Peut-on modéliser les Malformations cérébrales ?

Alfonso Represa

INMED, INSERM, Aix-Marseille University
Les malformations du développement cortical

Lissencephaly / agyria / pachygyria

Polymicrogyria

Subcortical band heterotopia

Periventricular nodular heterotopia

Focal cortical dysplasia

Schizencephaly

Hemimegaencephaly

25-40% des épilepsies réfractaires de l’enfant et l’adolescent

Leventer et al., Dialogues Clin Neurosci 2008
Cortical development disorders: clinical manifestations

- Epilepsy
- Developmental delay
- Mental retardation

- Account for 30-40 pharmaco-resistant epilepsies in children
- More than 25 syndromes resulting from abnormal neuronal migration have been described.
- Autism, schizophrenia, dyslexia can be related to neuronal migration defects
A developmental and genetic classification for malformations of cortical development (Barkovich et al, Brain 2012)

(I) MALFORMATIONS SECONDARY TO ABNORMAL NEURONAL AND GLIAL PROLIFERATION OR APOPTOSIS

(A) SEVERE CONGENITAL MICROCEPHALY (MIC), pre-migrational reduced proliferation or excess apoptosis

(B) MEGALENCEPHALY (MEG) including both congenital and early postnatal

(C) CORTICAL DYSGENESIS WITH ABNORMAL CELL PROLIFERATION BUT WITHOUT NEOPLASIA

(D) CORTICAL DYSPLASIAS WITH ABNORMAL CELL PROLIFERATION AND NEOPLASIA

(II) MALFORMATIONS DUE TO ABNORMAL NEURONAL MIGRATION

(A) MALFORMATIONS WITH NEUROEPENDYMYAL ABNORMALITIES: PERIVENTRICULAR HETEROOTOPIA

(B) MALFORMATIONS DUE TO GENERALIZED ABNORMAL TRANSMANTLE MIGRATION (radial and non-radial)

(C) MALFORMATIONS PRESUMABLY DUE TO LOCALIZED ABNORMAL LATE RADIAL OR TANGENTIAL TRANSMANTLE MIGRATION

(III) MALFORMATIONS DUE TO ABNORMAL POSTMIGRATIONAL DEVELOPMENT

(A) MALFORMATIONS WITH PMG OR CORTICAL MALFORMATIONS RESEMBLING PMG.

(B) CORTICAL DYSGENESIS SECONDARY TO INBORN ERRORS OF METABOLISM

(C) FOCAL CORTICAL DYSPLASIAS (WITHOUT DYSMORPHIC NEURONS) DUE TO LATE DEVELOPMENTAL DISTURBANCES

(D) POSTMIGRATIONAL DEVELOPMENTAL MICROCEPHALY
Neocortical histogenesis

1. division des progéniteurs
2. migration multipolaire
3. migration radiaire
4. croissance dendritique
5. synaptogenèse spinogenèse

Ohtaka-Maruyama and Okado, Front Neurosci 2015
Cytoarchitectonic organization of neocortical cells

Cajal “La Cellule”
t. VII, 1er fascicule 1891

Peters et Jones, *Cellular Components of the Cerebral Cortex* 1984
Anton-Sanchez et al., *Front Neuroanat* 2014
Neocortical histogenesis: human foetal development

Dr Antoinette Gelot, Département de Neuropathologie, Hôpital Trousseau, Paris
Neocortical histogenesis: human foetal development

Silbereis et al., Neuron 2016
Environmental factors and conditions which can potentially influence the developing brain

Therapeutic drugs
- Retinoic acid
- Anti-thyroid drugs
- Oestroprogestative drugs
- Testosterone and derivatives
- Anti-mitotic drugs
- Lithium and psychotropic drugs
- Benzodiazepines
- Anti-epileptic drugs
- Recreational drugs
- Tobacco
- Caffeine
- Ethanol
- Cocaine
- Heroin
- L.S.D.
- Marijuana
- Physical and chemical agents
- Dioxins
- Heavy metals
- Organic solvents
- Ionizing radiations
- Head trauma
- Repeated head shaking
- Maternal factors and conditions
- Sexual hormones

Catecholamines
- Thyroid hormones
- Diabetes
- Non treated phenylketonuria
- Coagulopathies
- Peptides (vasoactive intestinal peptide)
- Hormones from placenta and decidua
- Hypoxic-ischemic conditions
- Chorioamnionitis
- Hyperthermia
- Infectious agents (human pathogens)
- Herpes simplex virus I and II
- Varicella zoster
- Human cytomegalovirus
- Benign lymphocytic choriomeningitis virus
- Rubella virus
- Parvovirus B19
- Coxsackie virus (B group)
- Pestivirus
- HIV
- Influenza virus
- BK and JC viruses
- Toxoplasma gondii
- Listeria monocytogenes
- Treponema pallidum (syphilis)

Gressens et al, 2001
Fetal alcohol syndrome

Dr S Mattson et coll. 1994
<table>
<thead>
<tr>
<th>Genes involved</th>
<th>Type of malformation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TSC1</strong></td>
<td>Abnormal proliferation/differentiation (Tuberosclerosis, Megalencephaly and FCDs)</td>
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<tr>
<td><strong>TSC2</strong></td>
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<td><strong>AKT3</strong></td>
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<td><strong>PIK3CA</strong></td>
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<td><strong>PIK3R2</strong></td>
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<td><strong>MTOR</strong></td>
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<td><strong>DEPDC5</strong></td>
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<td><strong>EZH2</strong></td>
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<td><strong>NPRL3</strong></td>
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<tr>
<td><strong>ARX</strong></td>
<td>Abnormal migration : lissencephaly, Pachyrhizia, Subcortical band heterotopia</td>
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<td><strong>YWHAE</strong></td>
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<td><strong>DYNC1H1</strong></td>
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<td><strong>LIS1</strong></td>
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<td><strong>ACTB</strong></td>
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<td><strong>KIF5C</strong></td>
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<td><strong>DCX</strong></td>
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<td><strong>TUBG1</strong></td>
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<td><strong>RELN</strong></td>
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<td><strong>ACTG1</strong></td>
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<td><strong>KIF2A</strong></td>
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<td><strong>TUBA1A</strong></td>
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<tr>
<td><strong>TUBG1</strong></td>
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<td><strong>TUBB2</strong></td>
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<td><strong>VLDLR</strong></td>
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<td><strong>CDK5</strong></td>
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<td><strong>FLNA</strong></td>
<td>Abnormal migration : Periventricular Heterotopia</td>
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<td><strong>FMR1</strong></td>
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<td><strong>ARGEF2</strong></td>
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<td><strong>ERMARD</strong></td>
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<td><strong>DCHS1</strong></td>
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<td><strong>FAT4</strong></td>
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<td><strong>LRP2</strong></td>
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<td><strong>FCMD</strong></td>
<td>Cobblestone, Syndrome Walker-Warburg, Syndrome Muscle-eye-brain</td>
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<td><strong>GPR56</strong></td>
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<td><strong>POMGnT1</strong></td>
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<td><strong>B3GNT1</strong></td>
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<td><strong>TMEM5</strong></td>
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<td><strong>FKRP</strong></td>
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<td><strong>SNAP29</strong></td>
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<td><strong>GTDC2</strong></td>
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<td><strong>LAMB1</strong></td>
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<td><strong>LARGE</strong></td>
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<td><strong>B3GALNT2</strong></td>
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<td><strong>POMT2</strong></td>
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<td><strong>ISPD</strong></td>
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<tr>
<td><strong>SRPX2</strong></td>
<td>Abnormal cortical organization/Gyration, polymicrogyria</td>
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<td><strong>GPR56</strong></td>
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<td><strong>KIAA1279</strong></td>
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<td><strong>NSDHL</strong></td>
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<tr>
<td><strong>SNAP29</strong></td>
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Polymicrogyria due to *TUBB2B*

*Jaglin et al. Nat Genet 2009*
Animal models?

- Same etiology
- Same anatomic phenotype
- Same clinical manifestations
1) « Lesion » models

Rat MAM (systemic injection at GD15)  
Microgyria (local injection of Ibotenate at birth)

*Represa, Epilepsies 2009*
1) Lession models: Antiepileptic drugs affects cortical development (in utero exposure to Valproate or to Vigabatrin)


Neuronal migration is activity dependent
2) Genetic models

1) Gènes avec mutations identifiées chez l’humain: ils sont associés ou pas à de malformations
Codent des protéines s’associant aux microtubules (DCX, LIS1, KIF2A, EML1) ou des isoformes de tubuline (TUBA1A, TUBG1),

2) Gènes sans mutations identifiées chez l’humain
Codent des protéines à fonctions diverses (Afadin, Cdh2, N-Cadherin, α-E-catenin, Rapgef2, RhoA, Wnt3a, alpha2-chimaerin, REST)

3) Gènes inconnus
Rat Tish, souris BXD29-Tlr4lps-2J/J
2) Genetic models: Doublecortin loss of function

- DCX patient
  - Gleeson et al., 1998

- DCX KO mice
  - Corbo et al. 2002
2) Genetic models: subcortical band heterotopias

Embryonic (E14) Knockdown of Dcx

- **Injection**
- **Electroporation**

**A** mU6-DCX RNAi / CAG-mRFP Blue Nissl

**B** mU6-DCX RNAi / CAG-DCX-GFP Blue Nissl

4-OHT at PO

**Mismatch (ctl)**

**Dcx-KD**

- Br -2.4
- Br 0
- Br +1.2
- Br +2

**SBH**

✓ Altered cortical lamination and SBH

Bai et al., Nat Neurosci 2003
Manent et al., Nature Medicine 2009
2) Genetic models: Control mice brain is Lissencephalic
2) Genetic models: Ferret brain gyrification
2) Genetic models: Genetic manipulation of ferret brain using in utero electroporation
Contribution of animal models to our understanding of epileptogenesis and identifying new therapeutic strategies
Exploring human postsurgical samples and animal models for deciphering epileptogenesis (TSC and FCD)
Recordings of resected human brain tissue from TSC/FCD patients

Relative expression of NMDAR subunits in wild type and Tsc1 +/- mice

Reference genes: HPRT / cyclophilin A

Acute antiepileptic effects of NR2C/D antagonists and dapamycin in a mouse model of TSC
Neuronal network changes: exploring the Dcx Knockdown rat model of SBH

**Juvenile Dcx-KD rats (< P30)**

- PTZ
- PTZ

**MM**

**DCX-KD**

**Adult Dcx-KD rats (>P200)**

Adult DCX-KD rat  
SBH Patient
Epilepsy focus in SBH syndrome

**SEEK**

- In 2 patients: epileptic activity recorded in both the heteropic band and the normotopic cortex
- In 1 patient: discharges starting at a distance and secondarily propagating to the heterotopic band and the normotopic cortex
- In 2 patients: no epileptiform activity in the heterotopic band

**EEG-FRMI**

- In 1 patient: changes in the MRI signal at both the heterotopic band and the normotopic cortex during interictal and ictal activities

*Bernasconi et al., 2001; Mai et al., 2003; Lo Russo et al., 2003*

*Tyvaert et al., 2008*
Genesis and Propagation of interictal events in the cortex of DCX-KD rats

*SBH* is always recruited (98%)

*SBH* is neither active at the beginning nor the end of the epileptiform event

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Petit, Jalabert et al., Annals of Neurology 2014
Functional connectivity of cortical neurons in normotopic cortex

Laminar profile of L2/3 inputs

Laminar profile of L5 inputs

Mism. DCX-KD

Jalabert et al, preliminary data
Epileptogenesis in DCX-KD rats: increased glutamatergic drive and synchronized activity of normotopic neurons

Spontaneous Glu PSCs ($V_h=-70\text{mV})$

Spontaneous GABA PSCs ($V_h=-40\text{mV})$

correlated spontaneous calcium events

Mismatch

Dcx-KD

$15\text{pA}$

$500\text{ms}$

GABA PSCs (Hz)

Glu PSCs (Hz)

Percent active

Cell no.

Percent correlated
In vivo manipulation of neuronal activity in Dcx-KD rats and seizure outcome

Conditional expression of inward rectifying K channels

Hyperpolarization $\rightarrow$ Reduced excitability

Petit, Jalabert et al., Annals of Neurology 2014
Suppression of neuronal network excitability

Kir2.1

K^+

Hyperpolarization (neuronal silencing)

Dcx-KD - normally active SBH

Dcx-KD - silenced SBH

Dcx-KD - silenced SBH and normotopic cortex

PTZ 1  PTZ 2  PTZ 3  PTZ 4

Δν/2 \( \text{min} \)

Proportion of animals (%)

Latency to seizure (sec)

Petit, Jalabert et al., Annals of Neurology 2014
Conclusions: Epileptogenesis in MCDs arises from cell autonomous and/or cortical network changes

- mTOR and NR2C receptor expression (TSC & FCD human samples, TSC1+/- mice)
- Synaptogenesis and neuronal circuitry altered in DCX-KD rats, a model of grey matter heterotopia
- Therapeutic vistas: i) identifying new targets for pharma agents and ii) genetic/molecular manipulation of neuron excitability
... and now, what do you think of animal models?

"Of COURSE this place is ruining your health... THAT'S THE IDEA!"
Alfonso Represa team
Carlos Cardoso
Jean-Bernard Manent
Françoise Watrin
Antoine de Chevigny
Surajit Sahu
Vanessa Plantier
Fanny Martineau
Véronique Brévaut-Malaty
Antoinette Gélot

Collaborators:
INMED (Aniksztejn, Burnashev, Crepel)
Jamel Chelly
Renzo Guerrini
Fabio Benfenati
Albert Becker
Hiroshi Kawasaki
Fiona Francis

Provence-Alpes-Côte d’Azur