# The relevance of multidisciplinary interaction in MCD gene discovery

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Clinical geneticist

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Athens, EPNS 2019 Satellite Meeting

September 17th, 2019

# The perspective of the clinical geneticist on multidisciplinary collaboration in MCD research

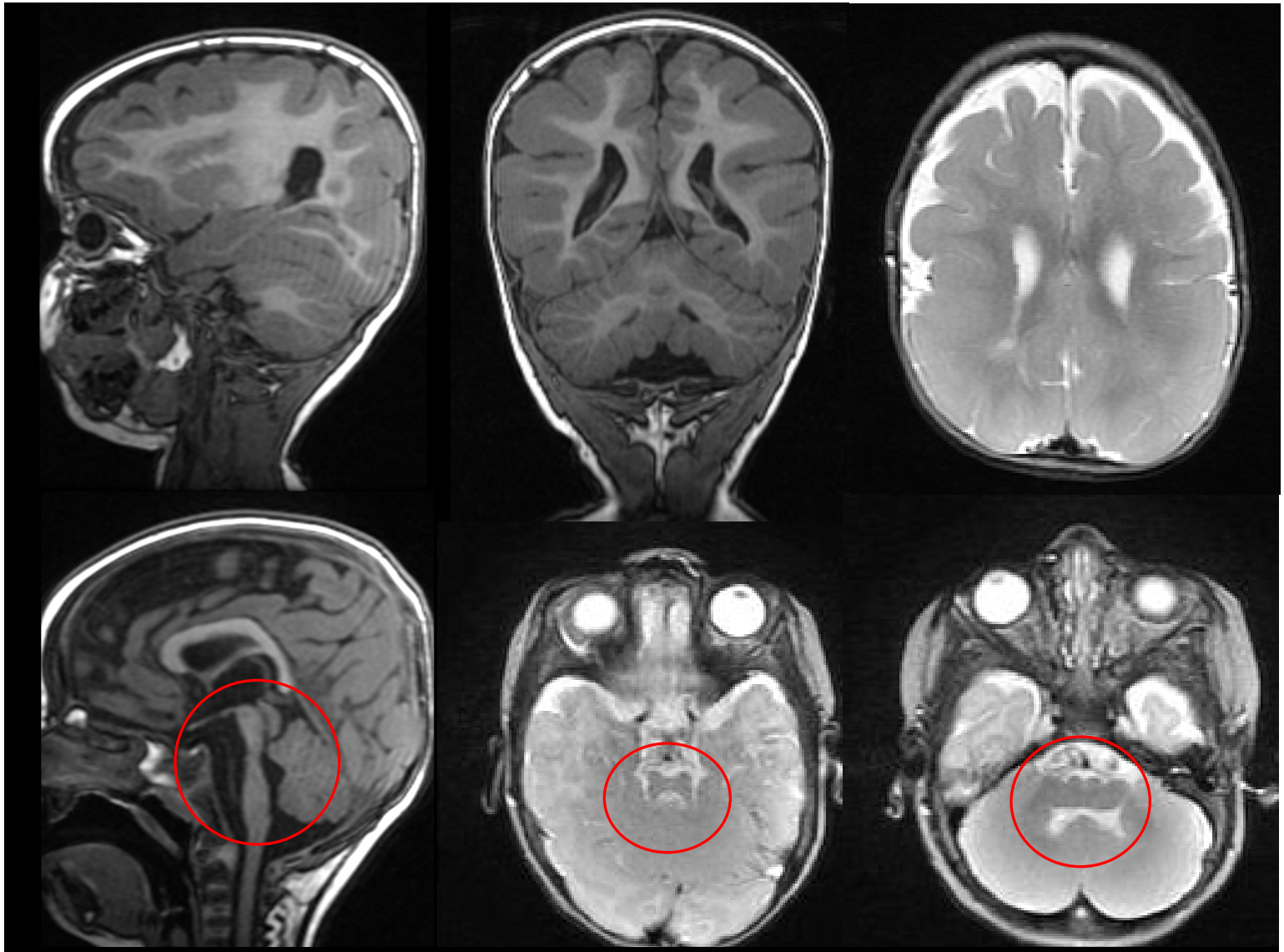
1) Interaction among neurologists, geneticists and neuroradiologists (*MACF1*)

2) Interaction with colleague dysmorphologists (*INTS8* and *INTS1*)

3) Interactions needed … when the exome lets you down (*SMPD4*)

# 1 – Interaction with neurologists, geneticists and radiologists

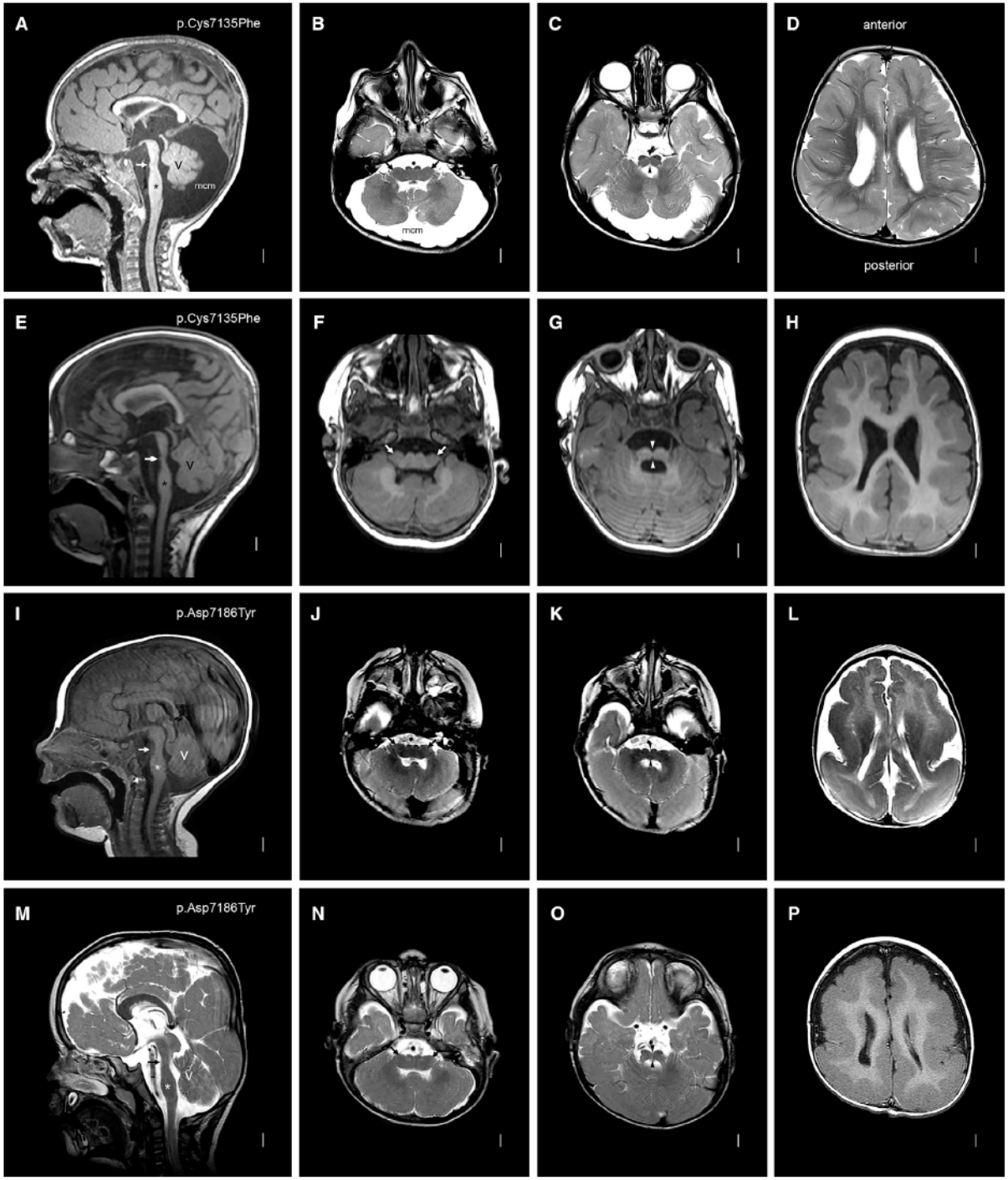
## (Bill Dobyns' multicenter review session)



10 yr male
Seizures
Spasticity
No speech

MRI:
-P>A pachygyria,
-Narrow pons,
-Mildly thick medulla

**Exome:**
**-NM_012090.5(MACF1):c.15530G>T, p.(Cys5177Phe), de novo.**




Bill Dobyns, Dan Doherty:

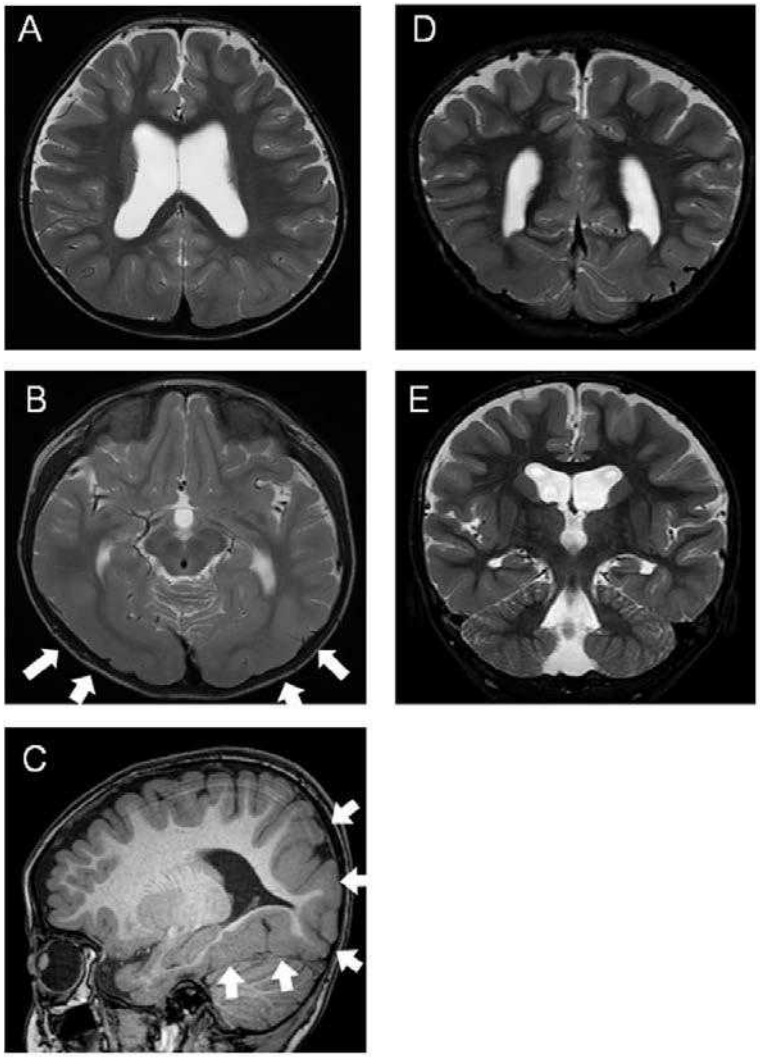
Distinctive and recognizable pattern in 8 unrelated individuals

# Pontine Malformation, Undecussated Pyramidal Tracts, and Regional Polymicrogyria: A New Syndrome

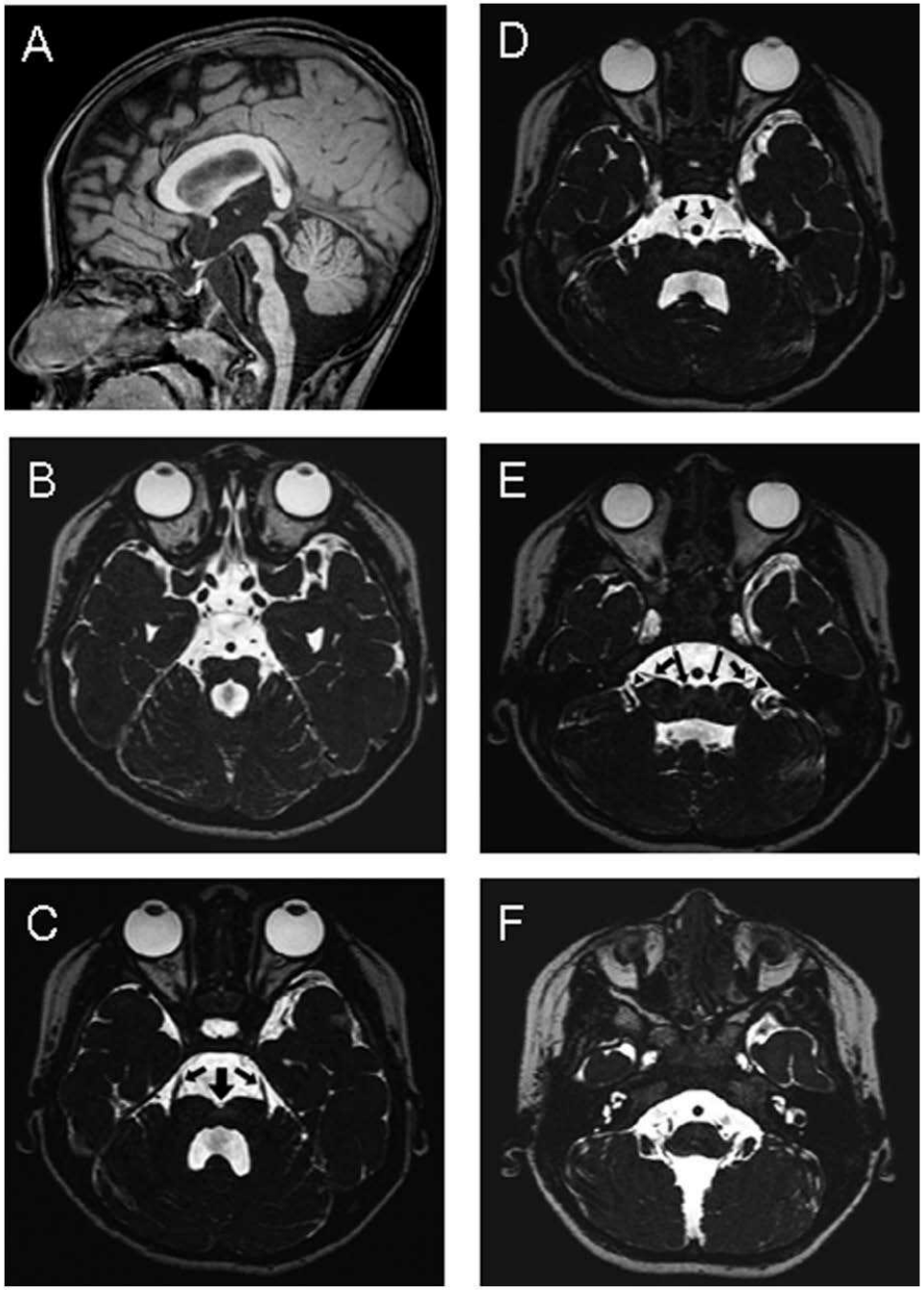
Kaori Irahara MD[a,b,*], Yoshiaki Saito MD PhD[a], Kenji Sugai MD PhD[a], Eiji Nakagawa MD,PhD[a], Takashi Saito MD PhD[a], Hirofumi Komaki MD PhD[a], Yasuhiro Nakata MD PhD[c], Noriko Sato MD PhD[c], Kazumi Baba MD[d], Toshiyuki Yamamoto MD PhD[e], Wai-Man Chan BS[f,g], Caroline Andrews MS[f,g], Elizabeth C. Engle MD[f,g], Masayuki Sasaki MD PhD[a]

Pediatric Neurology 50 (2014) 384–388

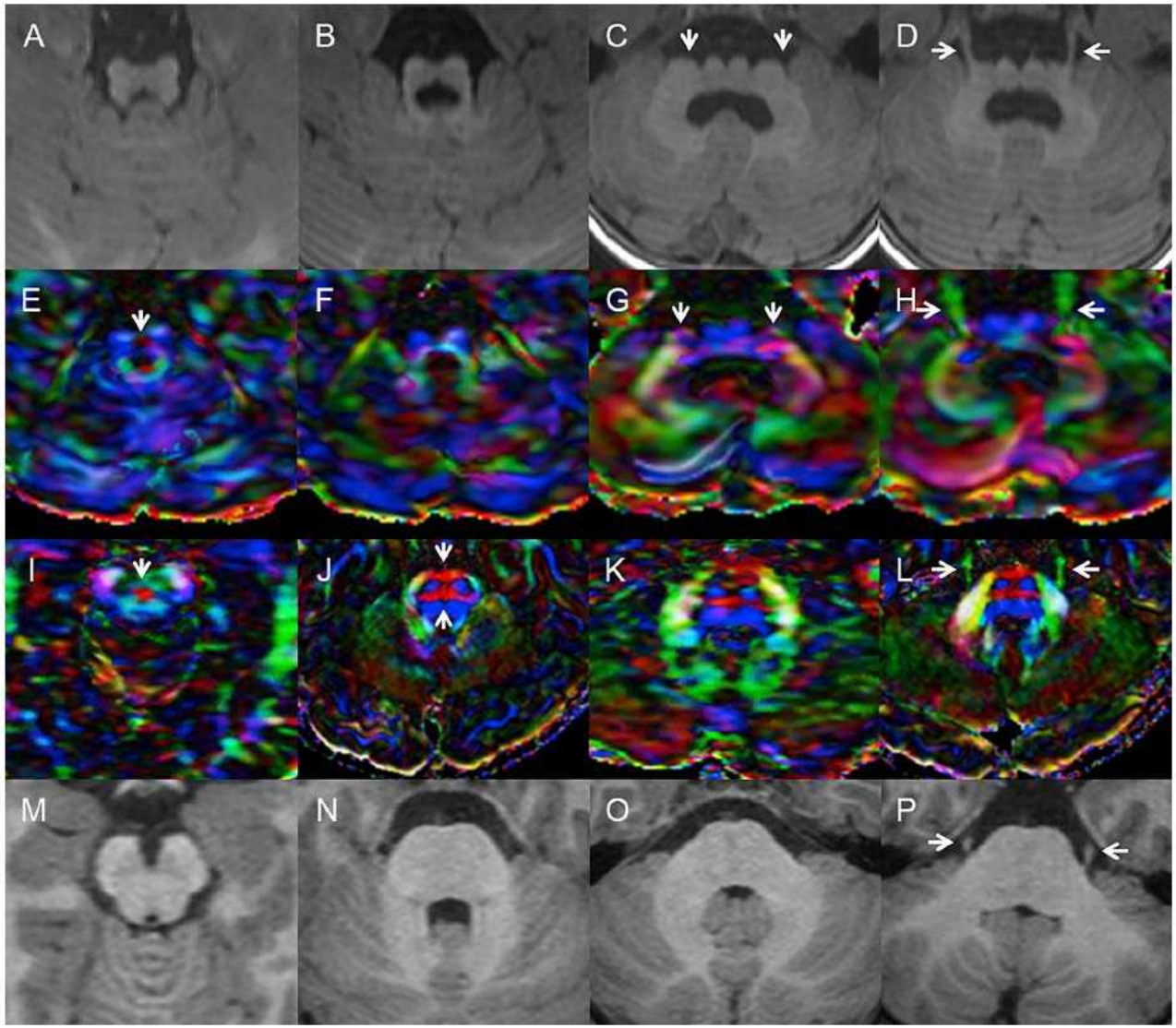
K. Irahara et al. / Pediatric Neurology 50 (2014) 384–388




K. Irahara et al. / Pediatric Neurology 50 (2014) 384–388



# DTI: defect in midline crossing tracts




*patient*

*control*

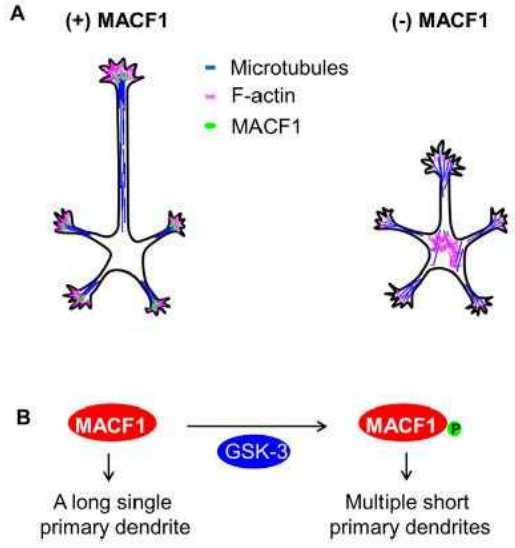
Marjolein Dremmen, Neuroradiologist

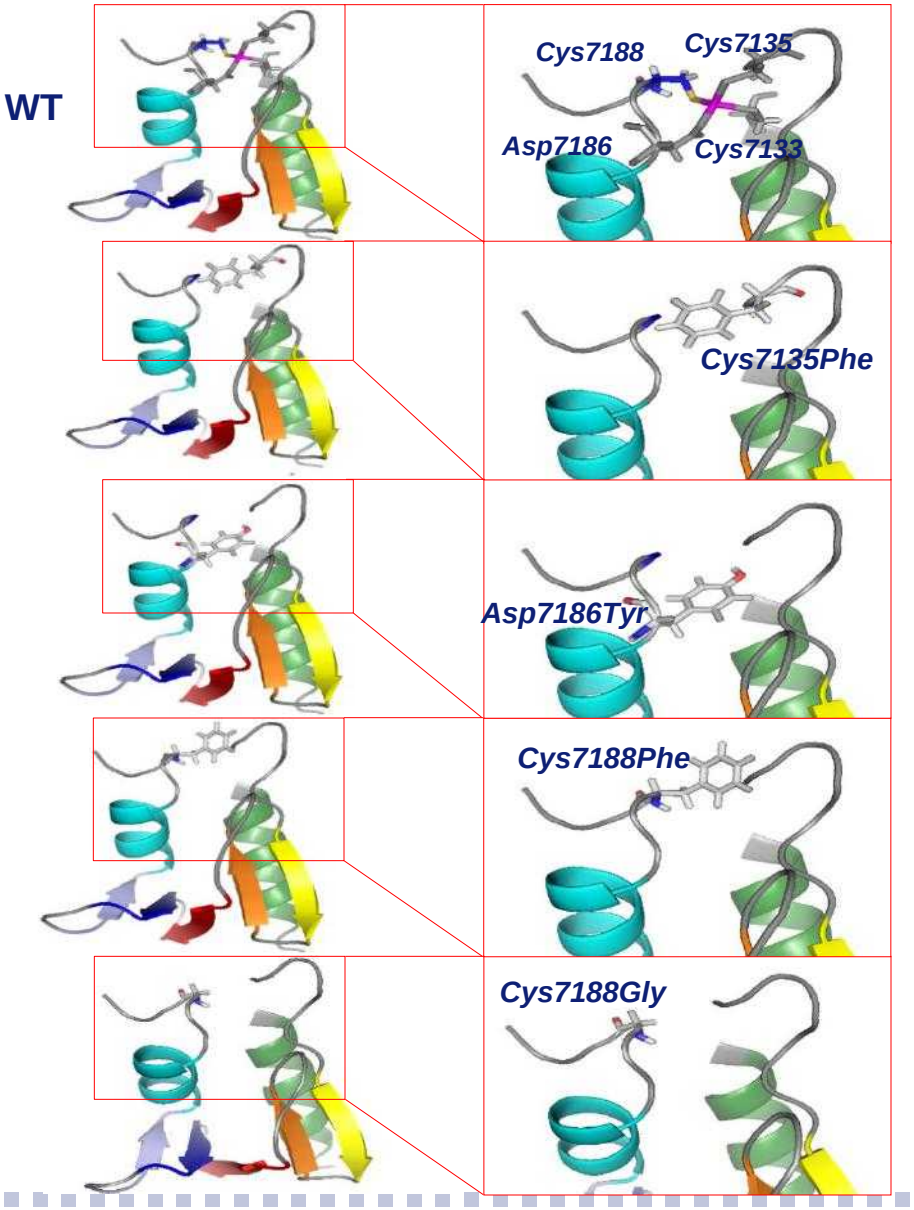
# *MACF1* Mutations Encoding Highly Conserved Zinc-Binding Residues of the GAR Domain Cause Defects in Neuronal Migration and Axon Guidance

William B. Dobyns,[1,2,3,24,*] Kimberly A. Aldinger,[3,24] Gisele E. Ishak,[3,4,24] Ghayda M. Mirzaa,[1,3] Andrew E. Timms,[5] Megan E. Grout,[1] Marjolein H.G. Dremmen,[6,7] Rachel Schot,[8] Laura Vandervore,[1] Marjon A. van Slegtenhorst,[8] Martina Wilke,[8] Esmee Kasteleijn,[8] Arthur S. Lee,[9,10] Brenda J. Barry,[9] Katherine R. Chao,[10] Krzysztof Szczałuba,[11] Joyce Kobori,[12] Andrea Hanson-Kahn,[13,14] Jonathan A. Bernstein,[14] Lucinda Carr,[15] Felice D'Arco,[15] Kaori Miyana,[16] Tetsuya Okazaki,[17] Yoshiaki Saito,[17] Masayuki Sasaki,[18] Soma Das,[19] Marsha M. Wheeler,[20,21] Michael J. Bamshad,[1,20,21] Deborah A. Nickerson,[20,21] University of Washington Center for Mendelian Genomics,[21] Center for Mendelian Genomics at the Broad Institute of MIT and Harvard,[10] Elizabeth C. Engle,[9,10,22,23] Frans W. Verheijen,[8] Dan Doherty,[1,3,24] and Grazia M.S. Mancini[8,24,*]

The American Journal of Human Genetics *103*, 1–13, December 6, 2018

Erasmus MC



# 2 – Interaction with dysmorphologists: reverse phenotyping

SPECIAL ARTICLE

Human Mutation
OFFICIAL JOURNAL
HGVS
HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

**Next-Generation Sequencing Demands Next-Generation Phenotyping**

Raoul C.M. Hennekam[1]* and Leslie G. Biesecker[2]

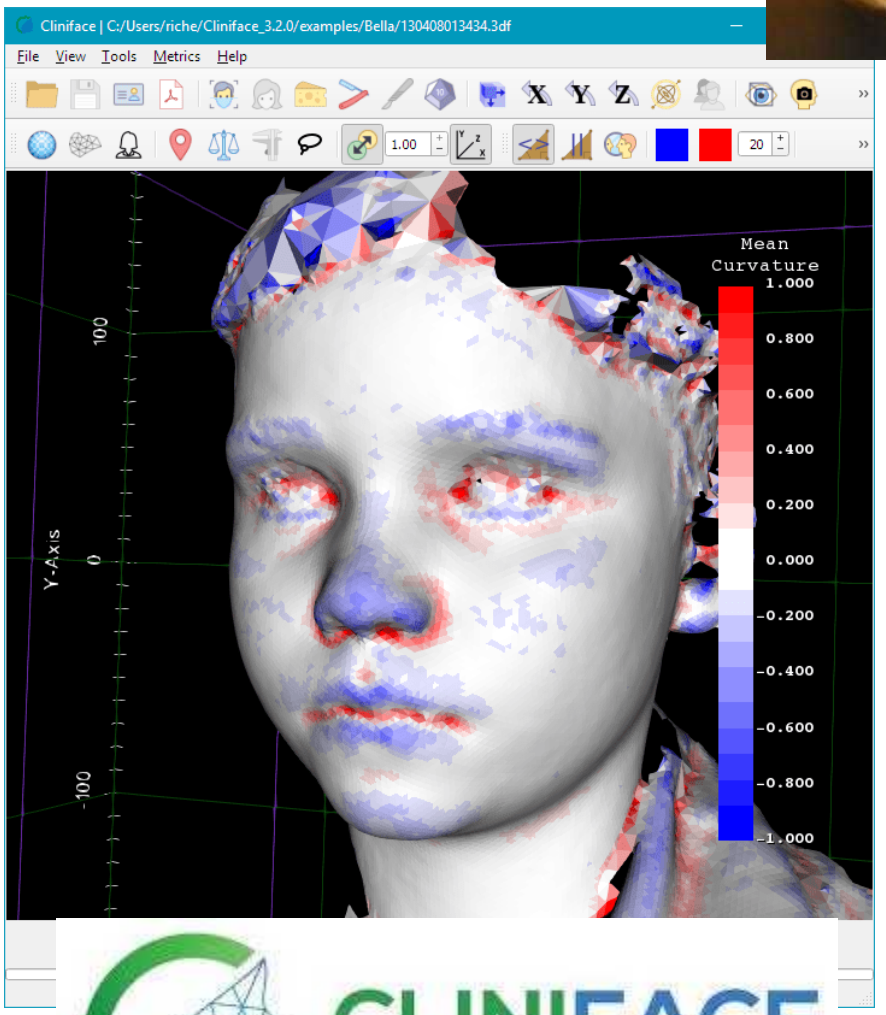
[1]*Department of Pediatrics and Translational Genetics, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands;*
[2]*Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland*

For the Deep Phenotyping Special Issue
Received 27 September 2011; accepted revised manuscript 8 January 2012.
Published online 13 February 2012 in Wiley Online Library (www.wiley.com/humanmutation).DOI: 10.1002/humu.22048

*Persona* = Latin word for the ancient resonating *(= per-sonare)* theater masks

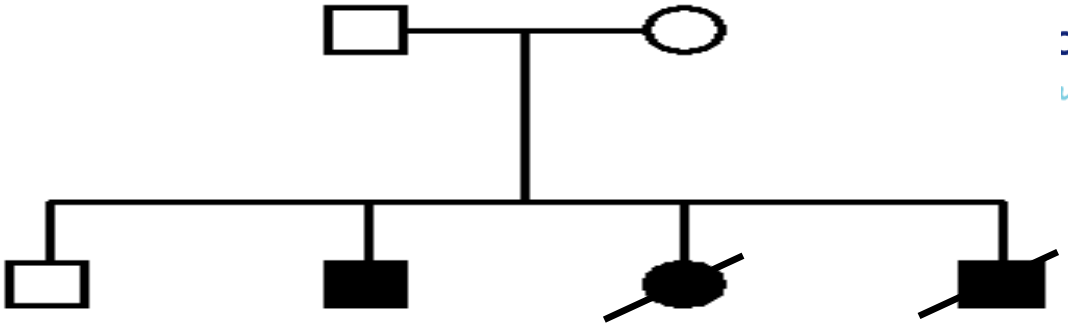
# Who needs the clinical dysmorphologist?

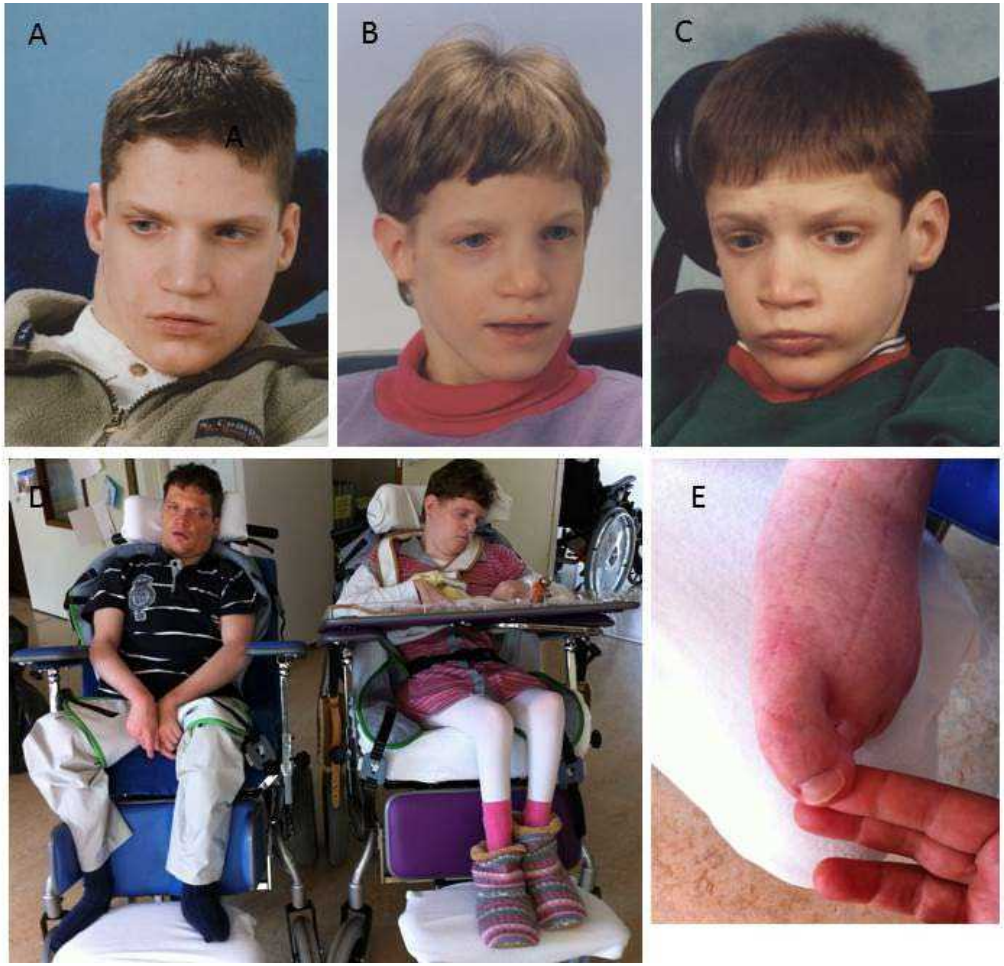

Face2gene




CLINIFACE
UNLOCKING FACIAL CLUES

*1983, 2003, 2013, 2017*






**Face dysmorphism**

**Abnormal toes**

Severe DD

Epilepsy

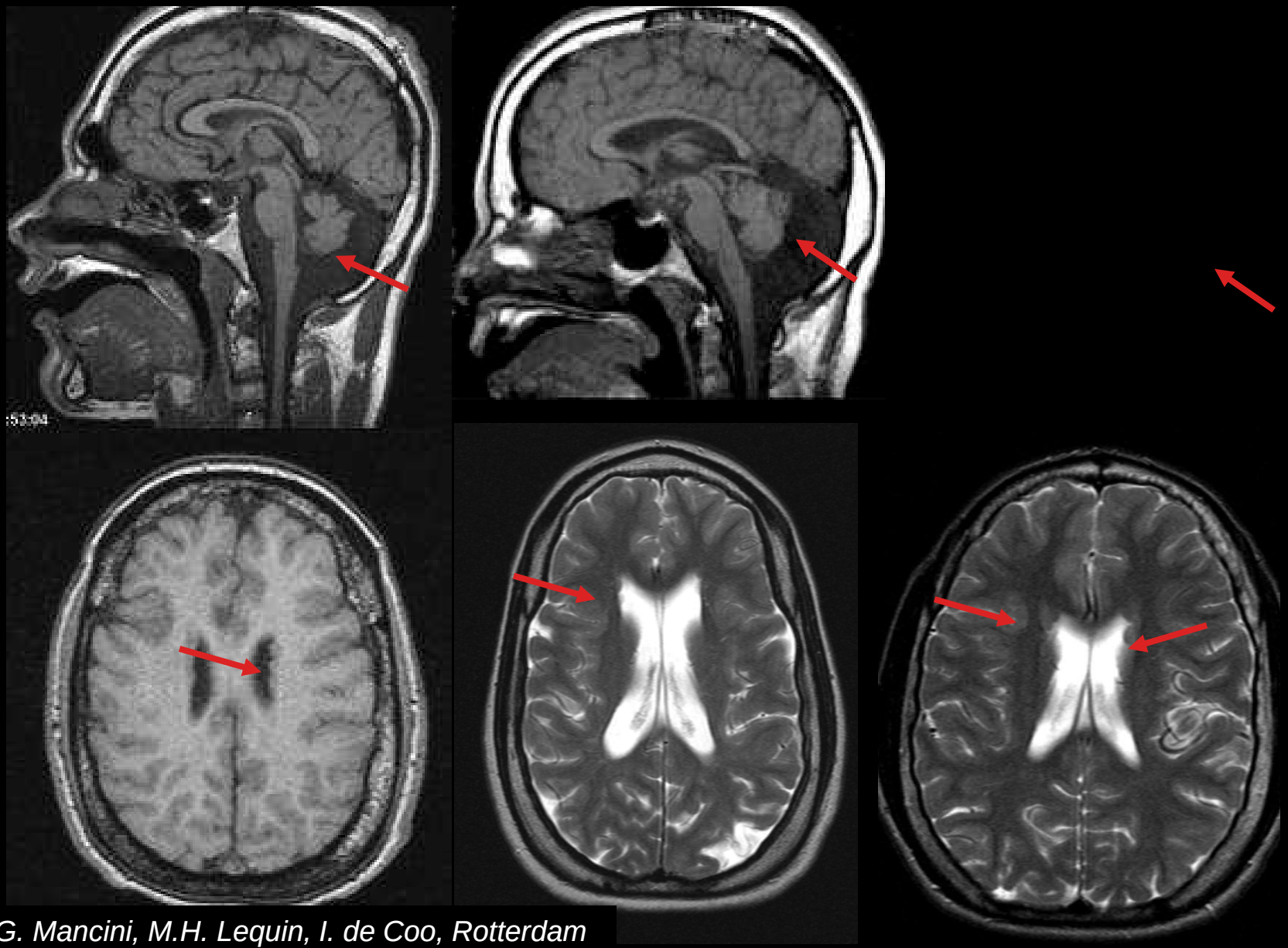
Blindness

Absent speech

Spasticity

*J. Hoogeboom, G. Mancini, R. Oegema*

# 2003, MRI: Cerebellar Hypoplasia and Periventricular Nodular Heterotopia

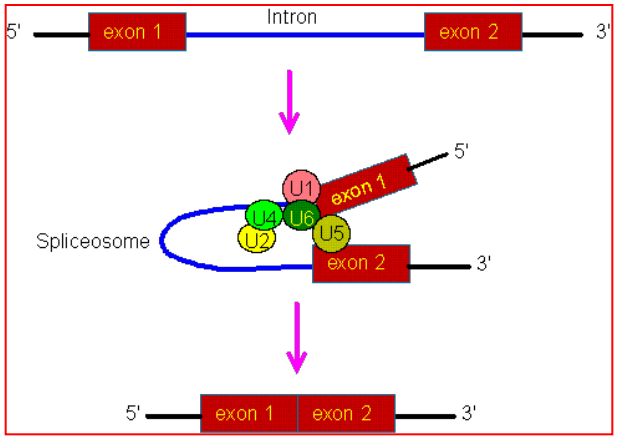


*G. Mancini, M.H. Lequin, I. de Coo, Rotterdam*

# WGS: INT, Integrator complex subunits

Oegema R et al.

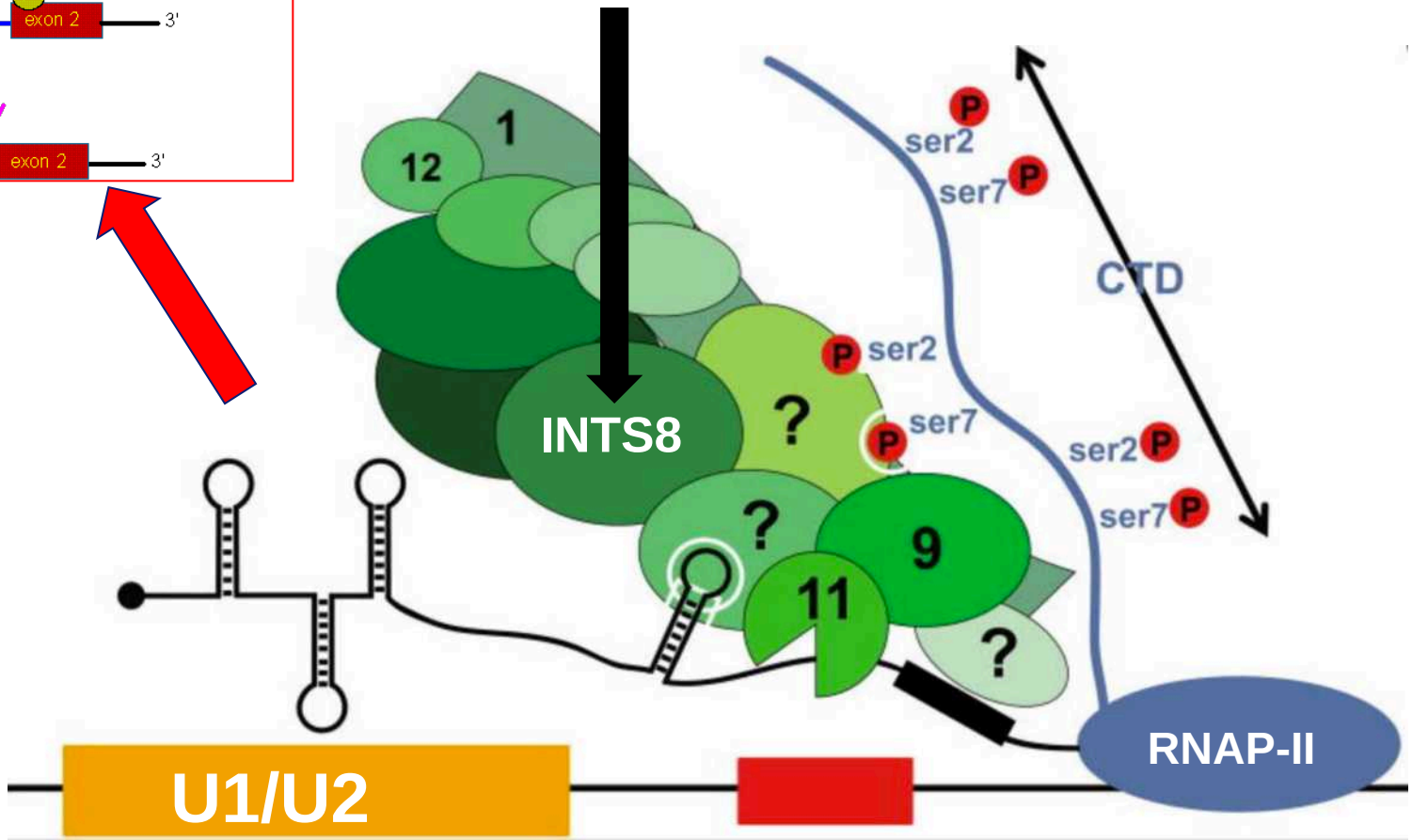
PLOS Genetics | https://doi.org/10.1371/journal.pgen.1006809 May 25, 2017




***INTS8:***

**- c.893A>G, p.Asp298Gly + affecting splicing**

**- c.2917_2925del, p.Glu972_Leu974del**




*D. Baillat and E. Wagner, Cell, 2005*

# Distinctive dysmorphic features

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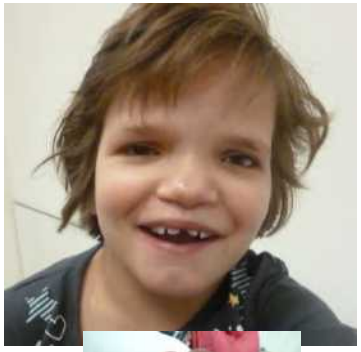
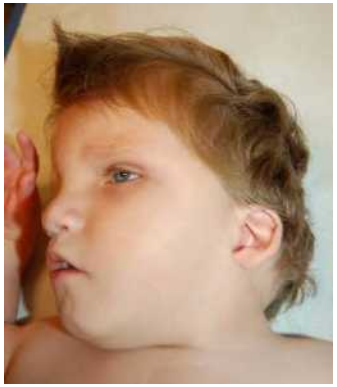
**Fam 1**



**Fam 2**

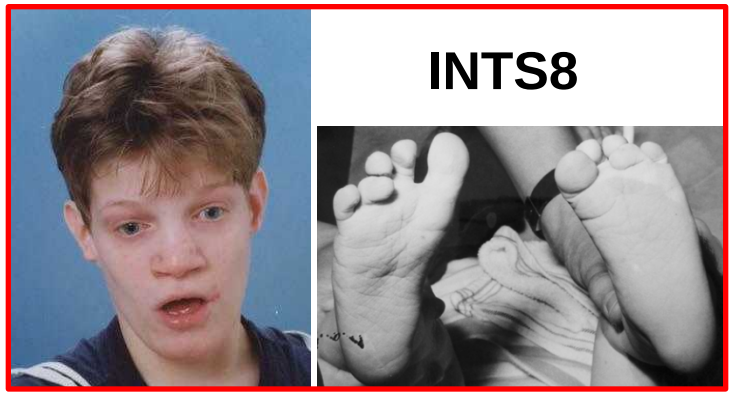
**Fam 3**

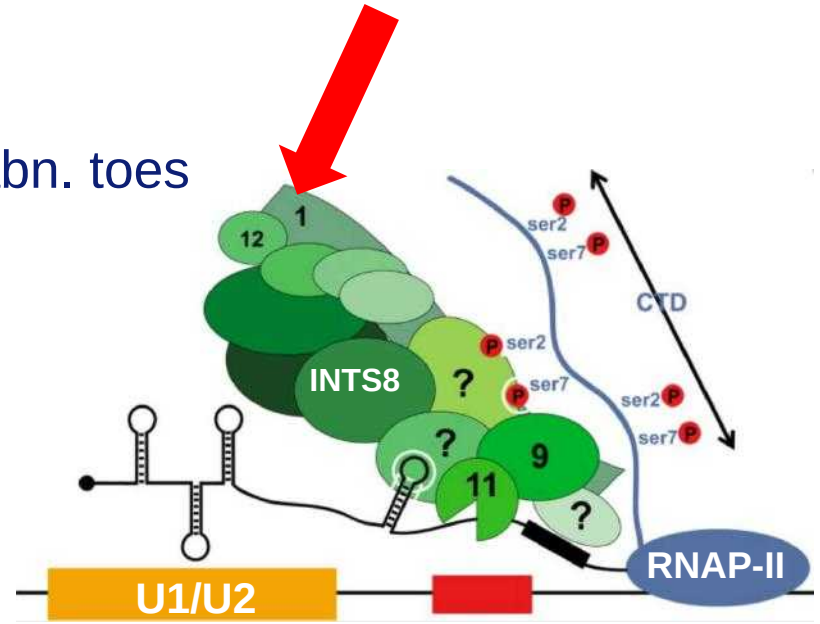


*Courtesy Dr A.S. Brooks*

***WES:***
***INTS1 / KIAA1440***
**c.5351C>A,**
**p.Ser1784***
**homozygous**

Severe ID, SS, cataract, dysmorphism, abn. toes




*WES: Marjon van Slegtenhorst, Martina Wilke*

# Integrator in cortical development

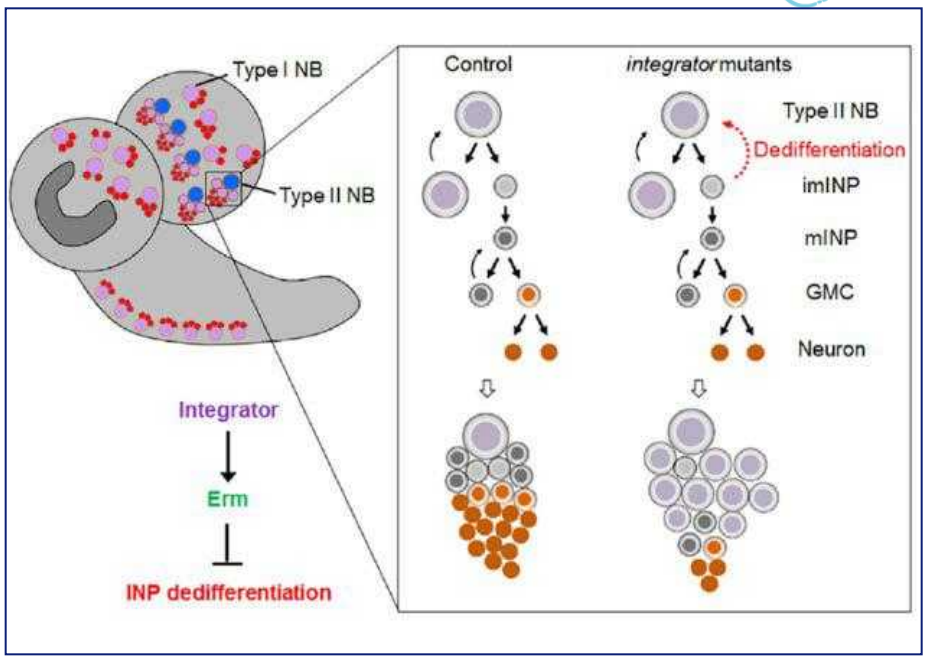
Erasmus MC

Cell Reports
Report

April 2019

**The Integrator Complex Prevents Dedifferentiation of Intermediate Neural Progenitors back into Neural Stem Cells**

Yingjie Zhang,[1,2,11] Chwee Tat Koe,[3,11] Ye Sing Tan,[1,11] Joses Ho,[4] Patrick Tan,[3,5,6,7] Fengwei Yu,[1,2,8] Wing-Kin Sung,[3,10] and Hongyan Wang[1,2,9,12,*]




- Neural progenitor-specific depletion of INTS1 and INTS8 induces ectopic type II neuroblasts and increased de-differentiation to INP
- Possibly, the PNH observed in affected individuals results from abnormal NP differentiation (rather than migration)

European Journal of Human Genetics
https://doi.org/10.1038/s41431-018-0298-9

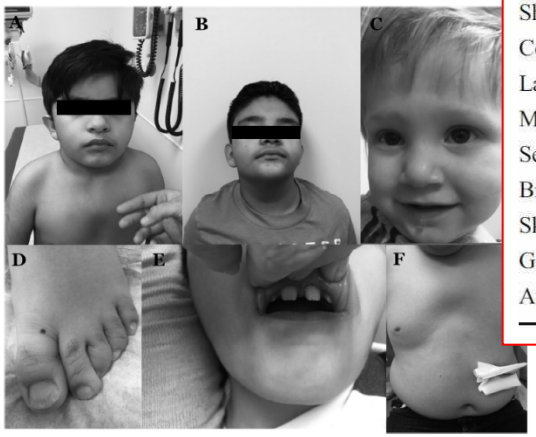
ESHG

ARTICLE

## Biallelic sequence variants in *INTS1* in patients with developmental delays, cataracts, and craniofacial anomalies

Max Krall[1] · Stephanie Htun[1] · Rhonda E. Schnur[2] · Alice S. Brooks[3] · Laura Baker[4] · Alejandra de Alba Campomanes[5] · Ryan E. Lamont[6] · Karen W. Gripp[4] · Care 4 Rare Canada Consortium · Dina Schneidman-Duhovny[7,8] · A. Micheil Innes[6] · Grazia M. S. Mancini[3] · Anne M. Slavotinek[1]

Received: 22 March 2018 / Accepted: 25 October 2018


Erasmus MC

Journal of Molecular Neuroscience
https://doi.org/10.1007/s12031-019-01393-x

Check for updates

## Biallelic *INTS1* Mutations Cause a Rare Neurodevelopmental Disorder in Two Chinese Siblings

Xuemin Zhang[1] · Yajian Wang[2] · Fang Yang[3] · Jiulai Tang[1] · Xiaoyan Xu[1] · Li Yang[1] · Xiu-An Yang[3] · De Wu[1]

Received: 22 April 2019 / Accepted: 29 July 2019


**Table 1** Clinical phenotype of individuals with CCFDN and mutations in INTS1

| | Our patients | | Reported patients with *INTS1* variants | Reported patients with *INTS8* variants | CCFDN patients |
|---|---|---|---|---|---|
| Patients | Proband | Brother | Eight patients | Three patients | |
| Diagnostic age | 5 years | 11 years | 21 months~19 years | 30~35 years | > 1 year |
| Gene, inheritance | *INTS1*, *AR* | *INTS1*, *AR* | *INTS1*, *AR* (8/8) | *INTS8*, AR (3/3) | *CTDP1*, AR |
| Cataract | + | + | + (8/8) | − (3/3) | + |
| Facial dysmorphism | + | + | + (8/8) | + (3/3) | + |
| Neuropathy (peripheral nervous) | − | − | NR | NR | + |
| Short stature | + | + | + (8/8) | + (3/3) | + |
| Cognitive delay | Severe | Severe | Severe | Severe (3/3) | Mild |
| Language development | No | No | No (8/8)* | No (3/3) | Yes |
| Motor impairment | + | + | + (7/8) | + (3/3) | + |
| Seizures | − | − | − (7/8) | + (3/3) | NR |
| Brain MRI scan | − | − | + (3/8) | + (3/3) | +/− |
| Skeletal/limb positive symptoms | + | + | + (5/8) | + (3/3) | + |
| Genitourinary | + | + | NR | NR | +/− |
| Autism spectrum disorder | + | − | + (4/5)[#] | NR | NR |

*On-line 19 Aug 2019*

# *CCFDN is caused by mutation in CTDP1*

Erasmus MC

## Partial deficiency of the C-terminal-domain phosphatase of RNA polymerase II is associated with congenital cataracts facial dysmorphism neuropathy syndrome

Raymonda Varon[1,16], Rebecca Gooding[2,16], Christina Steglich[1], Lorna Marns[2], Hua Tang[3], Dora Angelicheva[2], Kiau Kiun Yong[2], Petra Ambrugger[1], Anke Reinhold[1,4], Bharti Morar[2], Frank Baas[5], Marcel Kwa[5], Ivailo Tournev[6], Velina Guerguelcheva[6], Ivo Kremensky[7], Hanns Lochmüller[8], Andrea Müllner-Eidenböck[9], Luciano Merlini[10], Luitgard Neumann[1], Joachim Bürger[1,11], Maggie Walter[8], Kathryn Swoboda[12], P K Thomas[13], Arpad von Moers[4], Neil Risch[14,15] & Luba Kalaydjieva[2]

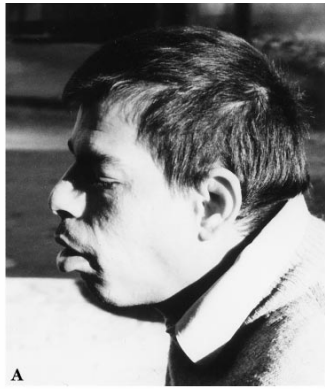
**Congenital cataracts facial dysmorphism neuropathy (CCFDN) syndrome (OMIM 604168) is an autosomal recessive developmental disorder that occurs in an endogamous group of Vlax Roma (Gypsies; refs. 1–3). We previously localized the**

Developmental abnormalities include congenital cataracts and microcorneae, hypomyelination of the peripheral nervous sys impaired physical growth, delayed early motor and intellectual d opment, facial dysmorphism and hypogonadism[1–3]. Central ner

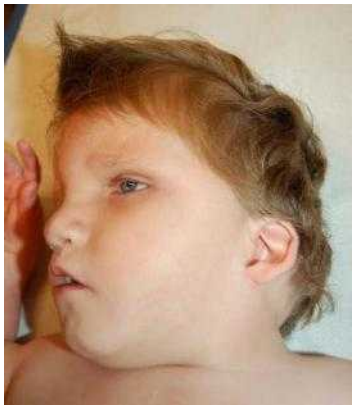
*Trends in Biochemical Sciences* May 2015, Vol. 40, No. 5

D. Baillat & E. Wagner

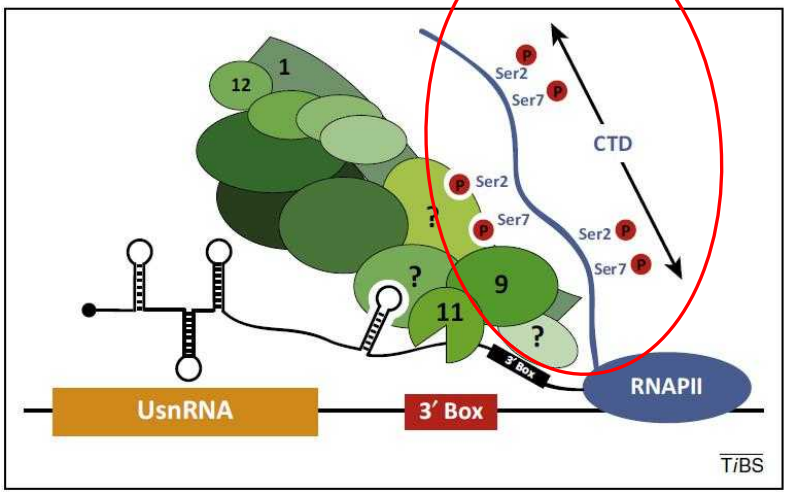
**CTDP1**



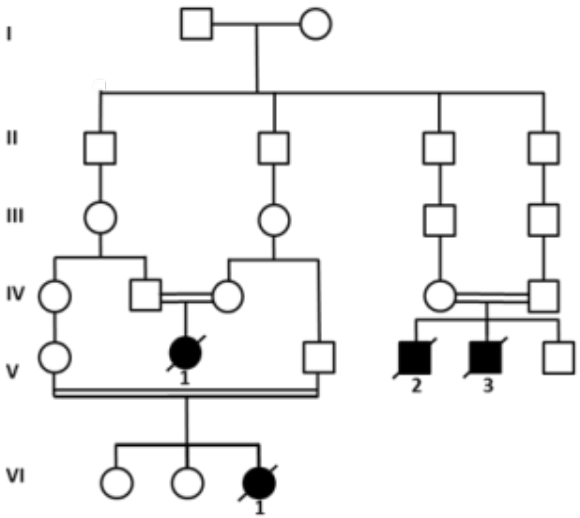

**INTS1**



**INTS8**



# 3 – Interactions…when the exome lets you down



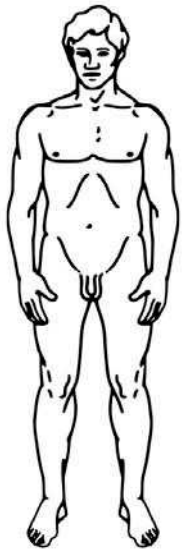

Since 2002 in follow-up, large consanguineous pedigree, all affected share the **same phenotype:**

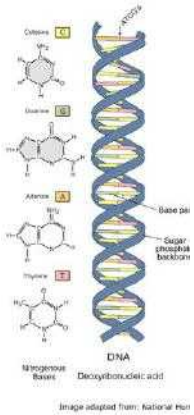
- Primary microcephaly with simplified gyral pattern and hypomyelination
- No developmental milestones
- Congenital arthrogryposis of hands and feet
- Early death

- **Linkage analysis**: one large region of homozygosity on chrom 2q22, shared by all affected > MUTATION LOCUS (300 genes)

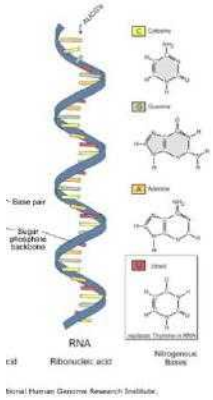
**2016 Exome sequencing: no mutation!**

# 2017 > RNAseq






DNA



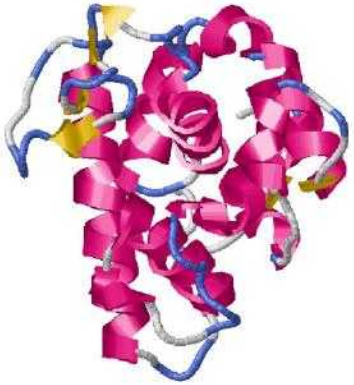

RNA

REVIEWS

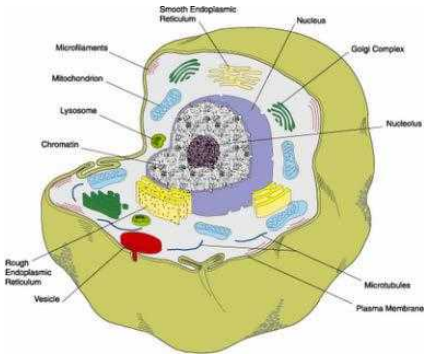
APPLICATIONS OF NEXT-GENERATION SEQUENCING

Translating RNA sequencing into clinical diagnostics: opportunities and challenges

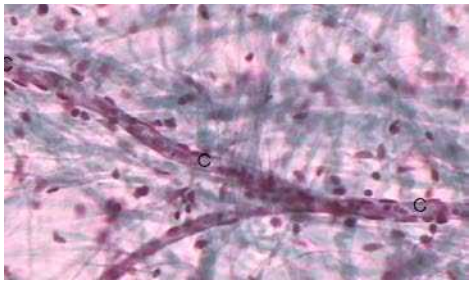
Sara A. Byron[1], Kendall R. Van Keuren-Jensen[2], David M. Engelthaler[3], John D. Carpten[4] and David W. Craig[2]



PROTEIN




CELL

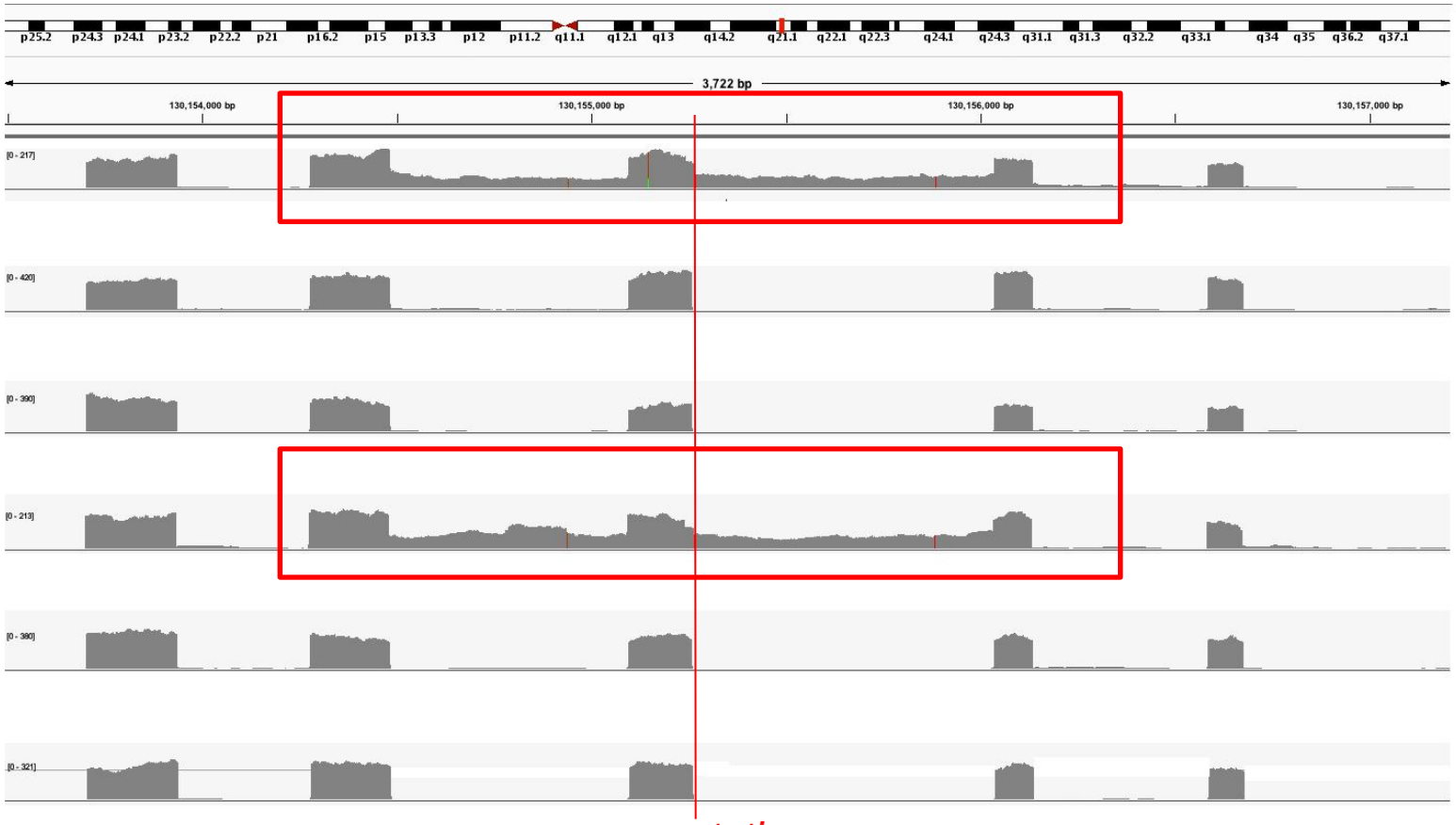


TISSUE

ORGAN

# RNASeq reads from ROH on chromosome 2q22

A **single nucleotide mutation in INTRON 14 of *SMPD4*** (chr2:130,155,051 G>T) creates a mutant splice acceptor site, close to exon 15




*Sample*

*#2*

*#1*

*#3*

*#4*

*#5*

*#6*

*mutation*

*17* *16* *15* *14* *13* **SMPD4 exons**

***Dr. Maarten Fornerod, Cell Biology***

# *SMPD4* recessive variants cause the same phenotype

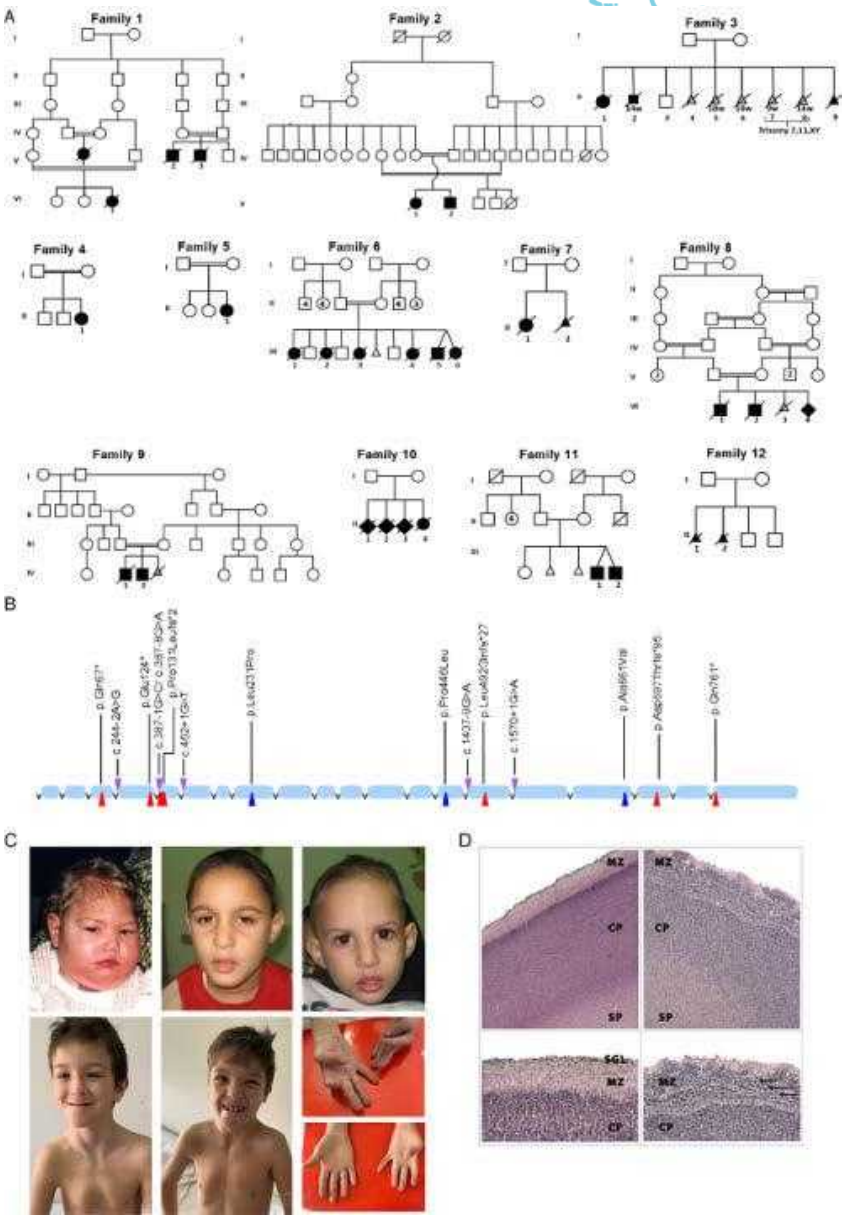
*Open access databases of genomic variants: https://www.genematcher.org/*

ARTICLE

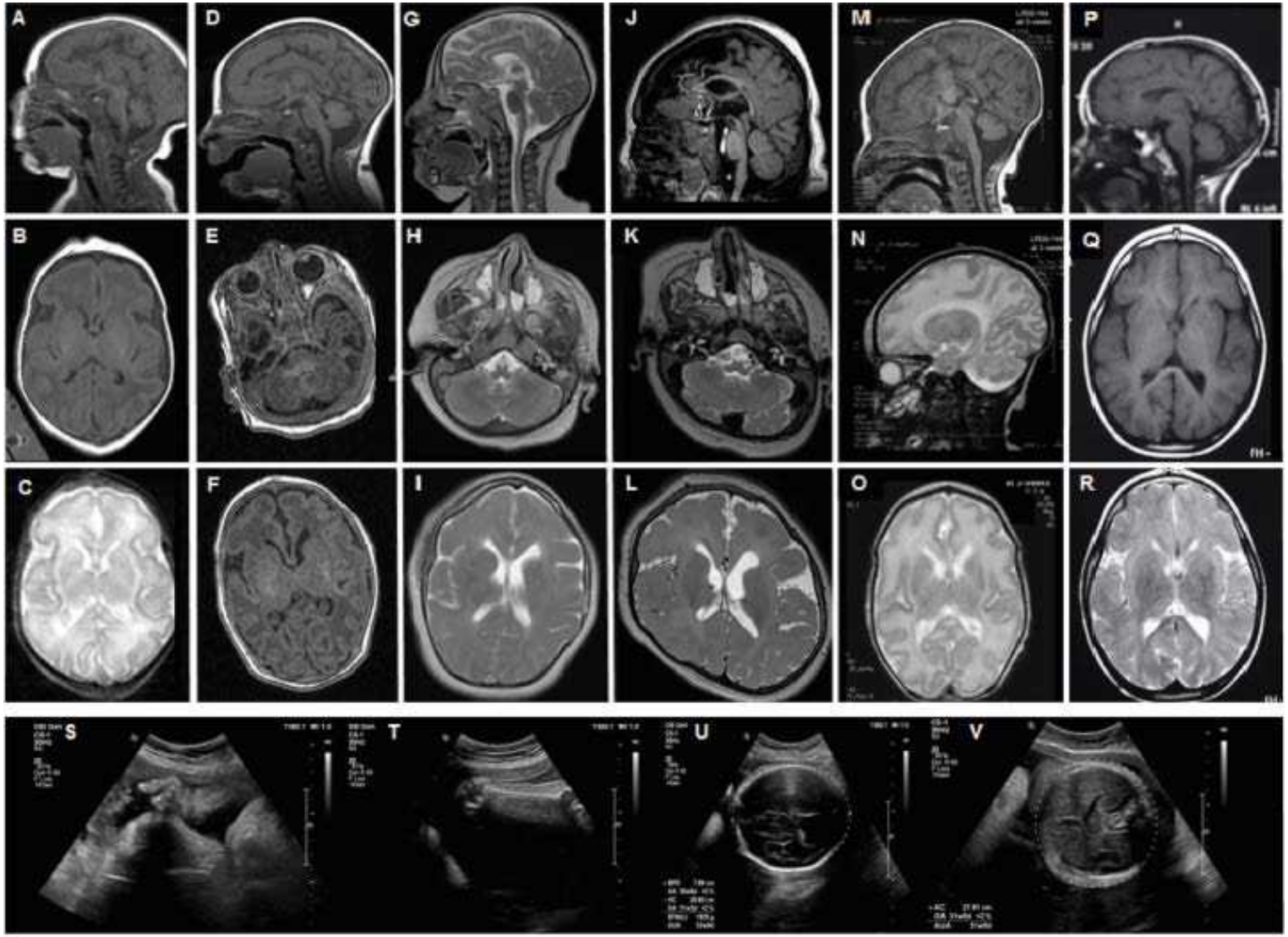
Loss of SMPD4 Causes a Developmental Disorder Characterized by Microcephaly and Congenital Arthrogryposis

Pamela Magini,[1,40] Daphne J. Smits,[2,40] Laura Vandervore,[2,3] Rachel Schot,[2] Marta Columbaro,[4] Esmee Kasteleijn,[2] Mees van der Ent,[5] Flavia Palombo,[6] Maarten H. Lequin,[7] Marjolein Dremmen,[8] Marie Claire Y. de Wit,[9] Mariasavina Severino,[10] Maria Teresa Divizia,[11] Pasquale Striano,[12,13] Natalia Ordonez-Herrera,[14] Amal Alhashem,[15,16] Ahmed Al Fares,[15,16] Malak Al Ghamdi,[17] Arndt Rolfs,[14] Peter Bauer,[14] Jeroen Demmers,[18] Frans W. Verheijen,[2] Martina Wilke,[2] Marjon van Slegtenhorst,[2] Peter J. van der Spek,[19] Marco Seri,[20] Anna C. Jansen,[3,21] Rolf W. Stottmann,[22] Robert B. Hufnagel,[23] Robert J. Hopkin,[22,24] Deema Aljeaid,[25] Wojciech Wiszniewski,[26,27] Pawel Gawlinski,[27] Milena Laure-Kamionowska,[28] Fowzan S. Alkuraya,[29] Hanah Akleh,[30] Valentina Stanley,[31] Damir Musaev,[31] Joseph G. Gleeson,[31] Maha S. Zaki,[32] Nicola Brunetti-Pierri,[33,34] Gerarda Cappuccio,[33,34] Bella Davidov,[35] Lina Basel-Salmon,[35,36,37] Lily Bazak,[35] Noa Ruhrman Shahar,[35] Aida Bertoli Avella,[12] Ghayda M. Mirzaa,[38,39] William B. Dobyns,[38] Tommaso Pippucci,[1] Maarten Fornerod,[5,41] and Grazia M.S. Mancini[2,41,*]

*AJHG 5 Sept 2019, online*

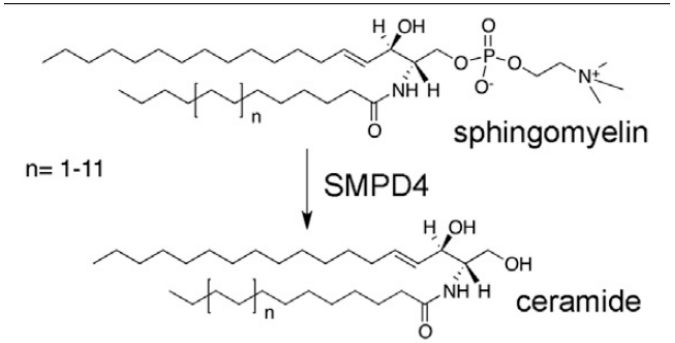


# SMPD4 defect: Microcephaly with simplified gyral pattern and hypomyelination

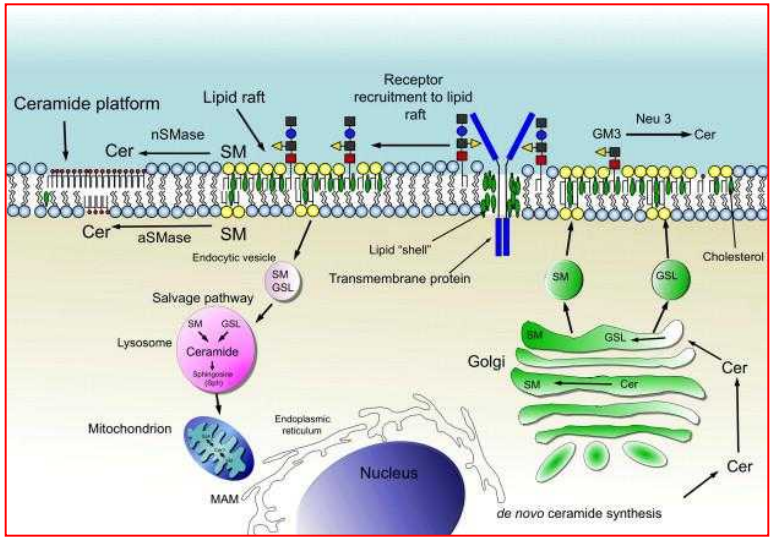


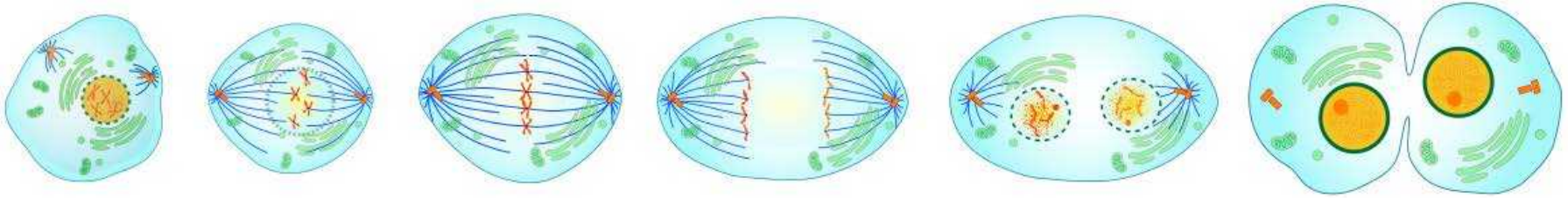

Magini P, Smits D et al AJHG Sept 2019

# Link between SMPD4, membrane and cell proliferation?




- SMPD4 is a neutral sphingomyelinase in the ER membrane
- Sphingomyelin is particularly abundant in de Outer Nuclear Membrane
- Dynamics of Nuclear Membrane important during cell division (mitosis)



*https://commons.wikimedia.org/wiki/File:Mitosis_cells_sequence.svg*

# SMPD4 sequence identical to NET13, Nuclear Envelope Transmembrane protein-13

Erasmus MC

## Nuclear envelope proteomics: Novel integral membrane proteins of the inner nuclear membrane

Mathias Dreger*†, Luiza Bengtsson*†, Torsten Schöneberg‡, Henning Otto*, and Ferdinand Hucho*§

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Edited by J. Richard McIntosh, University of Colorado, Boulder, CO, and approved August 10, 2001 (received for review April 24, 2001)

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Cell. Mol. Life Sci. (2010) 67:1353–1369
DOI 10.1007/s00018-010-0257-2

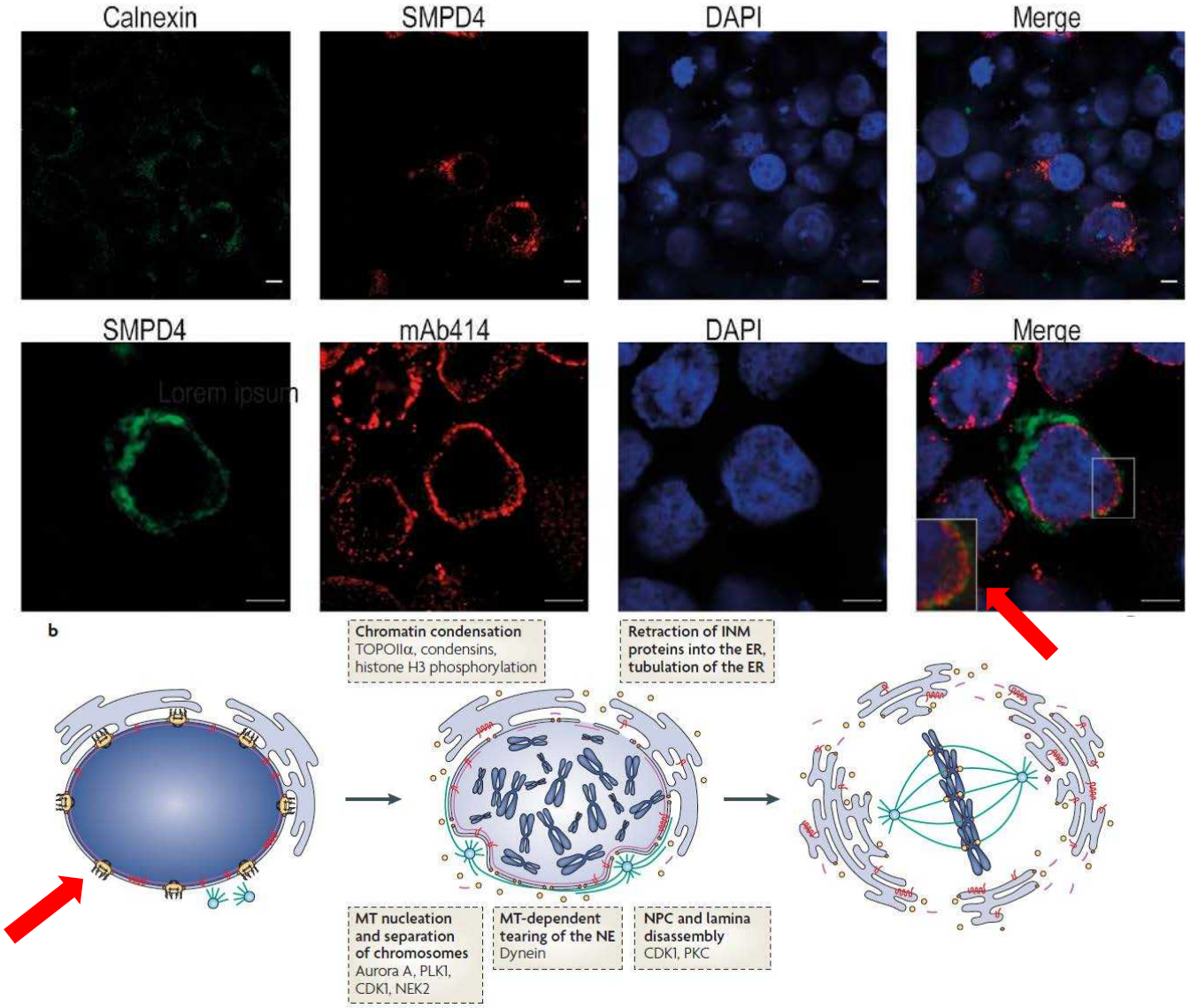
Cellular and Molecular Life Sciences

RESEARCH ARTICLE

## Cell-specific and lamin-dependent targeting of novel transmembrane proteins in the nuclear envelope

Poonam Malik · Nadia Korfali · Vlastimil Srsen · Vassiliki Lazou · Dzmitry G. Batrakou · Nikolaj Zuleger · Deirdre M. Kavanagh · Gavin S. Wilkie · Martin W. Goldberg · Eric C. Schirmer

# Link between nuclear membrane and mitosis



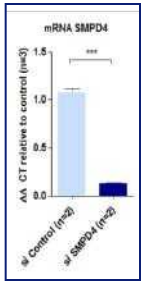

**Daphne Smits**

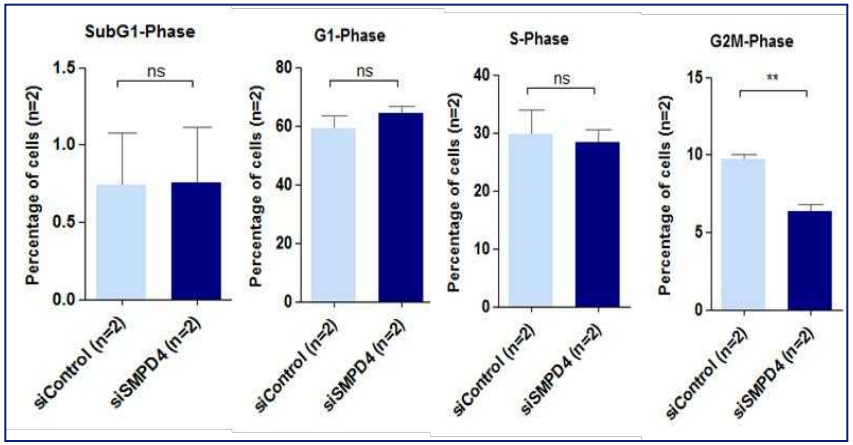
*Nuclear envelope breaks down during mitosis*

SMPD4 localizes at the nuclear envelope

# Loss of *SMPD4* results in cell cycle defects and apoptosis

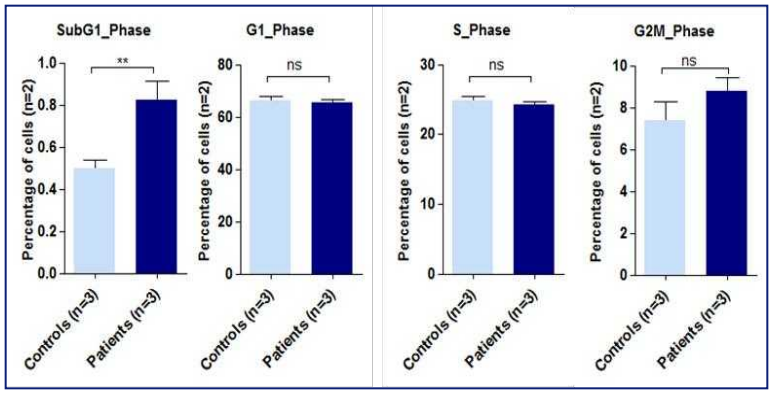
*Daphne Smits, Rachel Schot, Maura van Mook*



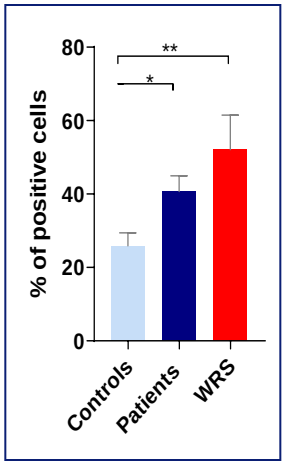




*Cell cycle phases (flow cytometry)*

*KD SMPD4*
- *↓ Dividing cells*
- *G1 delay*




*Cell cycle phases*



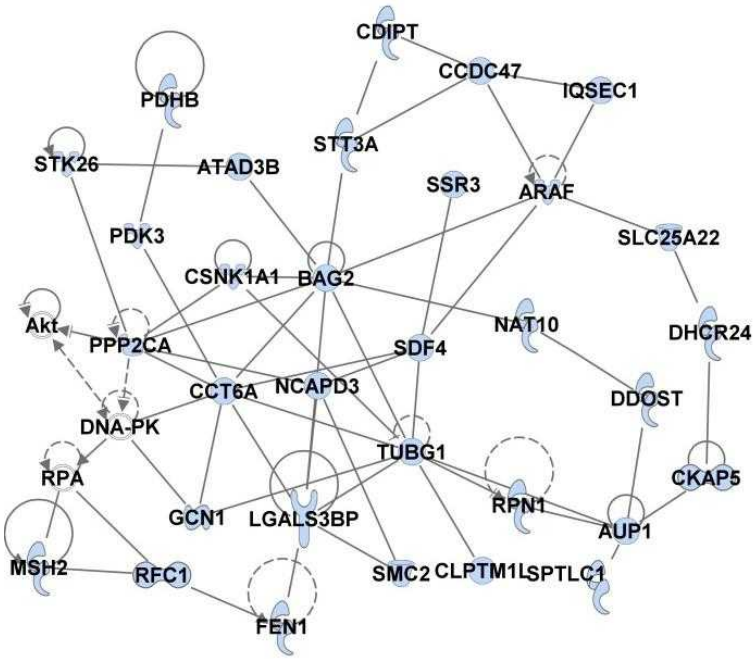

*Active Caspase assay,FLICA)*

*Patient fibroblasts*
- *Increased apoptosis*
- *No G1 delay*

# SMPD4 interacting proteins are localized in the ER and the nuclear envelope

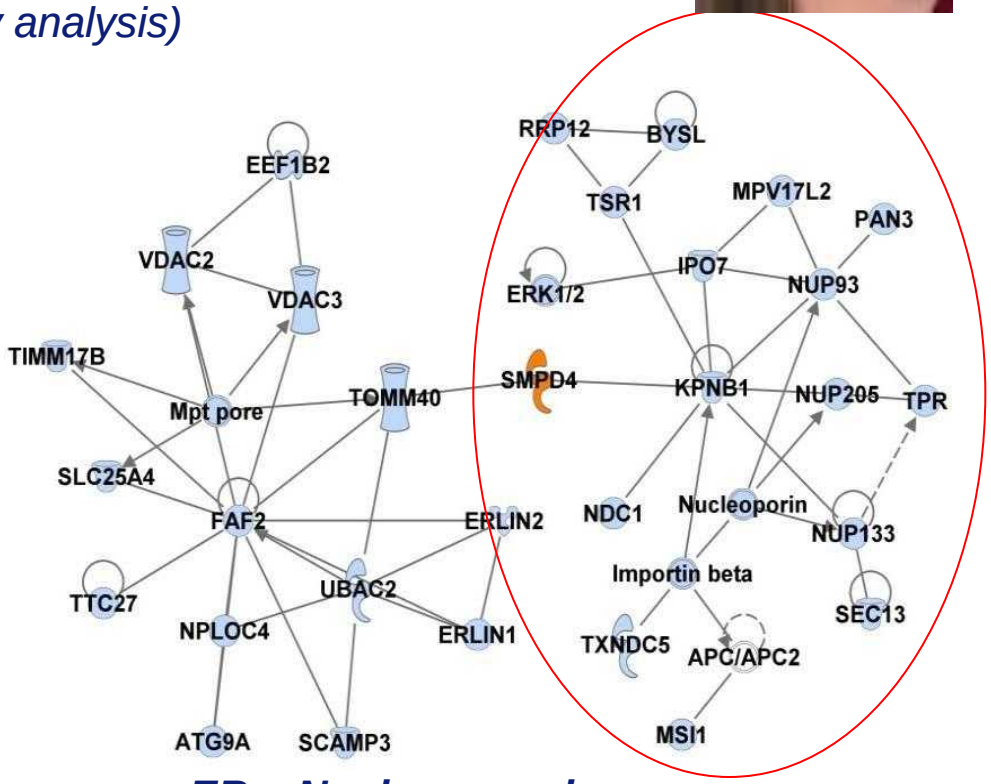
*Pulldown of Myc-SMPD4 → Mass spectrometry*

- *253 significant potential binding partners*
- *Network analysis in IPA (ingenuity pathway analysis)*




***ER membrane proteins***

*Involved in protein folding, protein synthesis, protein translocation*




***ER + Nuclear envelope***

*Several components of the nuclear pore complex*

**Magini, Smits et al AJHG 2019**

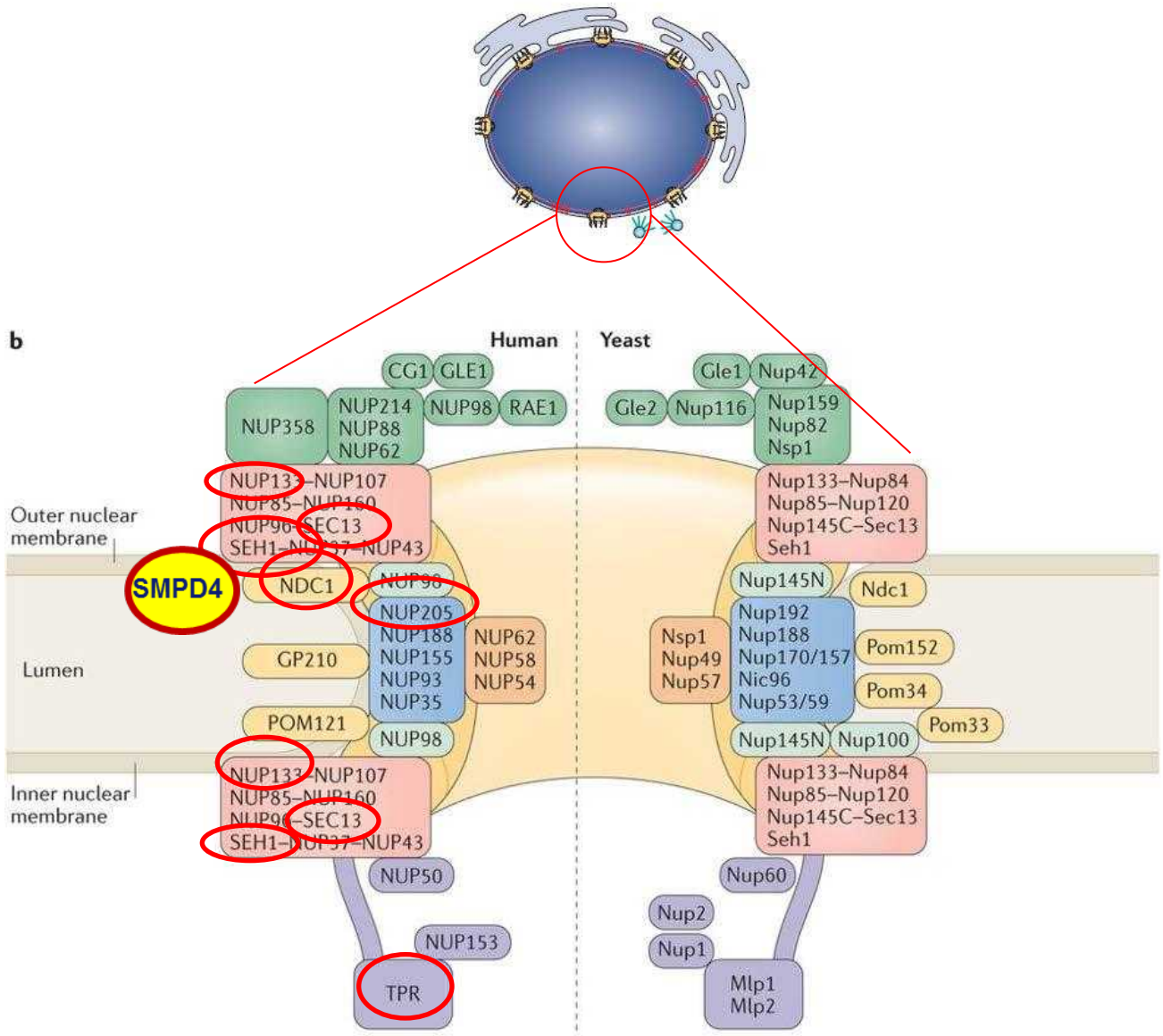
# SMPD4 localizes at the Nuclear Pore Complex

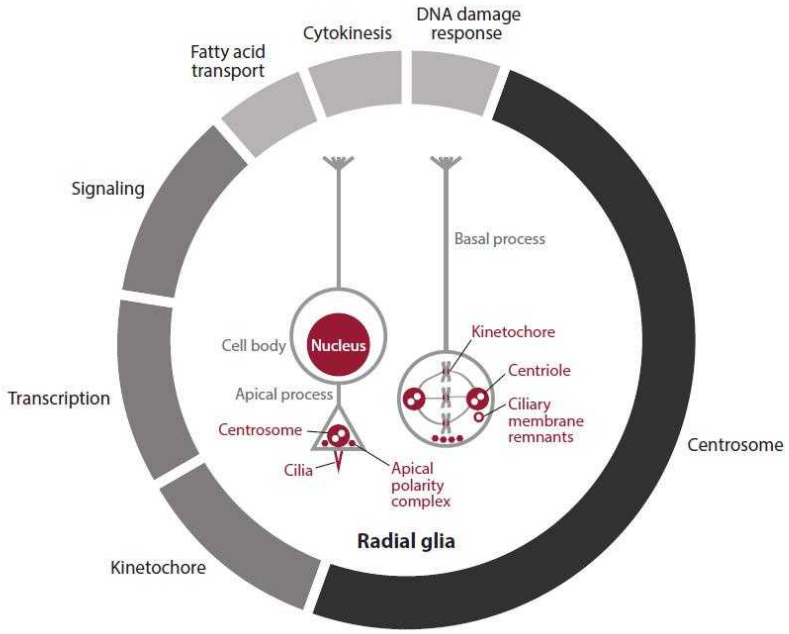
Erasmus MC

Cytoplasm

Nuclear envelope

Nuclear lumen






*Annual Review of Genomics and Human Genetics*

The Genetics of Primary Microcephaly

Divya Jayaraman,[1,2,3] Byoung-Il Bae,[4] and Christopher A. Walsh[1,5,6]

- SMPD4 is an outer Nuclear Membrane sphingomyelinase associated with the Nuclear Pore Complex

- Lack of SMPD4 leads to MIC, SGP and Hypomyelination during brain development,
- Linking lipid metabolism to neural progenitors proliferation and differentiation

# The perspective of the clinical geneticist on multidisciplinary collaboration in MCD research

Lessons learned:

***MACF1*** > periodic multidisciplinary MRI review sessions support recognition and classification of rare MCD phenotypes

***INTS8/1*** > accurate phenotyping supports genome data interpretation and leads to syndrome recognition

***SMPD4*** > searching beyond the exome and integrating new technologies is increasingly needed

***All*** > importance of long term follow up of patients with rare phenotypes

EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

Neuro-MIG

- **Erasmus MC:**

**Clinical Genetics**

Rachel Schot, Laura Vandervore, Daphne Smits, Marjon van Slegtenhorst, Martina Wilke, Frans Verheijen, Alice Brooks, Esmee Kasteleijn, Vincenzo Bonifati, Stefan Barakat, Maura van Mook

**Cell Biology**

Maarten Fornerod, Niels Galjart, Raymond Poot

**Pathology**

Peter van der Spek, Max Kros, Stefanie Brock

**Child Neurology:** Marie Claire de Wit

**Radiology:** Marjolein Dremmen

**Proteomics:** Jeroen Demmers, Dick Dekkers

ENCORE

ZonMw

- **Patients and families, Referring clinicians, Collaborators:**

Anna Jansen, UZ Brussel
Nadia Bahi-Buisson, Paris
Andrew Fry, Cardiff
Maarten H. Lequin, Utrecht
Renske Oegema, Utrecht
Daniela Pilz, Glasgow
Nataliya Di Donato, Dresden
Ghayda Mirzaa, Seattle
Bill Dobyns, Seattle
Jim Barkovich, UCSF
Pamela Magini, Bologna
Mariasavina Severino, Genova
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Tugba Kalayci, Istanbul
Maha Zaki, Cairo
Joseph Gleeson, La Jolla
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Maria Teresa Divizia, Genova
Wojciech Wiszniewski, Warsaw
Amal Alhashem, Riyadh
Bella Davidov, Ten Aviv
Nicola Brunetti Pierri, Napoli
Rolf Stottmann, Cincinnati
Alexandra Afenjar, Paris
Stephanie Coury, Boston
Boris Keren, Paris
Caroline Nava, Paris
Florence Renaldo, Paris
María José Sanchez, Murcia
Wen-Hann Tan, Boston **and many more….**