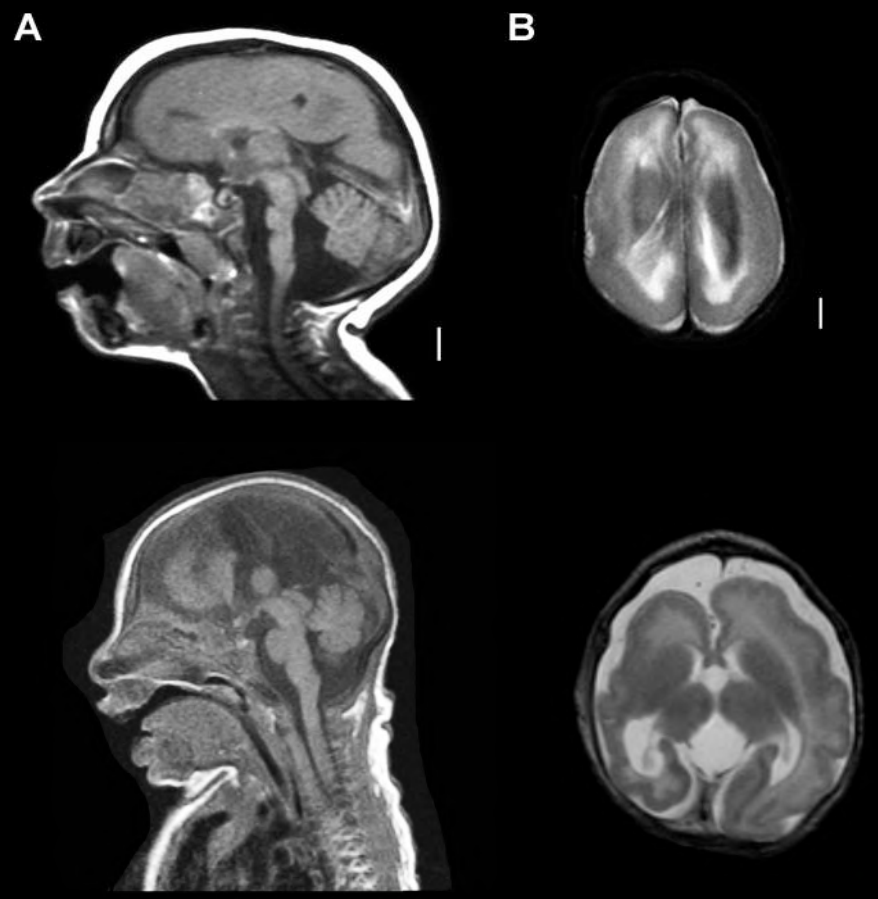
Scheck et al., unpublished

MSG vs. lissencephaly/pachygyria

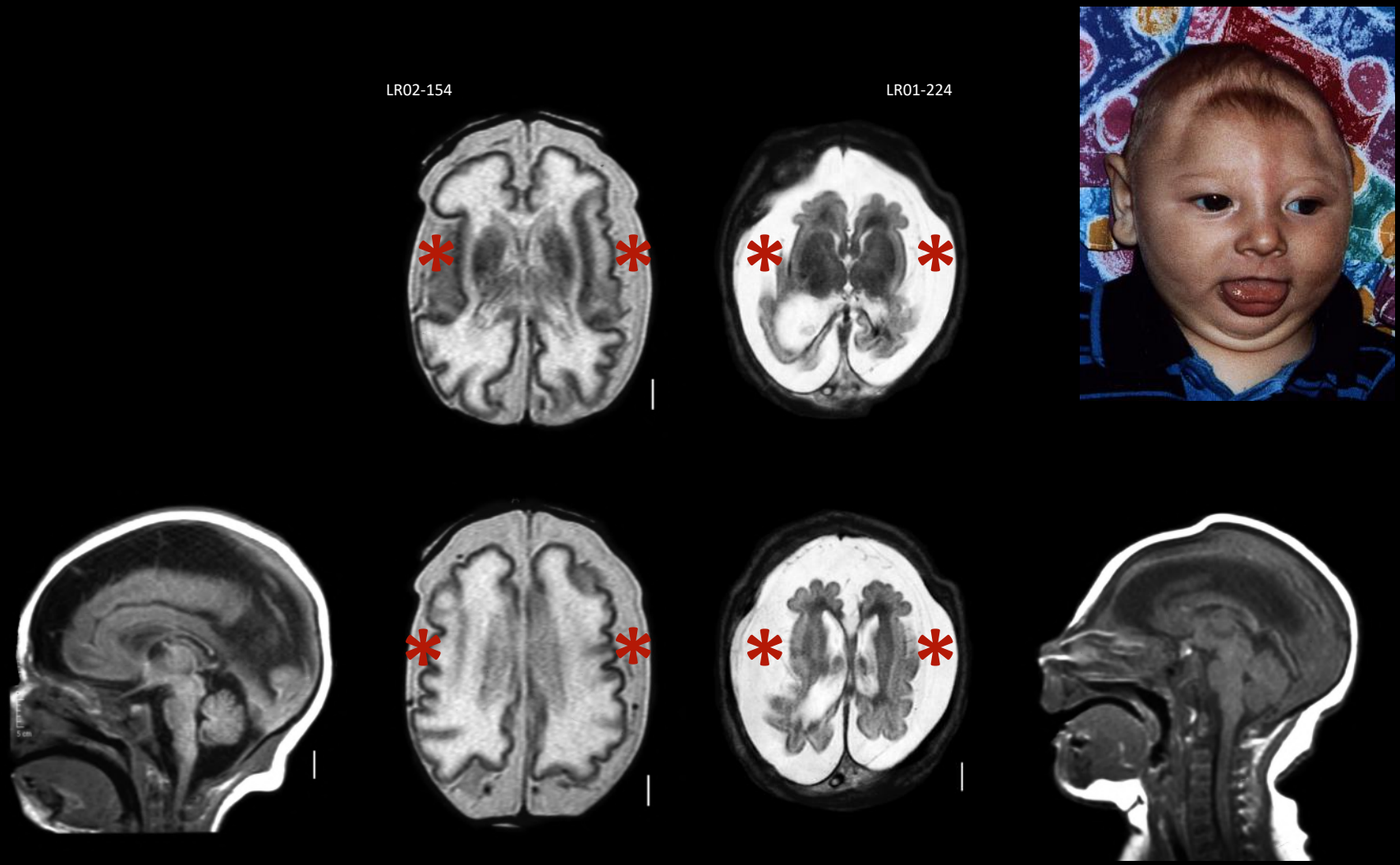
Microcephaly with simplified gyri (MSG)



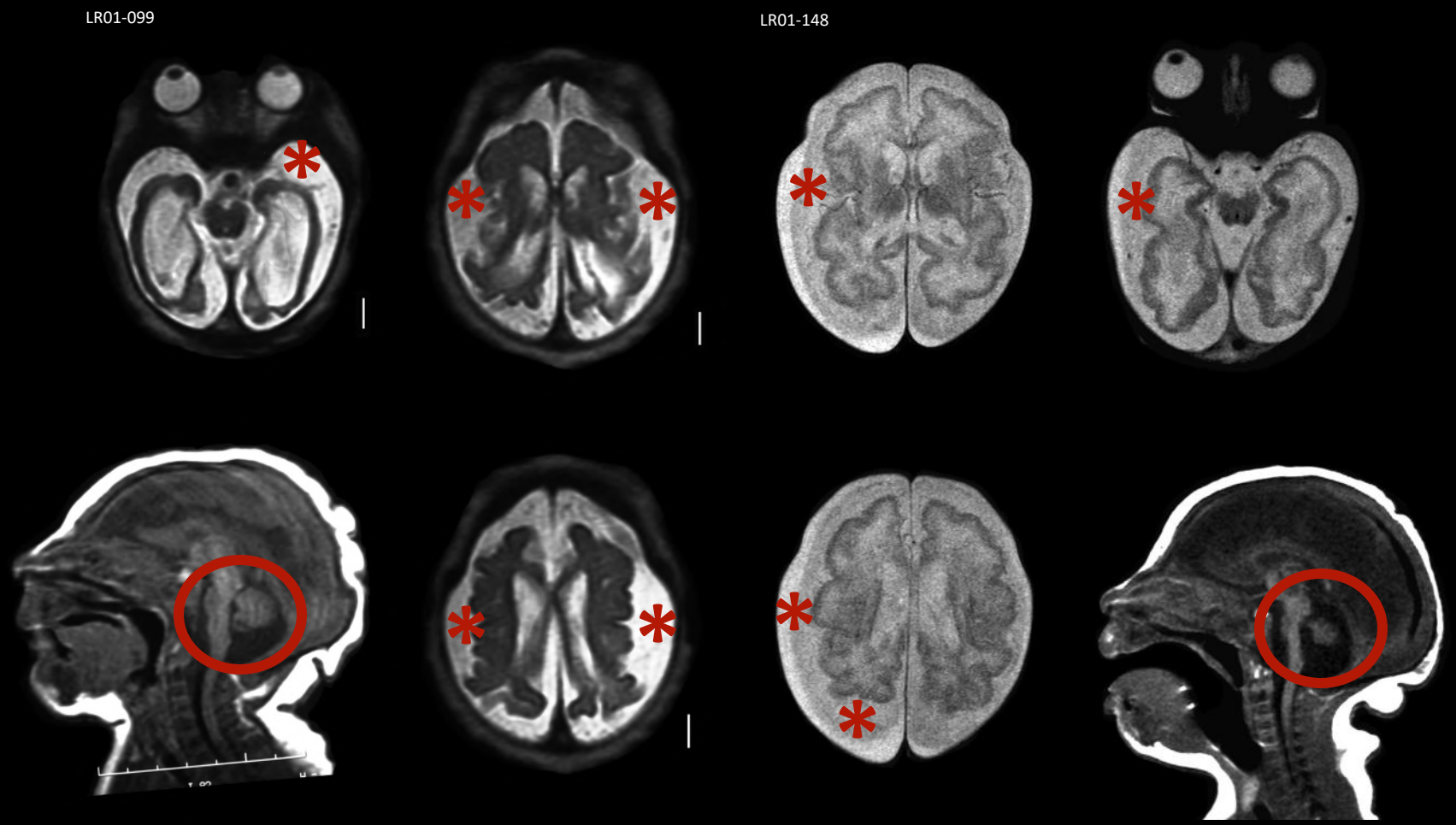
Microlissencephaly (MLIS)



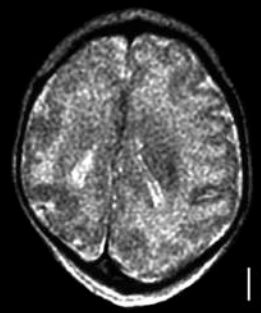
MIC with enlarged extra-axial space (XAX)



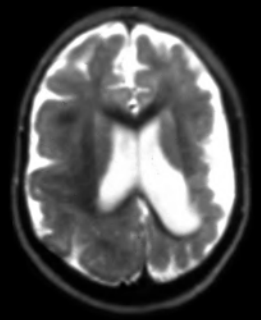
MIC with PCH and enlarged XAX



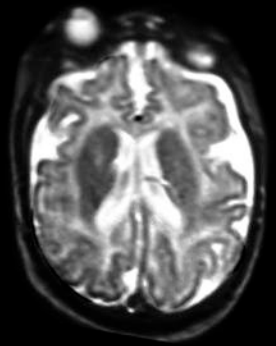
MIC with asymmetric PMG



LP98-078



LR04-062a1



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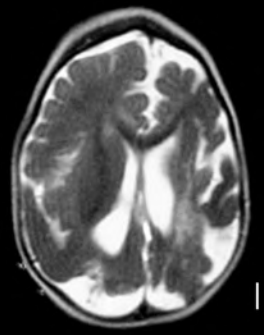

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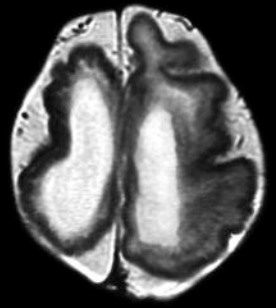
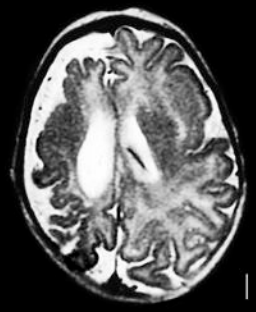
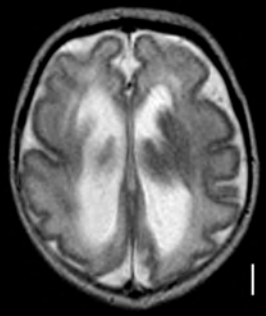


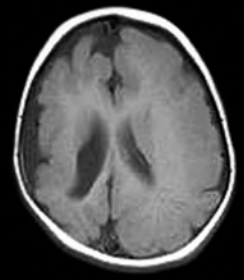
LP97-070a1



LP97-070a2



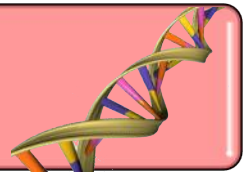
LP97-070a3



LR04-051a2

Causes of MIC

Genetic

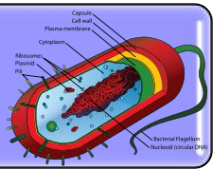


Single gene:
- Autosomal recessive primary (MCPH)
- Autosomal dominant

Chromosomal:
- Trisomy 13, 18
- Deletion syndromes (-5p, -4p, del 22q11.2)

Syndromes:
- Cornelia de Lange
- Holoprosencephaly
- Smith-Lemli-Opitz
- Seckel/MOPD syndrome

Acquired



Disruptive/injuries:
- Stroke (ischemic,hemorrhagic)
- Fetal death of a twin

Infections:
- TORCHES, HIV

Teratogens/Toxins:
- Alcohol
- Maternal PKU
- Poorly-controlled diabetes

Maternal Deprivation:
- Maternal malnutrition
- Placental insufficiency
- Maternal folate deficiency
- Maternal hypothyroidism

Mirzaa, Ashwal and Dobyns, 2011

MIC genes and pathways

DDR, chromosome stability and cell cycle regulation

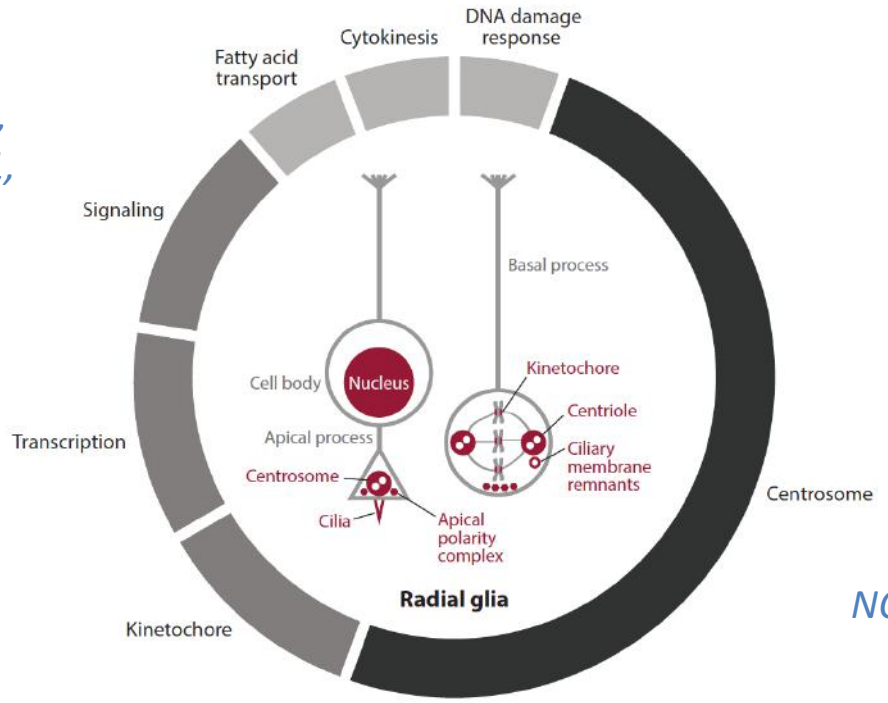
ATR, ATRIP, RBBP8, NPN, RAD50, MRE11A, PNKP, BRCA1, BRCA2, LIG4, NHEJ1, DDX11, PHC1, DNA2, XRCC2, XRCC4, RECQL3, DONSON, STAMBP, CKD6 ANKLE2, MFSD3A

Origin recognition complex

ORC1, ORC4, ORC6, CDT1, CDC6, GMNN, CDC45

Cellular trafficking, fatty acid metabolism, lipid binding proteins

WDFY3, COPB2



Centrosome formation, spindle orientation, microtubule organization, cytokinesis

MCPH1, ASPM, WDR62, CDK5RAP2, CASC5, CENPJ, SASS6, STIL, CEP152, CEP63, NDE1, NIN, PCNT, BUB1B, CENPE, KIF5C, KIF2A, KIF11, KIF14, TUBA1A, TUBG1, TUBB2B, TBCD, POC1A, ZNF335, CIT, NCAPD2, NCAPD3

Kinetochore

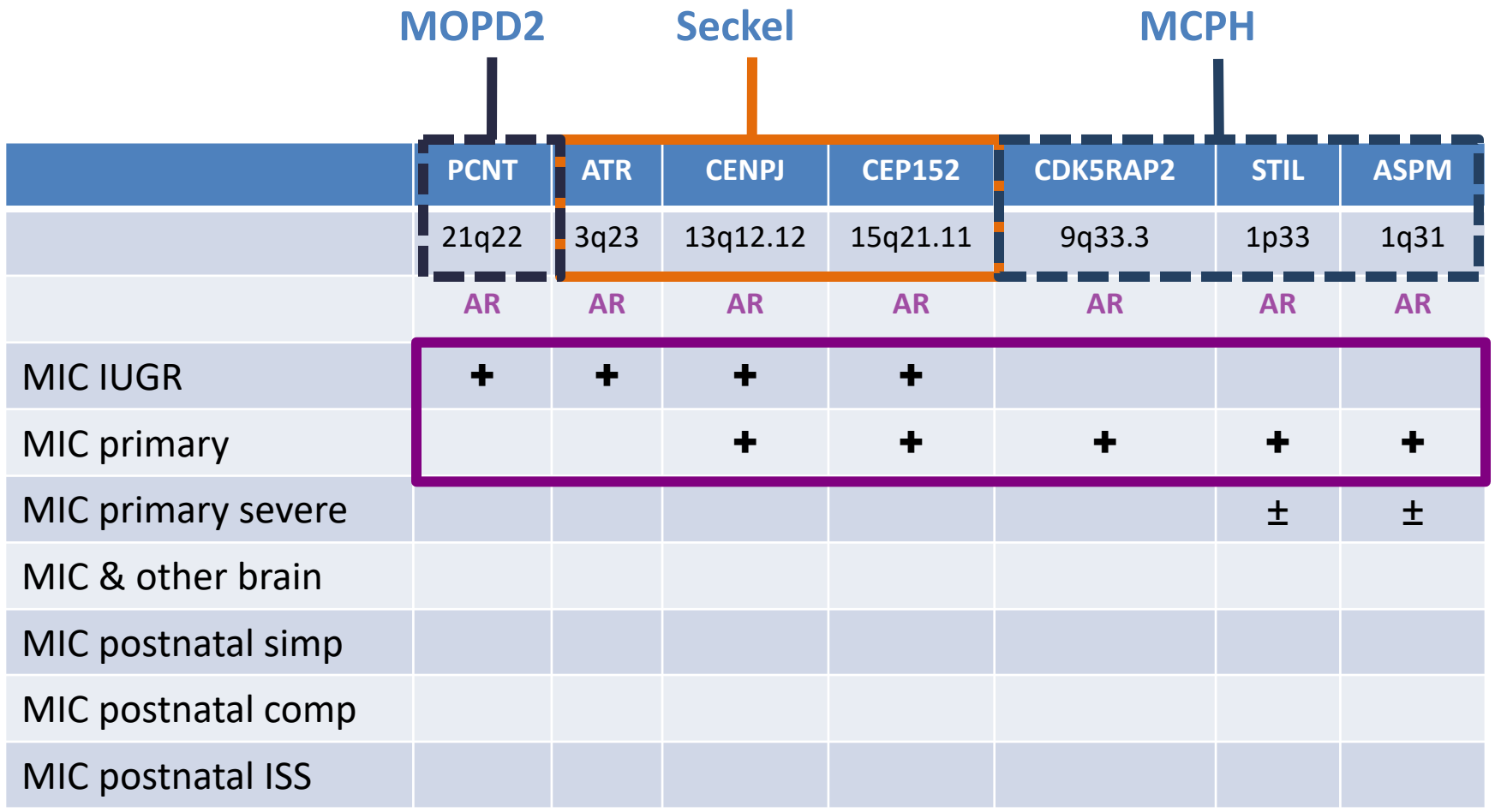
NCAPH, KATNB1, CEP135, CENPJ, CHAMP1

Mitotic-chromosome structure

NCAPD2, NCAPH, NCAPD3

Pirozzi, Nelson and Mirzaa, 2019; Jaymaran et al., 2018

Major groups of MIC syndromes



Scheck et al., unpublished

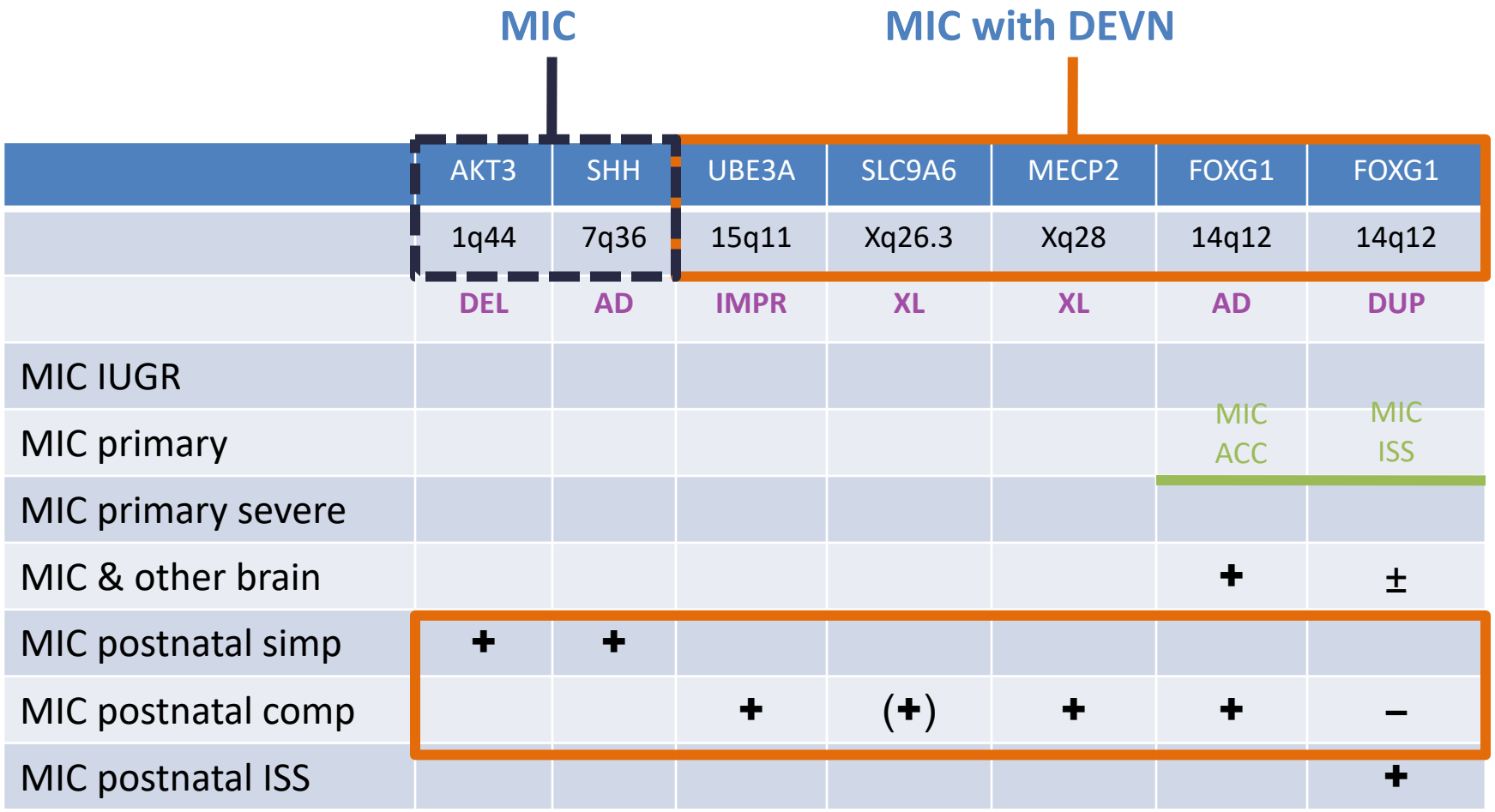
Major groups of MIC syndromes

MIC with MCD

	PNKP	WDR62	TBR2	TUBB2B	TUBA1A	ARFGEF2
	19q13.33	19q13.12	3p21	6p25.2	12q13.12	20p13
	AR	AR	AR	AD	AD	AR
MIC IUGR						
MIC primary						
MIC primary severe	+	+	+	+	+	+
MIC & other brain	+	+	+	+	+	+
MIC postnatal simp			MIC	MIC	MIC	
MIC postnatal comp			ACC	ACC	ACC	
MIC postnatal ISS	MIC PGY	MIC PMG	PMG CBLH	PMG CBLH	LIS CBLH	MIC PNH

Scheck et al., unpublished

Major groups of MIC syndromes



Scheck et al., unpublished

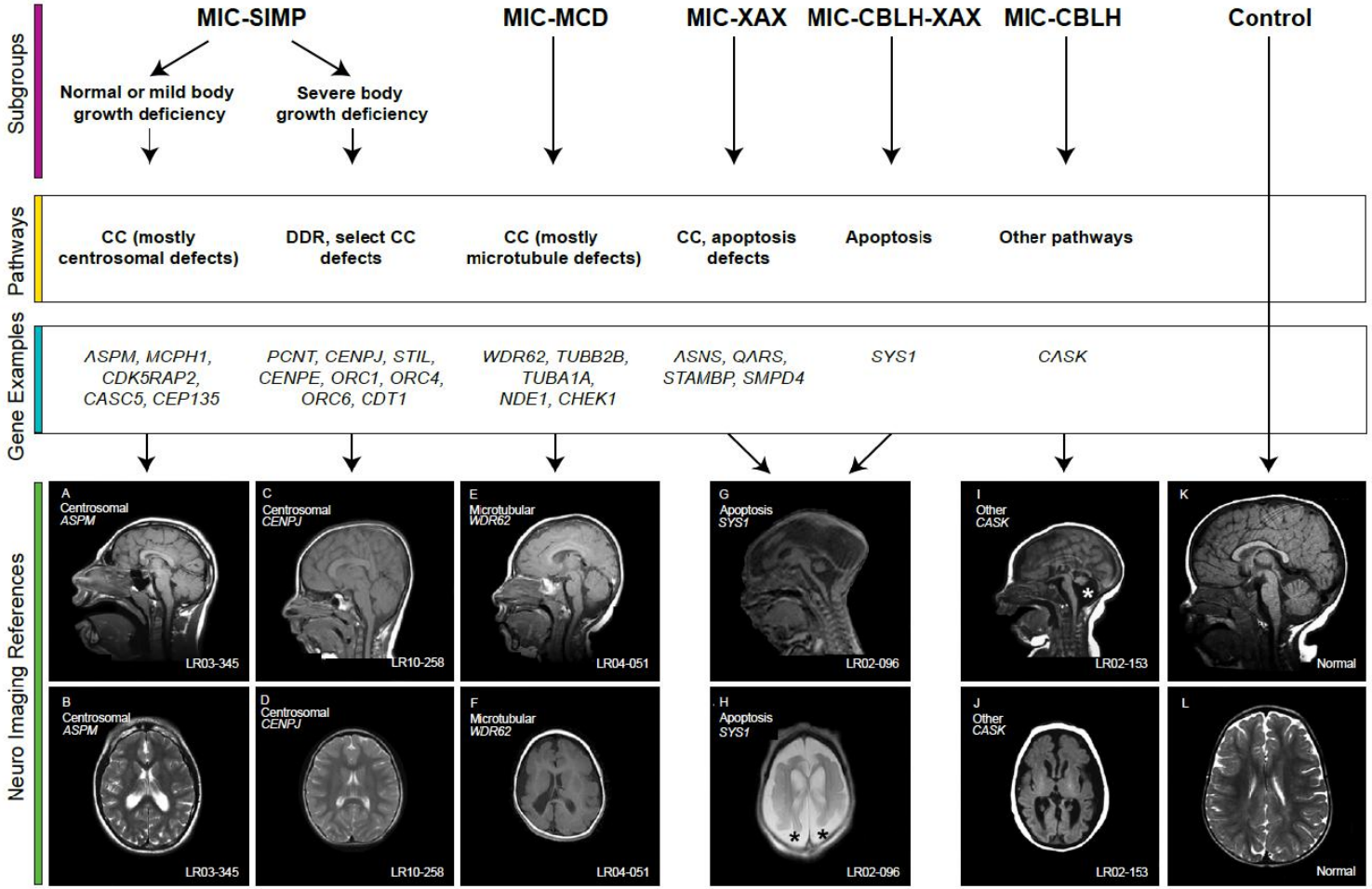
Major groups of MIC syndromes

MIC with DEVN

	TCF4	NRXN1	CNTNAP2	ZEB2	JARID1C	CASK
	18q21.2	2p16.3	7q36.1	2q22	Xp11.2	Xp11.4
	AD	AR	AR	AD	XL	XL
MIC IUGR						
MIC primary	MIC CBLH					MIC CBLH
MIC primary severe						
MIC & other brain	+					+
MIC postnatal simp						
MIC postnatal comp	+	+	+	+	+	+
MIC postnatal ISS						

Scheck et al., unpublished

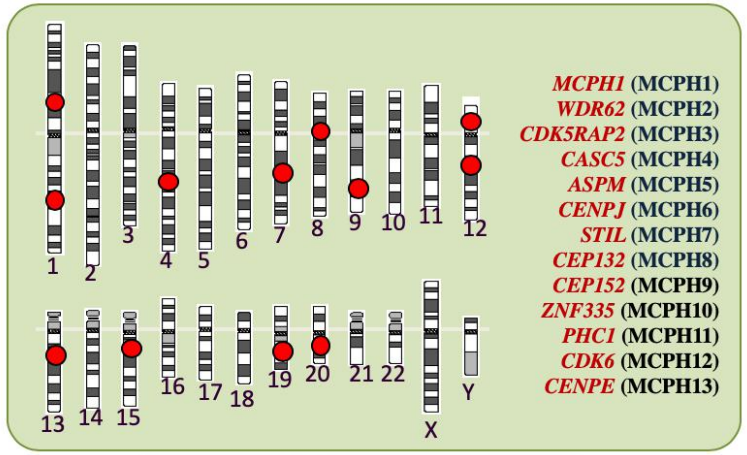
Correlation MIC genes – pathways and phenotypes

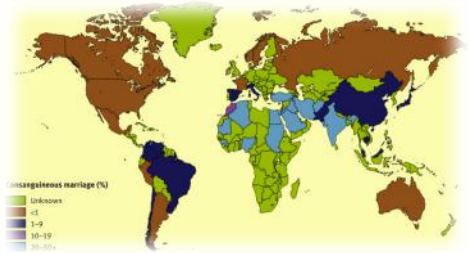


Mirzaa et al., unpublished

Evolution of MIC gene identification

Whole genome linkage analysis
Homozygosity mapping in large, often consanguineous, families
Positional cloning





1980s-2010

NGS
(multi-gene panels, ES)

DDR, chromosome stability and cell cycle regulation
ATR, ATRIP, RBBP8, NPN, RAD50, MRE11A, PNKP, BRCA1, BRCA2, LIG4, NHEJ1, DDX11, PHC1, DNA2, XRCC2, XRCC4, RECQL3, DONSON, STAMBP, CKD6 ANKLE2, MFSD3A

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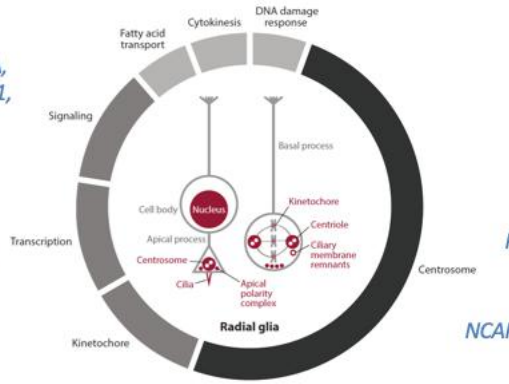

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Kinetochore
NCAPH, KATNB1, CEP135, CENPJ, CHAMP1

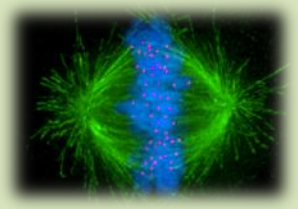
Mitotic-chromosome structure
NCAPD2, NCAPH, NCAPD3

2010 - present

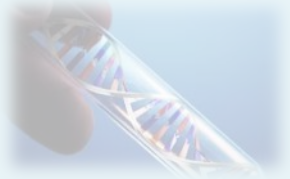
Outline

Overview of MIC phenotypes and causes

Update on new MIC genes and syndromes



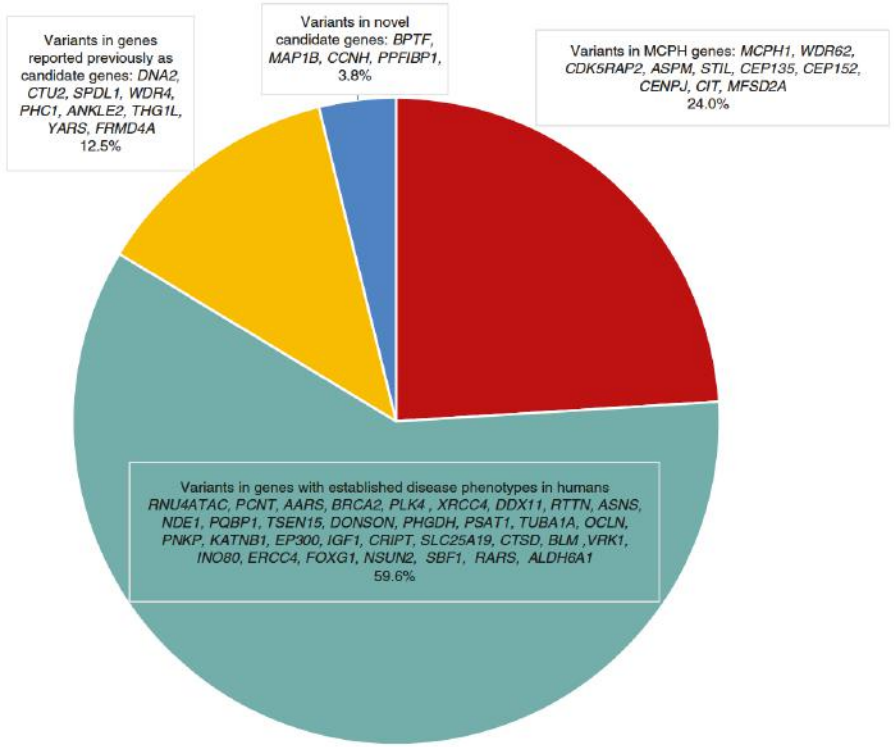
Future directions: diagnostics and mechanistic studies



Genomic and phenotypic delineation of congenital microcephaly

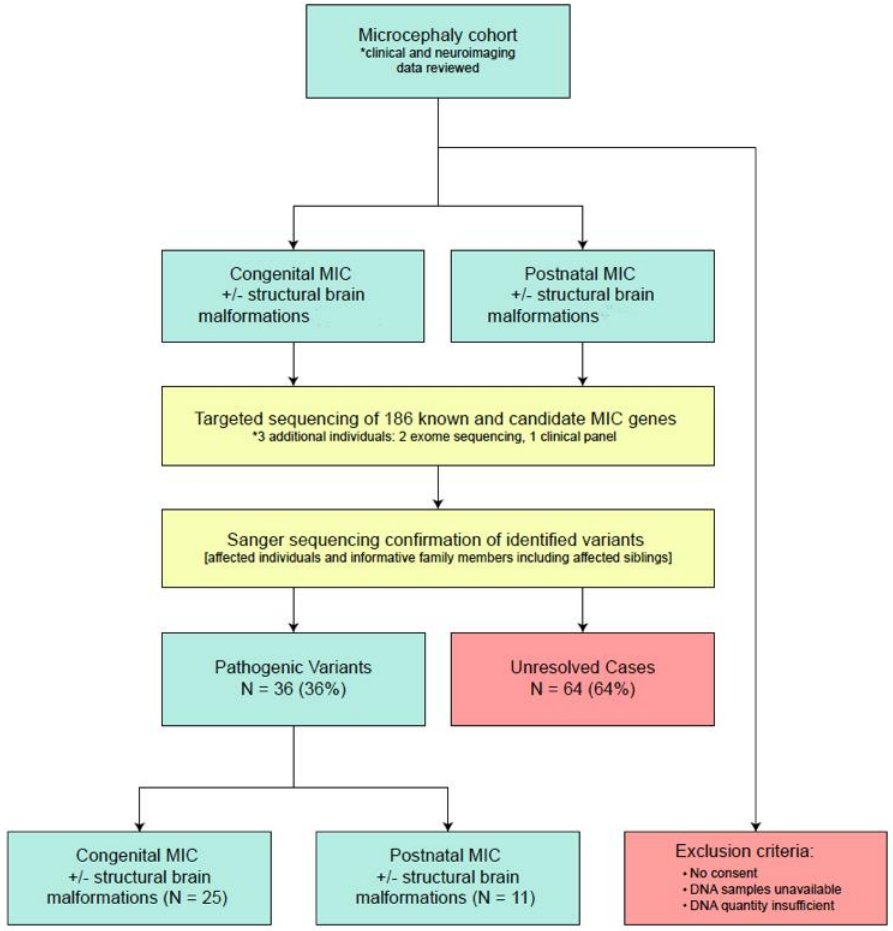
Ranad Shaheen, PhD, Fowzan S. Alkuraya, MD, ABMG et al.

- 137 families with MIC (OFC < 2 SD), w/wo cortical and non-cortical malformations
- Exome sequencing (1st tier)
  - Potentially causal variant in 104 patients (75%)
  - 37 novel likely disease causing variants in 27 disease genes
  - Pathogenic variants in 10 primary MIC genes (25/104; 24%)
    - Founder pathogenic mutation in *ASPM*



Shaheen et al., 2018

MIC: genomics in outbred populations



Congenital MIC

MIC (isolated)

Gene	N
ASPM	***8***
CEP135	*1*
CENPJ	*1*
RBBP8	*1*
KIF11	*1*
ADSL	*1*
ASNS	*1*

MIC++

Gene	N
TUBA1A	***2***
RTTN	*1*
WDR62	*1*
CDKL5	*1*

Postnatal MIC

MIC (isolated)

Gene	N
DYRK1A	*1*
MECP2	*1*
UBE3A	*1*

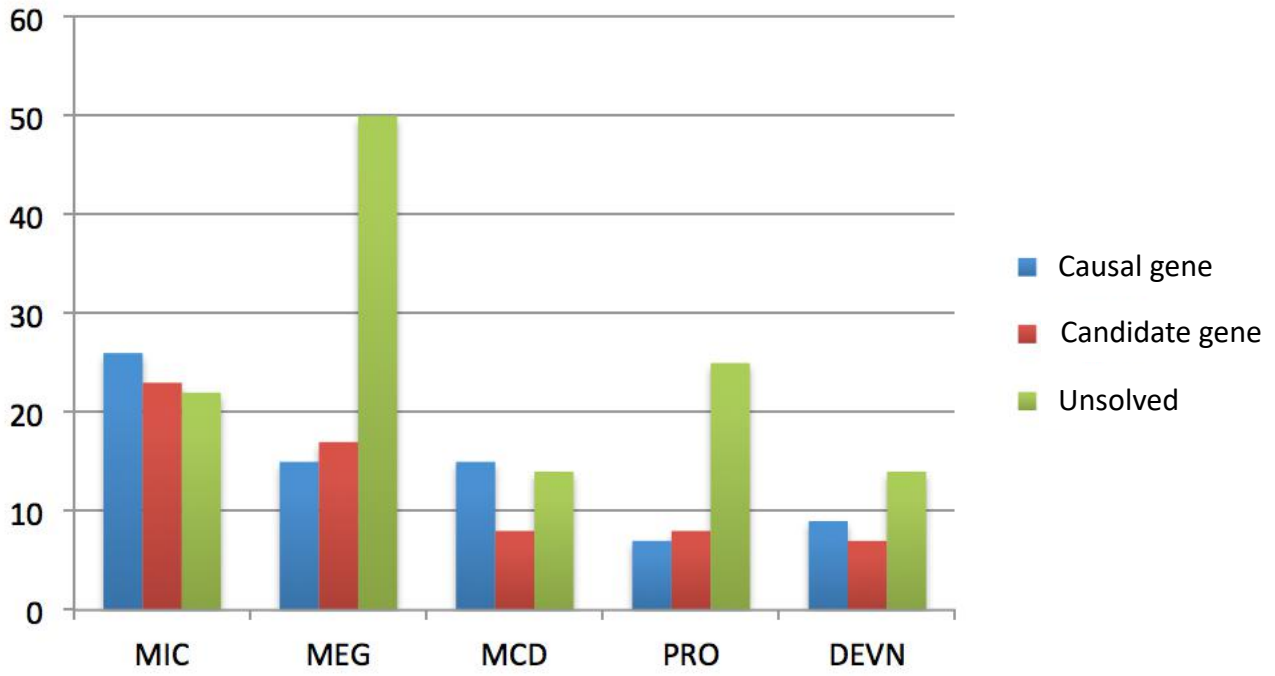
MIC++

Gene	N
RAB3GAP2	*1*
CASK	*1*
SMARCA2	*1*
SOX2	*1*
EFTUD2	*1*
FOXG1	*1*

++ *Additional developmental brain disorders: cortical, cerebellar, callosal abnormalities*

Scheck et al., unpublished

Exome sequencing of MIC cohort



Mirzaa GM & Timms AE, In preparation

Exome sequencing of MIC cohort

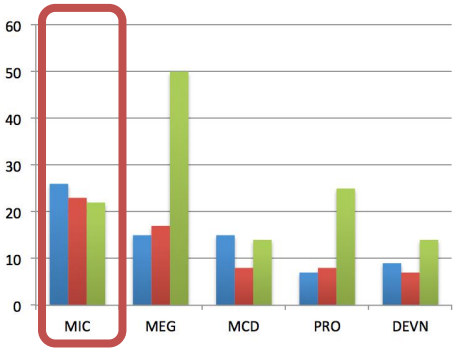


Table 5 – Molecular results of microcephaly (MIC) series

	1 (causal gene identified)			2 (candidate gene identified)		
Result category	**1a**	**1b**	**1c**	**2a**	**2b**	**4**
Explanation	*Known gene Phenotype fits*	*Known gene Phenotype expansion*	*Novel gene Novel disease*	*1 pt + functional evidence OR > 1 pt*	*Strong in silico candidate, 1 pt*	*Incidental findings*
MIC	*ASPM, KIF11, QARS, ASNS, TUBB2B, GRIN2B*	*SMARCB1, STXBP1, ACTG1, FIG4, CREBBP, DYNC1H1, ZEB2, GPSM2, ERCC4, VRK1, FAT4, TUBGCP4, PDHA1*	*NDE1, KATNB1, ANKLE2, CENPE, SMPD4*	*CHEK1, PCHD1, WDR91, PTCHD2*	*SHANK3, ZIC4, NRXN, PTCHD2, WDR25, CDC20, DDX31, ITSN1, NOTCH2, WDR27,*	*SCN9A, CACNA1A, G6PD*

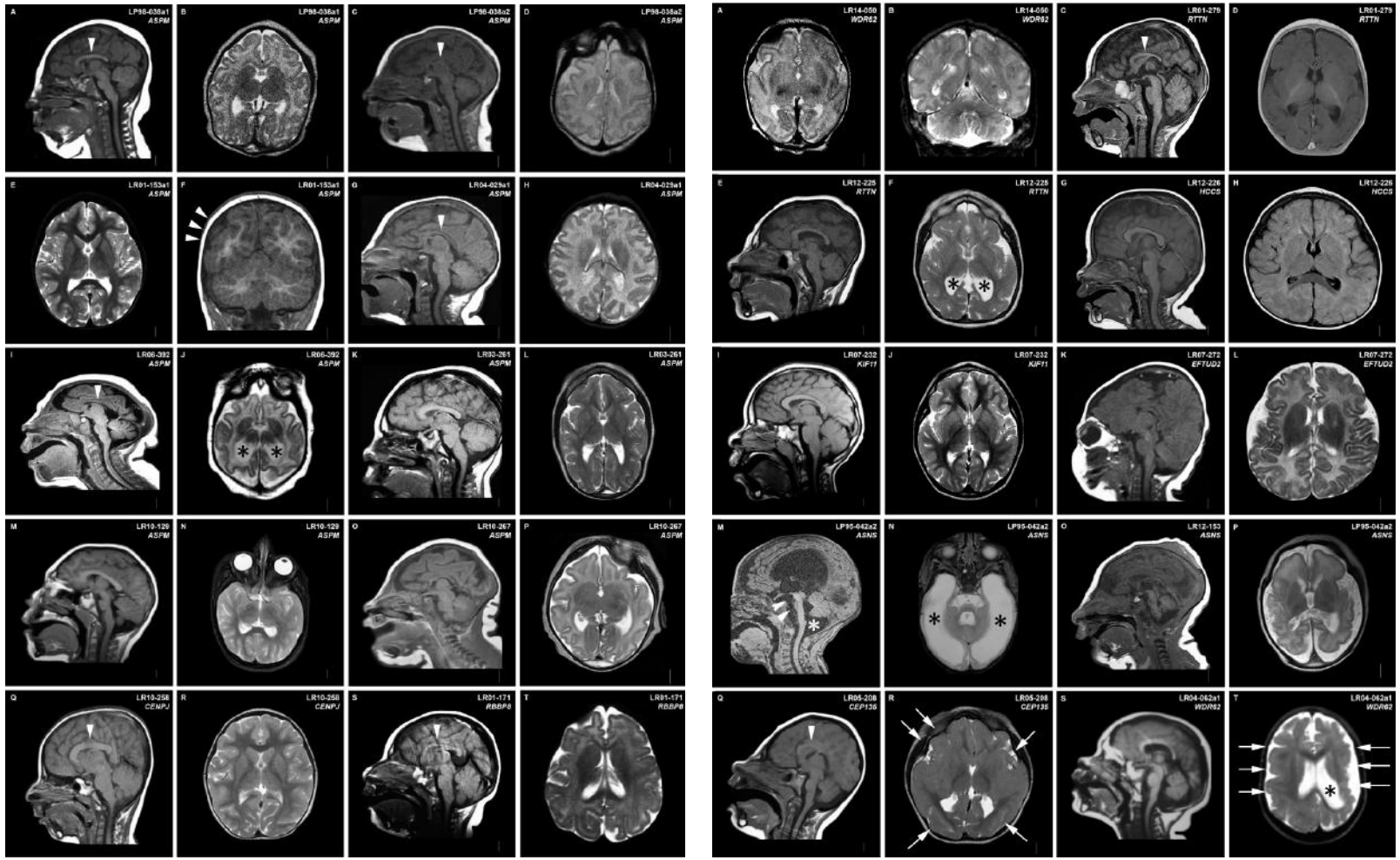
Mirzaa GM & Timms AE, In preparation

MIC: genomics in outbred populations

Insights and findings:

- Novel MIC gene: *CENPE*
- Novel MIC gene: *SMPD4*

- The second report of *RBBP8* related MIC
- Expansion of *ANKLE2* related MIC
- Expansion of *CEP135* related MIC
- Expansion of *WDR62* related MIC
- Additional report of *ASNS*-related MIC
- Expansion of *QARS*-related MIC



Scheck et al., unpublished

CENPE related microcephaly

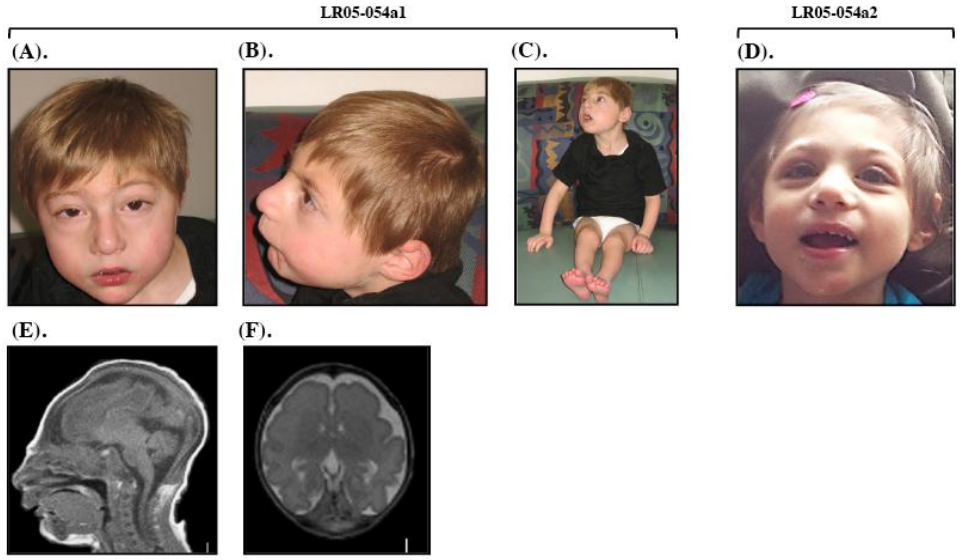
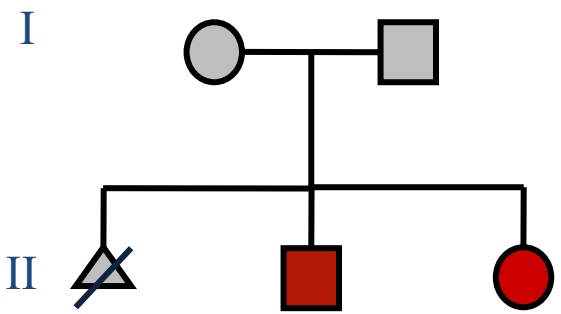
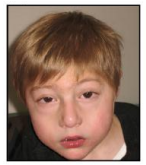
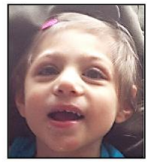


Figure 1



LR05-054a1 LR05-054a2

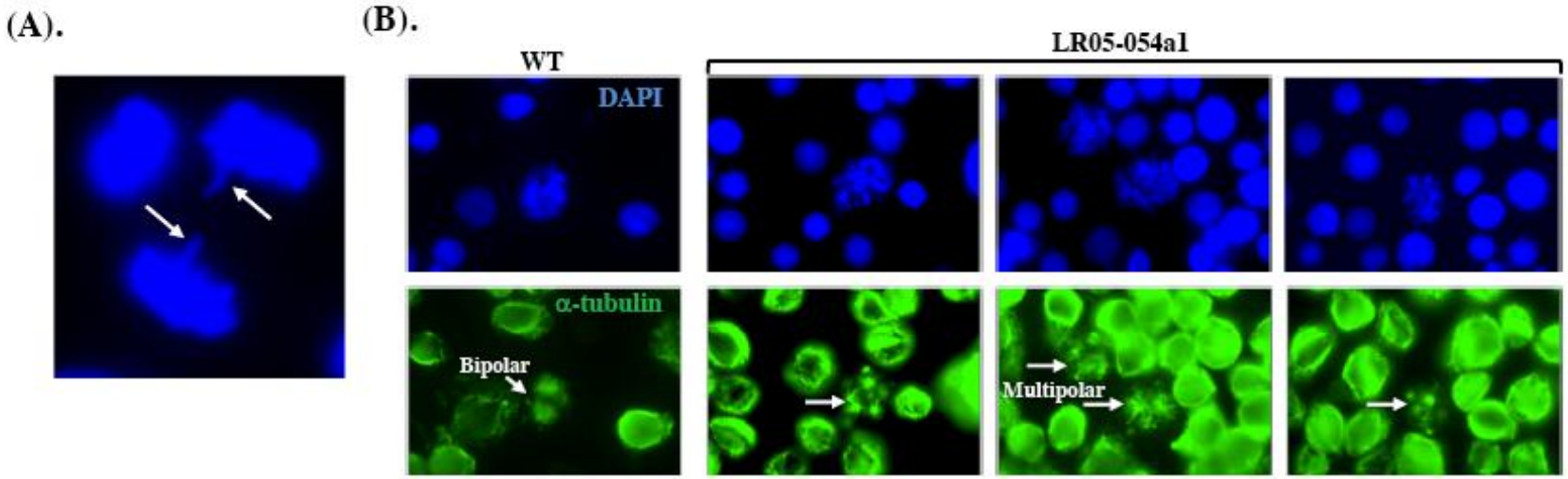


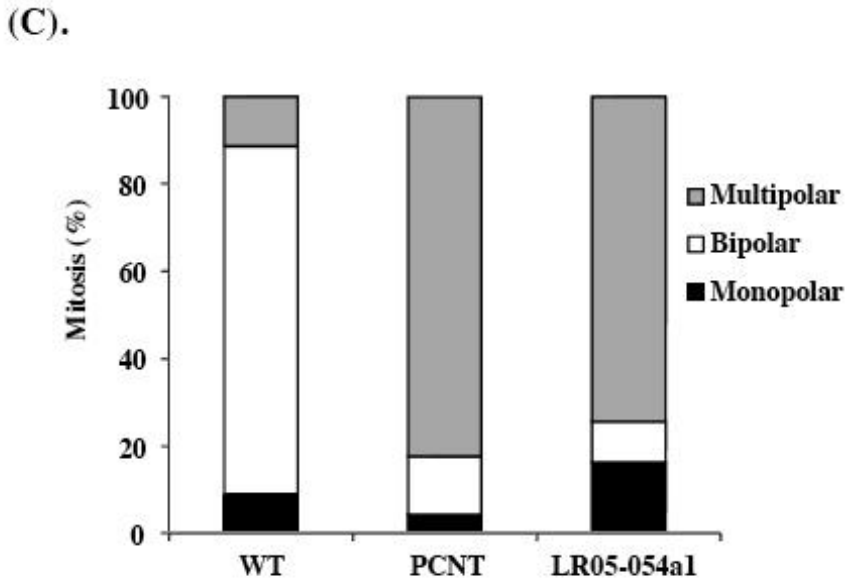


	LR05-054a1	LR05-054a2
Gender	Male	Female
Age last assessed	8 years	3 years
OFC (SD) - Birth	–5	– 3
Weight (SD) - Birth	– 2	– 3
Length (SD) - Birth	– 4	– 5
OFC (SD)	–7 (5 years)	– 7 (3 years)
Weight (SD)	–5 (5 years)	– 3.5 (3 years)
Length (SD)	–7 (5 years)	–3 (3 years)
Facial features	Apparent MIC, prominent nose, sloping forehead, relatively large ears, mild micrognathia	Apparent MIC, low sloping forehead, round face, proportionately large ears and nose
Extremities	Mildly small hands and feet	Small hands and feet (– 2 SD below mean)
Cardiac abnormalities	Congenital restrictive cardiomyopathy	None
Seizures	Tonic seizures, onset 7 months	None
Development	Severe DEV delay, nonverbal	Less severe DEV delay, 65% language delay at 1 year 4 months
Neuroimaging features	Simplified gyral pattern, thin cortex, mildly disproportionate cerebellar hypoplasia	ND
Outcome	Deceased at 8 years (pneumonia)	Alive

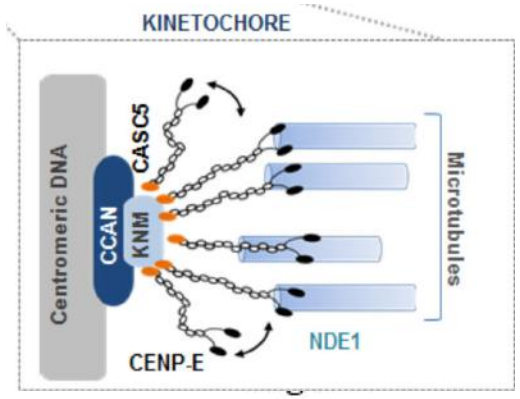
Mirzaa et al., 2014

CENPE related microcephaly



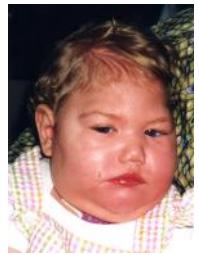


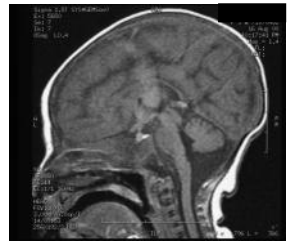
Affected mitotic LCLs exhibit altered spindle dynamics

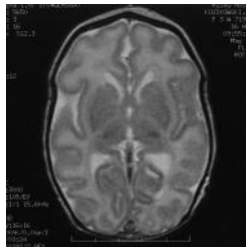


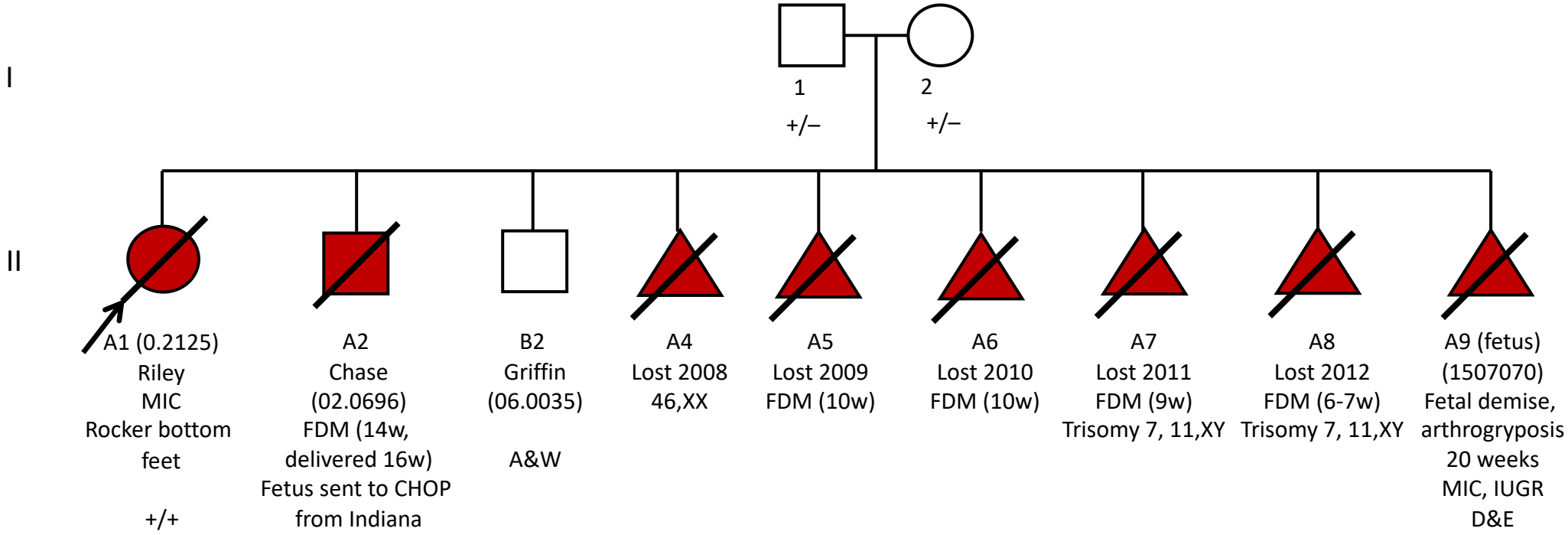
Mirzaa et al., 2014

LR00-144 *SMPD4*





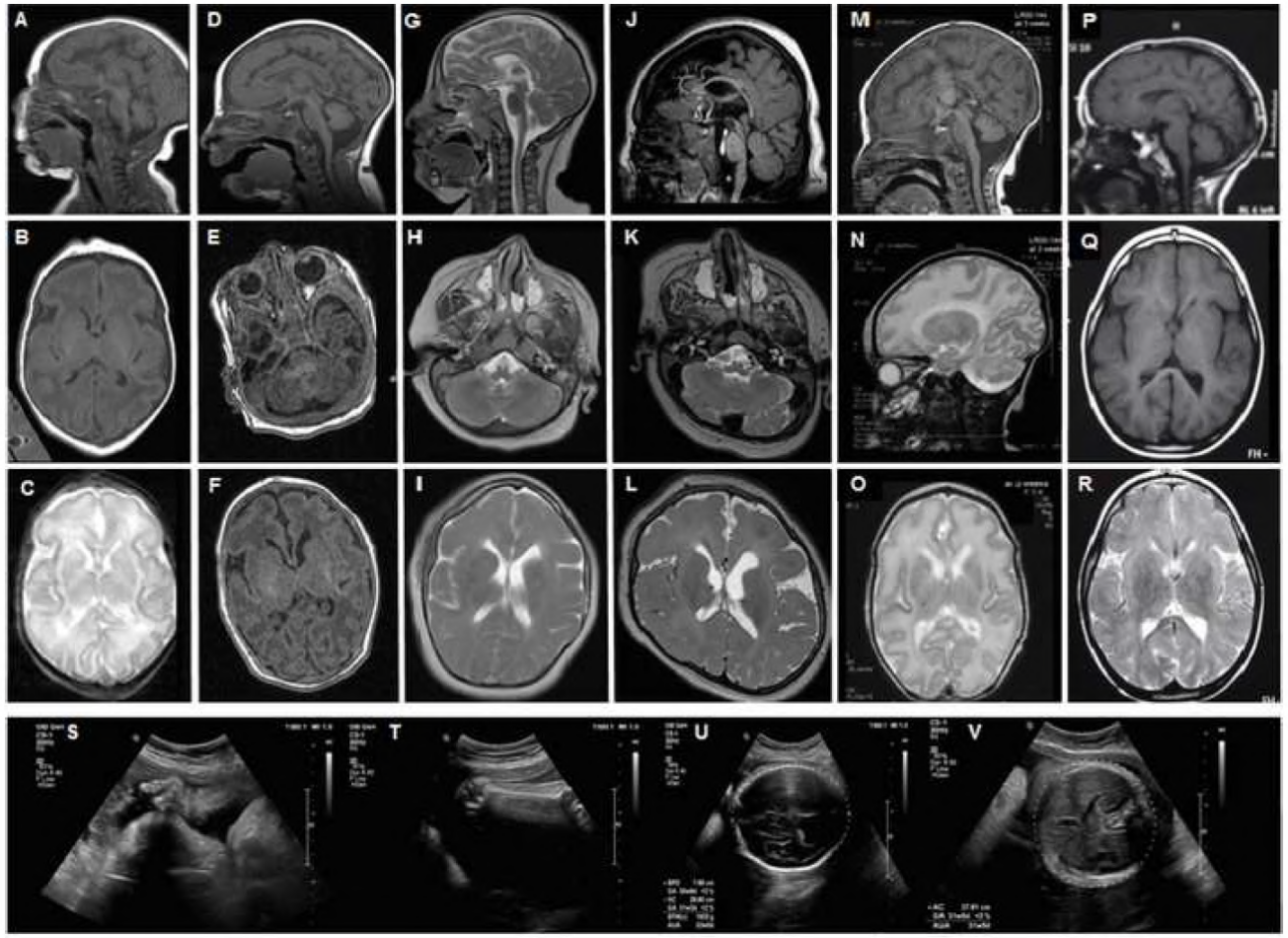


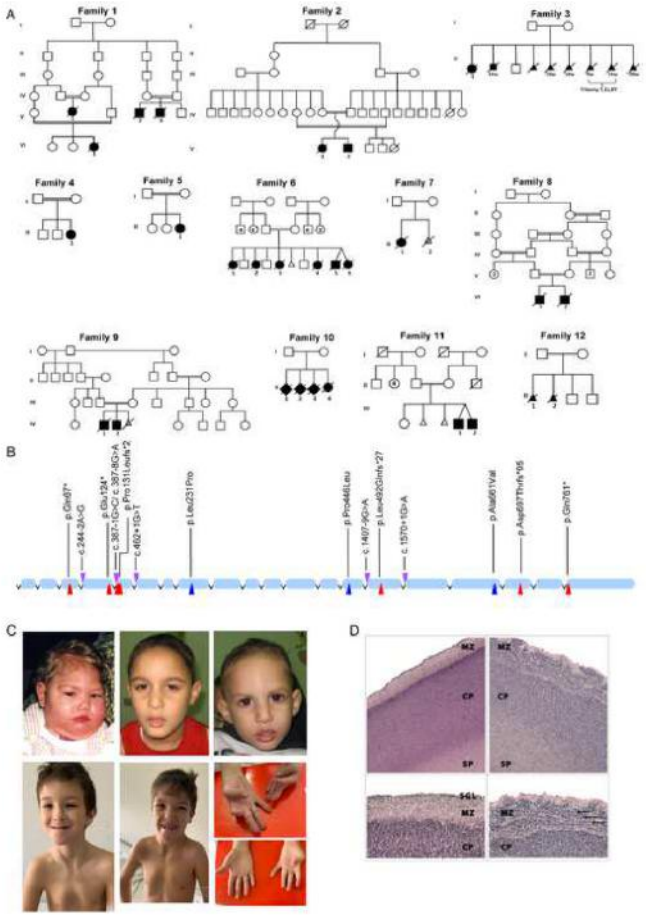


SPMD4	gDNA	Mutation info	AAF proband	AAF father	AAF mother
1	chr2:g.130930851C>A	NM_017751.2:c.462+1G>T	66/132	0/141	48/113
2	chr2:g.130932531G>A	NM_017751.2:c.199C>T, p.Gln67*	70/104	63/117	0/85

Magini et al., 2019

SMPD4-related microcephaly





Magini et al., 2019

RBBP8 related microcephaly

"Jawad Syndrome"

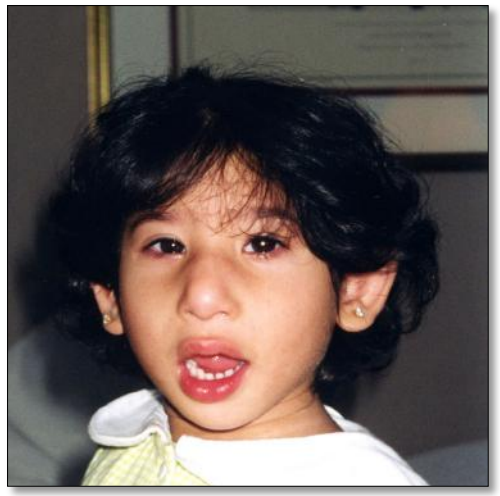
- 2.5 year old Pakistani (consanguineous union)

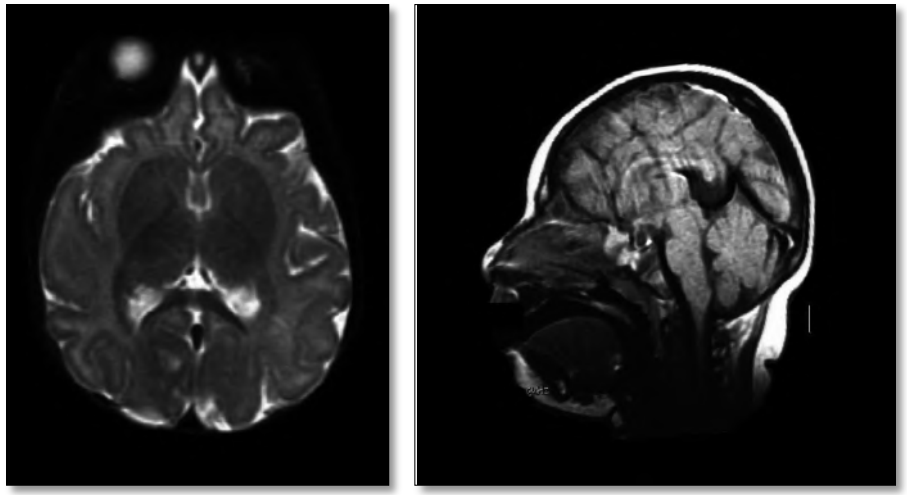
- Growth
  - Birth OFC –6 SD
  - OFC –8 SD at 2.5y

- Moderate ID
  - Walked at 16m
  - 15 words at 2.5y

- Neurological exam
  - No seizures
  - Normal tone
  - Normal DTRs

RBBP8: p.R307G (homozygous)





LR01-17 at 8m
Diffuse MSG, mildly foreshortened frontal lobes, mildly thin CC

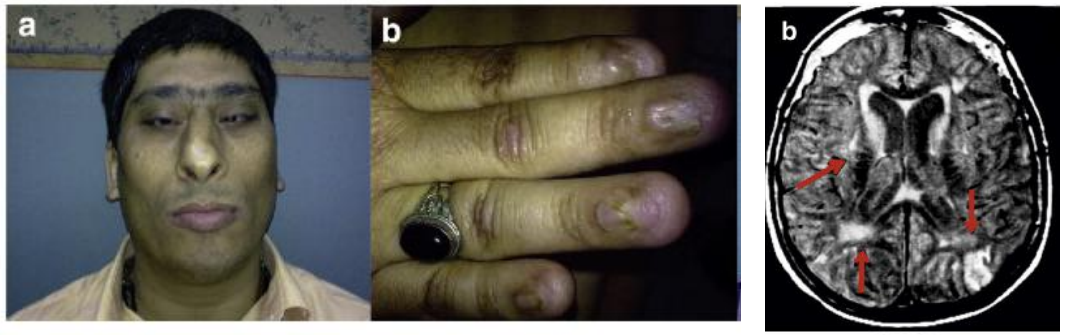
Scheck et al., unpublished

RBBP8 related microcephaly

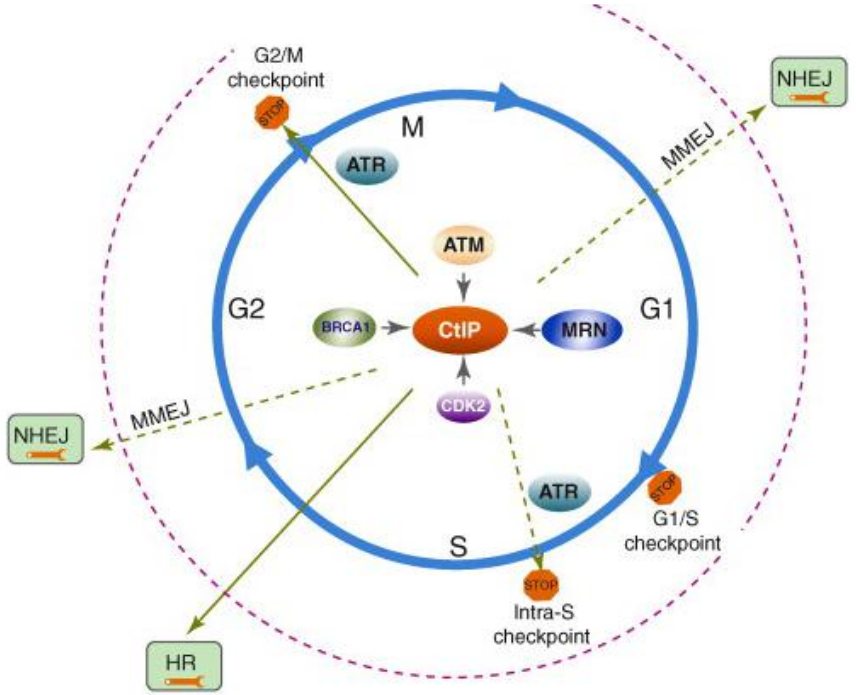
"Jawad Syndrome"

- Two brothers of Pakistani ancestry
- Congenital microcephaly
- Moderately severe ID

- Digital anomalies:
  - Syndactyly
  - Nail anomalies

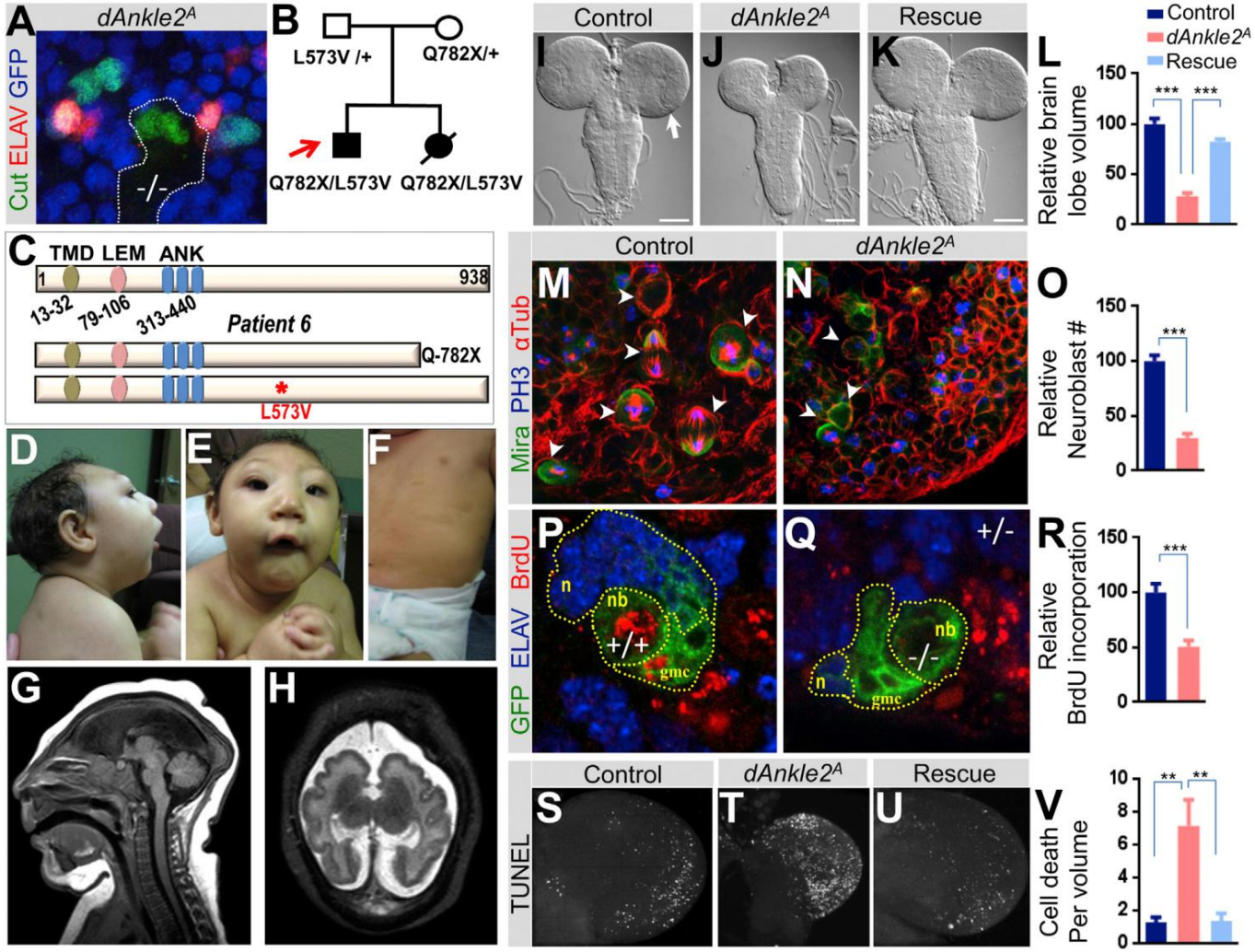


Ubiquitously expressed retinoblastoma-binding protein, a nuclear protein involved in the regulation of cell proliferation (DSB pathway)



Qvist et al., 2014; Agha et al., 2014

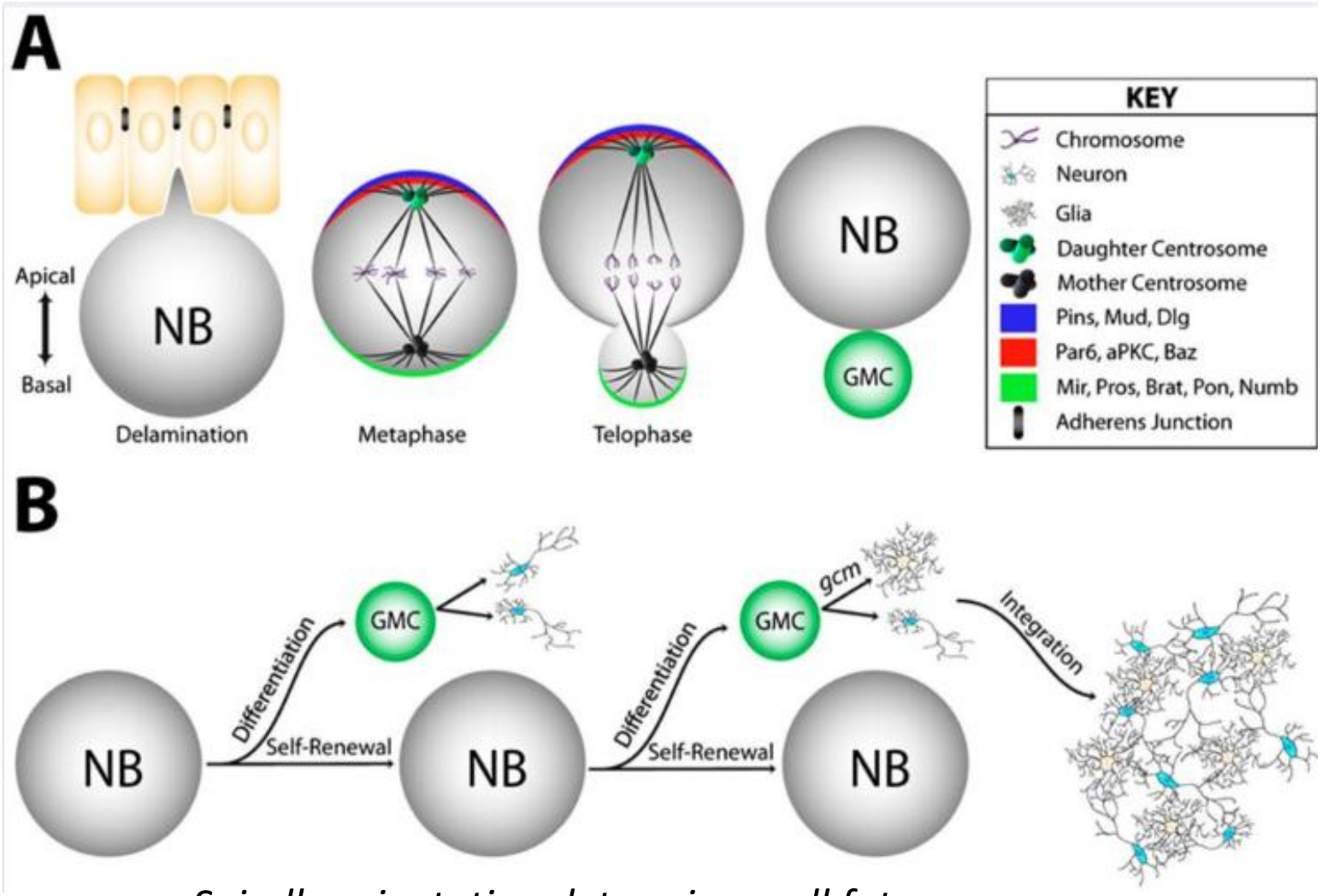
ANKLE2 related microcephaly



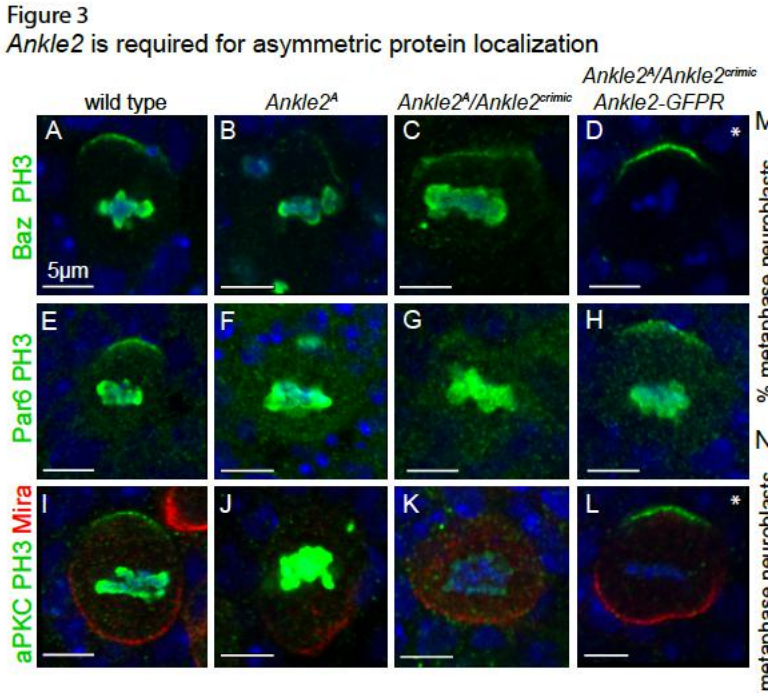
- *Ankle2* localizes to nuclear envelope and ER
  - Modulates VRK1 activity
  - Involved in mitotic nuclear envelope reassembly
- *Ankle2* mutants
  - Fewer neuroblasts
  - Disrupts neuroblast cell division and distribution of asymmetrically localized proteins, spindle orientation and centriole number.

Yamamoto et al., 2014

In *Drosophila* Neuroblasts Divide Asymmetrically



Spindle orientation determines cell fate



Dewey et al., 2015; Link et al., 2019

ANKLE2-related microcephaly

- Two Iraqi sisters of consanguineous parents
- Shared features
  - Mild growth abnormalities (stature –2-3 SD)
  - Severe congenital microcephaly (HC –8-10 SD)
  - Scalp rugae, mild dysmorphic facial features
  - Hypopigmented skin macules and diffuse café-au- lait lesions.
  - Severe neurodevelopmental delays
  - Ambulate and communicate verbally
- Homozygous *ANKLE2* variant on a targeted NGS panel.
  - c.686T>G, (p.Val229Gly)

Thomas et al., in preparation

ANKLE2-related microcephaly

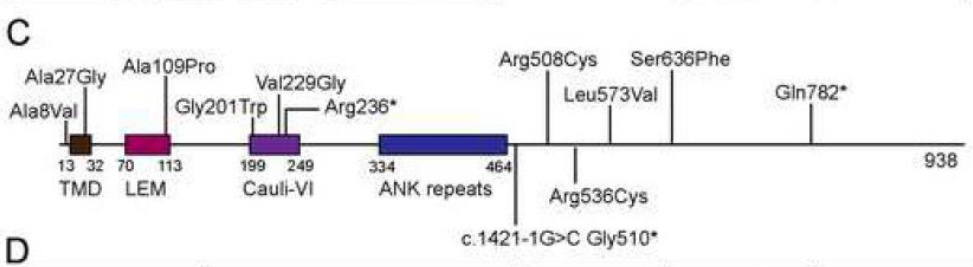
- A girl of outbred Northern European ancestry
- Clinical features
  - Congenital microcephaly (OFC –6 SD)
  - Mildly dysmorphic
  - Ambulatory, verbal
  - Mild-moderate neurodevelopmental delays
  - No scalp rugae or pigmentary skin changes

- Biallelic *ANKLE2* variants identified by exome
  - Maternal missense variant c.325G>C (p.Ala109Pro)
  - Paternal splice variant c.1421-1G>C
  - Novel variants, classified as likely pathogenic.

Thomas et al., in preparation

ANKLE2-related microcephaly

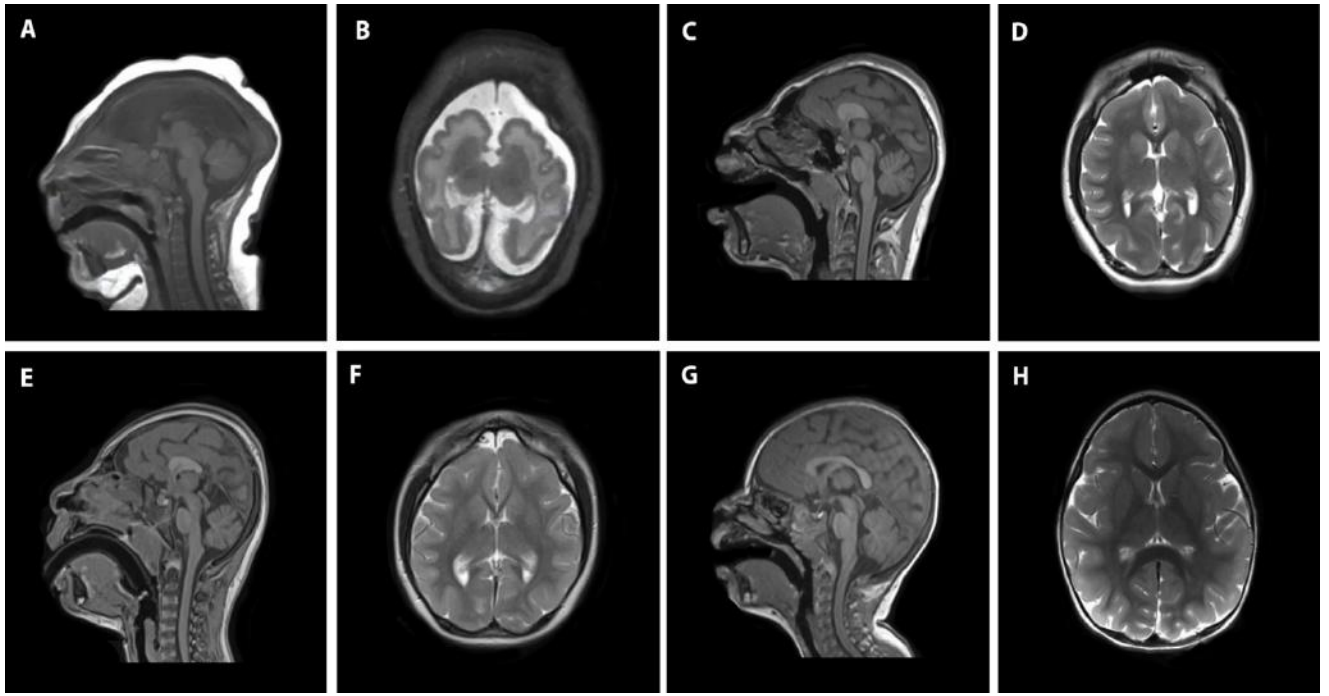
C

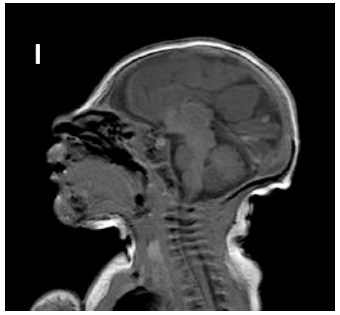


D

Family	*ANKLE2* variant(s)	Congenital Microcephaly	Brain MRI abnormalities	Skin pigmentary abnormalities
LR17-511 2 affected siblings	p.Val229Gly (homozygous)	+	+	+
LR18-033	p.Ala109Pro / c. 1421-1G>C (splicing variant)	+	+	-
BGL-1	p.Arg236* / p.Arg536Cys	+	+	+
BGL-2	p.Ala8Val / p.Ala27Gly	+	+	NS
BGL-3	p.Arg508Cys / p.Ser636Phe	NS	NS	NS

NS: not specified





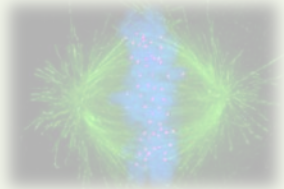
A-B. F**amily 1** diffuse undersulcation of the cortical gyral pattern, marked microcephaly, increased extra-axial space, agenesis of the corpus callosum. **C-D**. **Family 5 (**older child; **E-F.** younger child) diffuse simplification of the cortical gyral pattern, partial agenesis of the corpus callosum, relatively preserved brainstem and cerebellum.
G-H. F**amily 6** mild microcephaly, simplification of the cortical gyral pattern, mild thinning of the corpus callosum. **I. Family 7** short frontal lobes, simplified gyral pattern and corpus callosal agenesis.

Link et al., 2019 (in press)

Outline

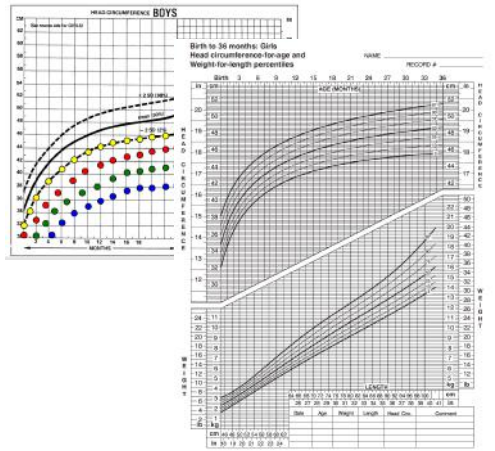
Overview of MIC phenotypes and causes

Update on new MIC genes and syndromes



Future directions: diagnostics and mechanistic studies

Microcephaly approach to evaluation



Growth

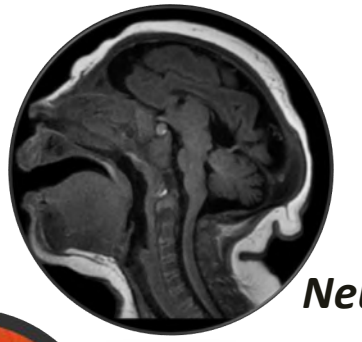
- *OFC*
- *Weight*
- *Length*

Dysmorphic features

- *Face*
- *Limbs, digits*
- *Genitalia*
- *Skin*
- *Body proportions*

Prenatal hx

- *Pregnancy*
- *Labor*



Neuroimaging

Neurodevelopmental Inventory

- *Neurological examination*
- *Epilepsy*
- *Autistic features*
- *Sleep*
- *Mood*
- *Behavior*
- *Food behavior*
- *Abnormal movements*

Ophthalmologic Exam

- ***Chromosomal breakage studies***
- ***Skeletal survey***
- ***Immune function tests***

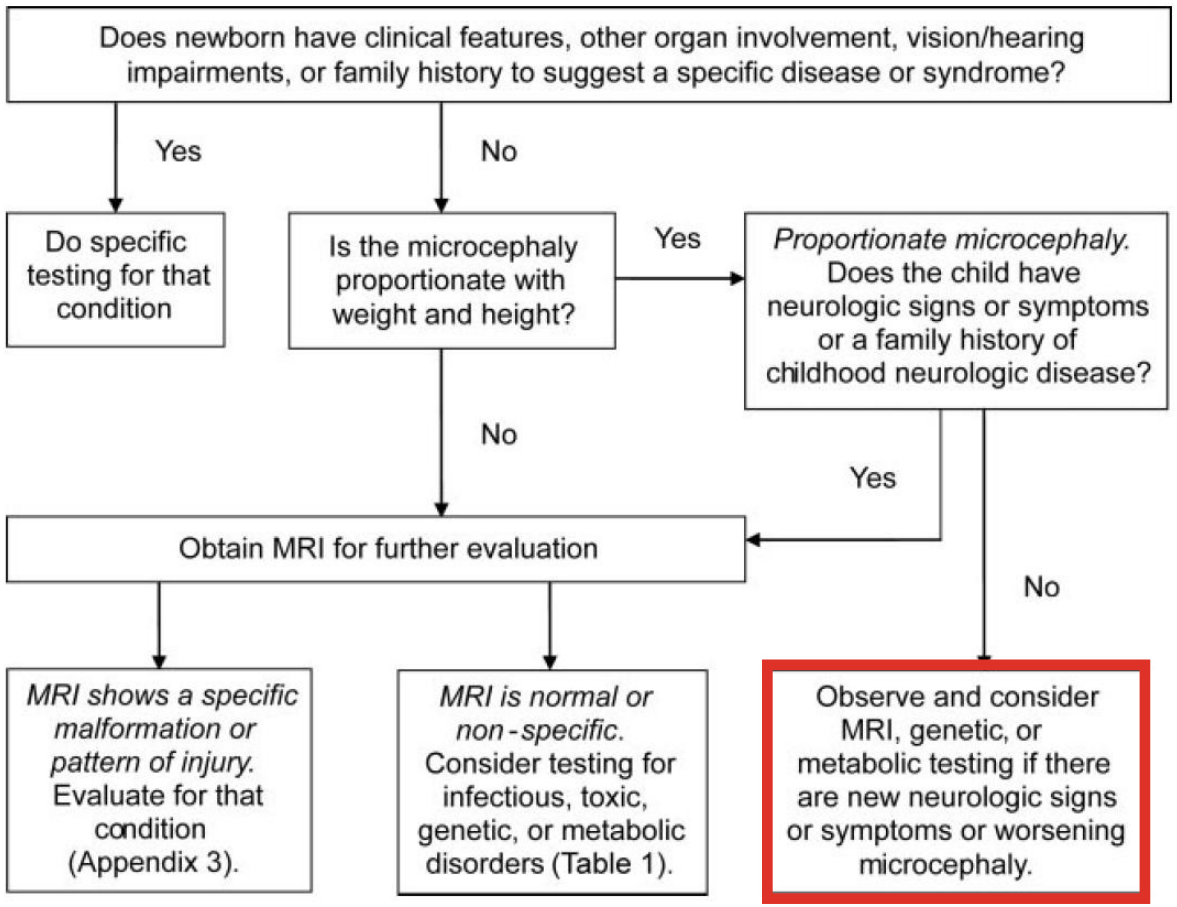
SPECIAL ARTICLE

AMERICAN ACADEMY OF NEUROLOGY

Practice Parameter: Evaluation of the child with microcephaly (an evidence-based review)

Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society

Figure 1 Evaluation of congenital microcephaly



Ashwal et al., 2009

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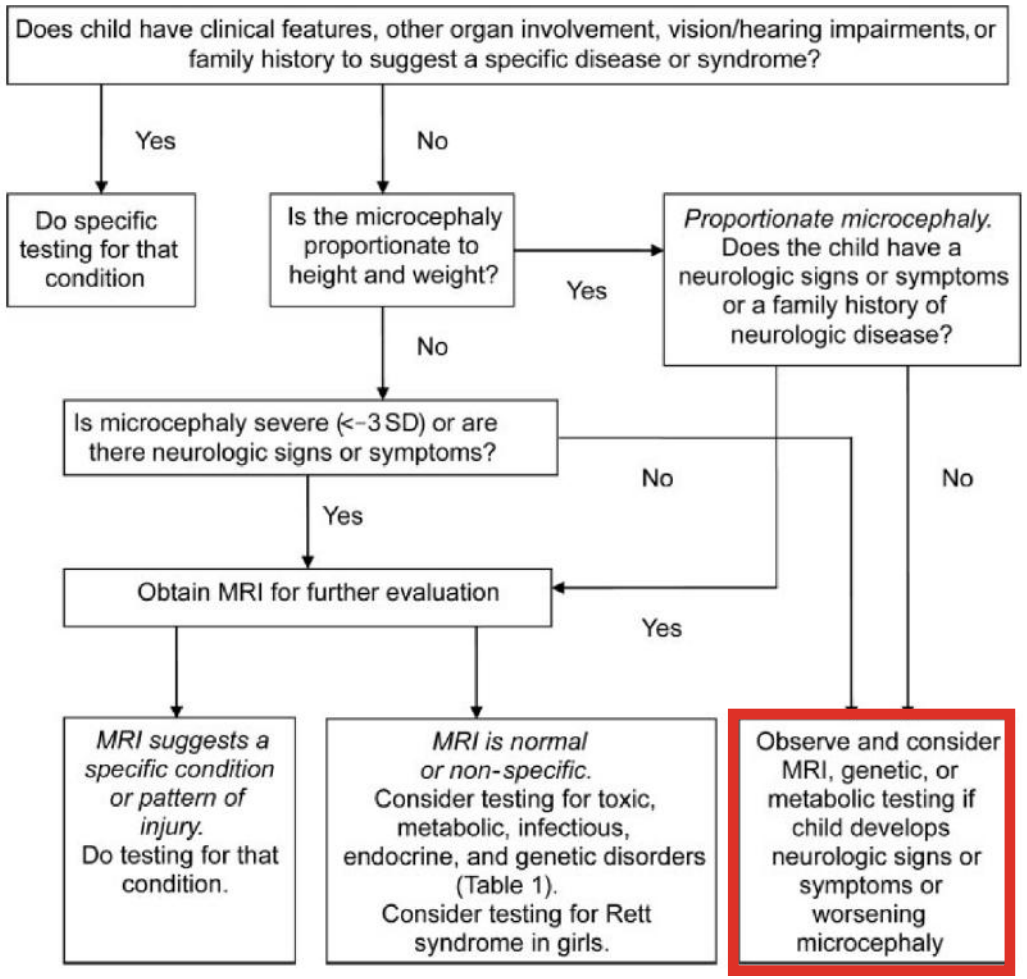
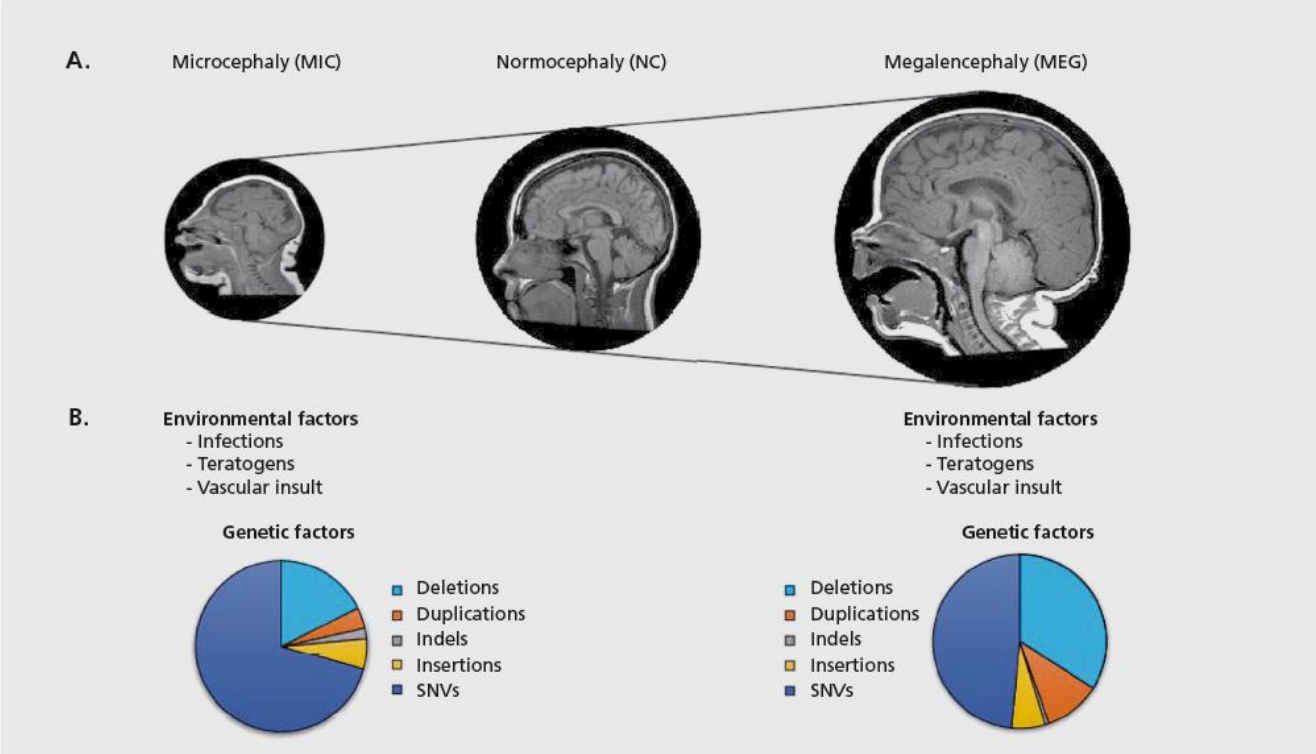
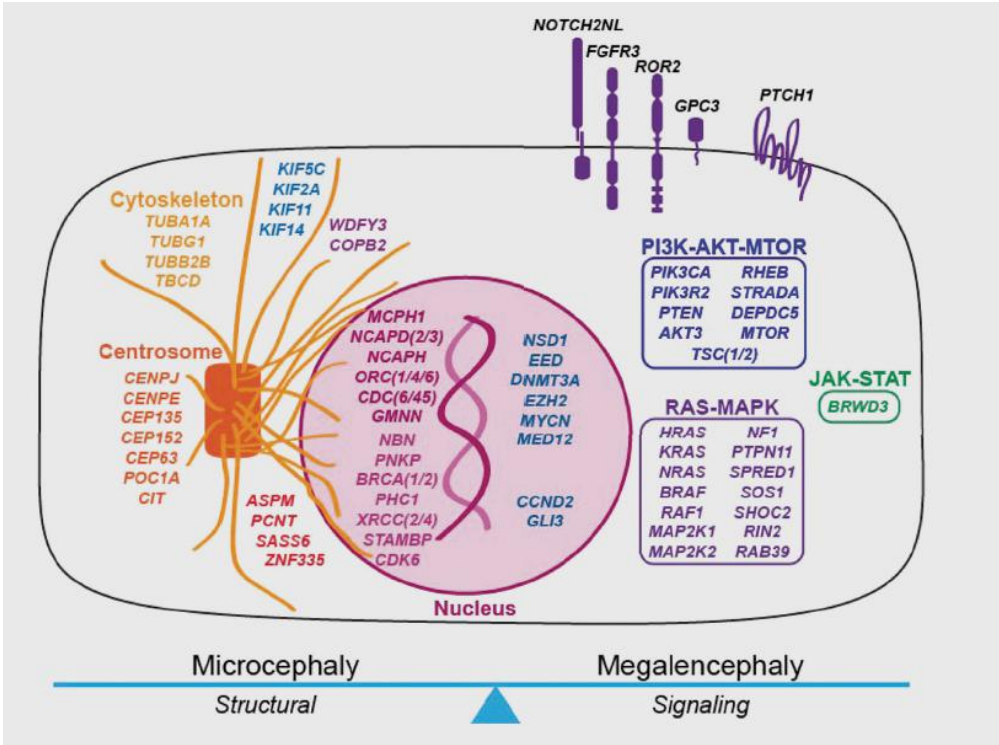
Figure 2 Evaluation of postnatal onset microcephaly



Ashwal et al., 2009

Overview of MIC-MEG causes





Pirozzi, Nelson and Mirzaa, 2019

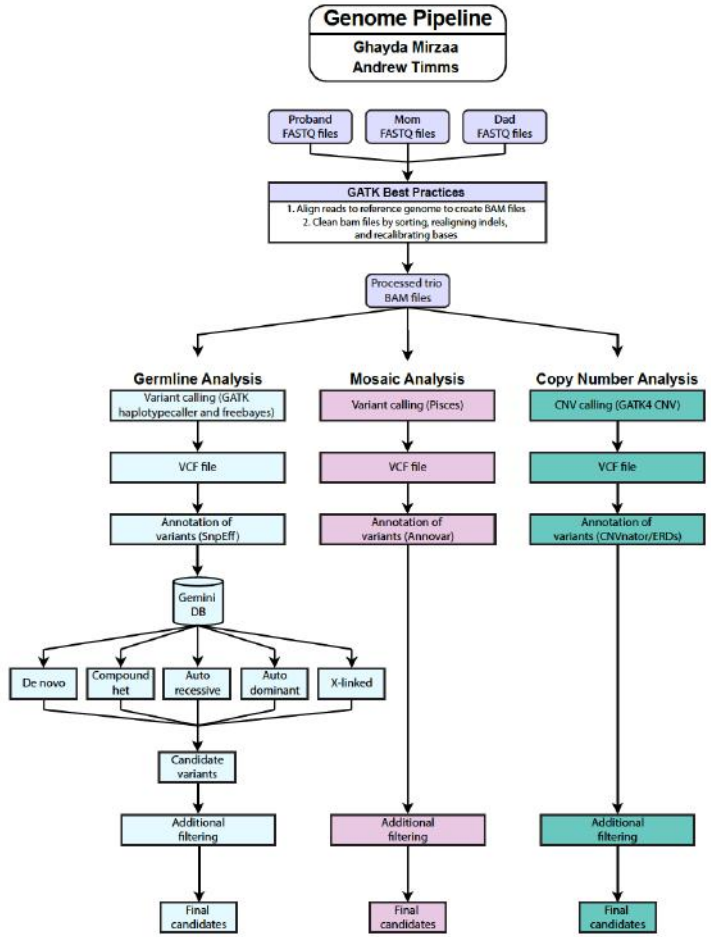
Experimental design

Targeted/Single-gene testing

Exome sequencing
Genome sequencing

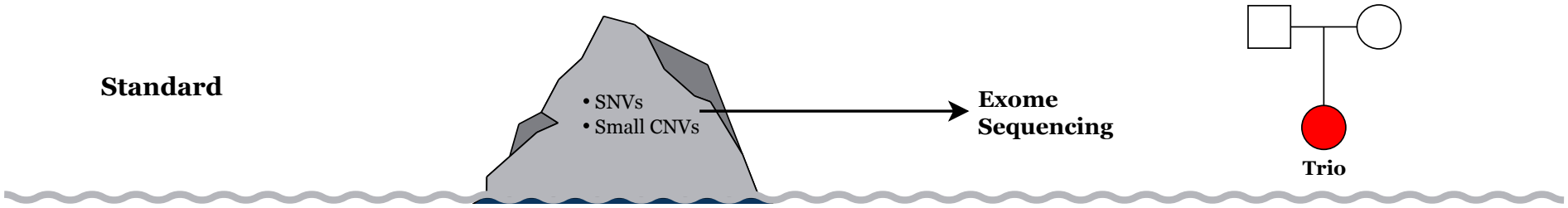
Genotype-phenotype correlation (clinical, MRI, EEG, IHC

Functional assays of MIC genes



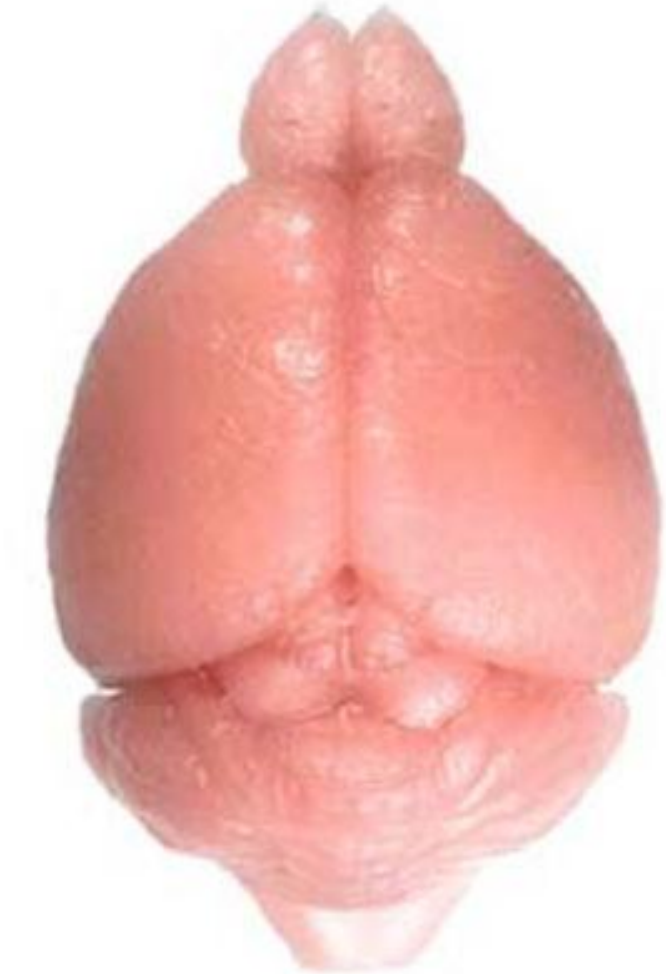
Mirzaa et al., 2016

The genetic landscape of developmental brain disorders

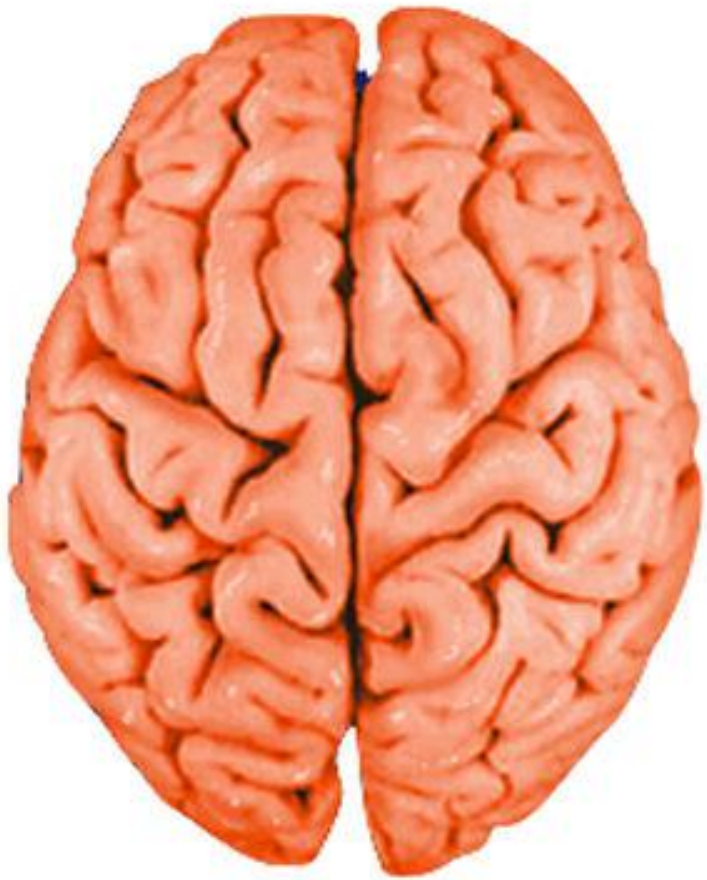


Studying mechanisms of microcephaly

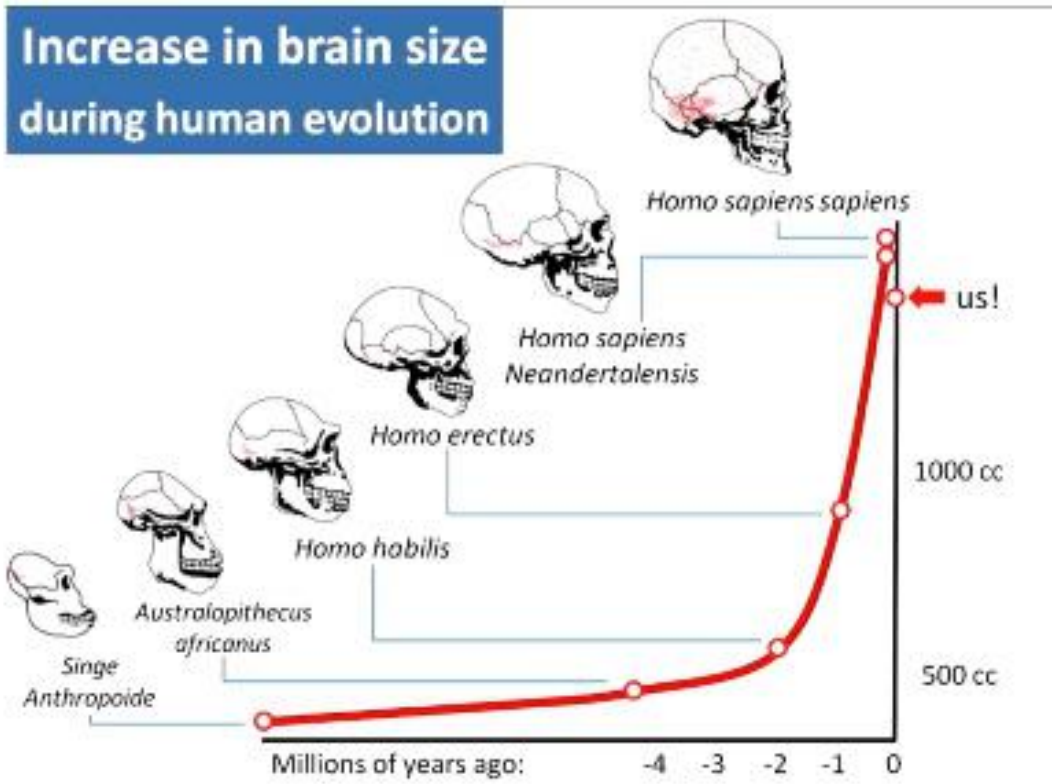
Mouse brain

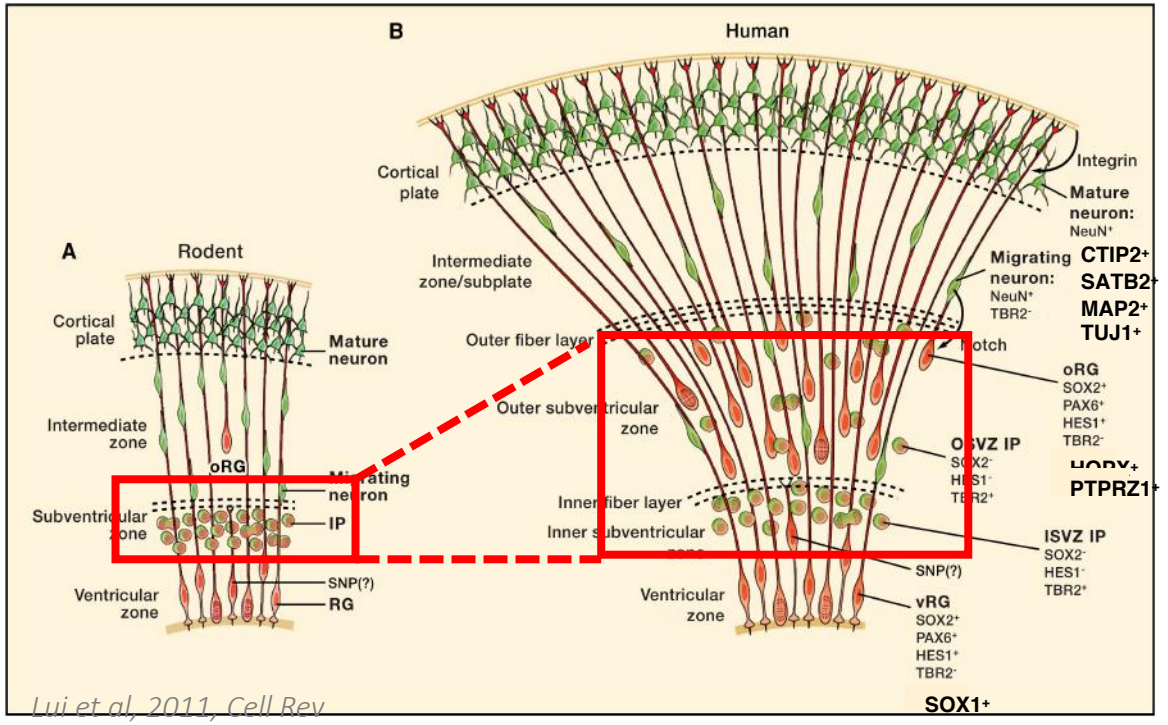


Human brain



Human brain size evolution





Lui et al, 2011, Cell Rev

Jaymaran et al., 2018

Acknowledgements

Patients & their families

CIBR – SCRI
Andrew Timms
William B. Dobyns
Andrew Piacitelli
Lorenzo Gonzalez
Vicky Cheng
Sarah Collins
Zach Thomson
Gaia Ruggeri
Jordan Zeiger

University of Sussex
Diana Alcantara
Mark O'Driscoll

Seattle Children's Hospital
Jeffery Ojemann
Jason Hauptman
Neurosurgical team

Edward Novotny
Russ Saneto
Sandra Poliachik
Jonathan Lopez
James Owens
Christopher Beatty
Pediatric Neurology team
Raj Kapur
Robert Hevner

Seattle Children's Research Institute
CENTER FOR INTEGRATIVE BRAIN RESEARCH

National Institute of Neurological Disorders and Stroke

Jordan's Guardian Angels