Leukodystrophies:
so much more than just diseases of myelin

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Nothing to disclose
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What is a leukodystrophy?

- 1980’s
- Genetic, progressive disorders primarily affecting myelin (myelin loss or insufficient myelination), either directly or through oligodendrocytes

Morell & Wiesmann, Neuropediatrics 1984; 15 (suppl): 62
Seitelberger, Neuropediatrics 1984; 15 (suppl): 53

- No known gene defects
- MRI had not entered clinical practice
- Data available from pathology, biochemical analyses of brain tissue and knowledge of several metabolic and enzymatic defects

- Curative treatment focused on stopping myelin loss and on remyelination
1980’s: introduction of MRI

- Very high sensitivity for white matter abnormalities
- Replaced neuropathology

MRI pattern recognition

Next generation sequencing

Most leukodystrophies are due to defects in gene encoding proteins specific for cell types other than the oligodendrocytes
Oligodendrocyte development, myelination, myelin maintenance and regeneration: teamwork required

Oligodendrocyte progenitor
Jagged1

(pre)myelinating oligodendrocyte

LIF

miR-219
miR-338

Oligodendrocyte

TH
Nkx2.2

YY1, MRF
HDAC

NRG1
laminins

NG2 glia

ATP adenosine

Astrocyte

Microglia

Neuron

Myelin Sheath
Oligodendrocyte development, myelination, myelin maintenance and regeneration: teamwork required

New definition of leukodystrophy: genetic white matter disorder due to a defect in any of the white matter structural components
A new classification of leukodystrophies

Myelin disorders
Astrocytopathies
Leuko-axonopathies
Leuko-microgliopathies
Leukovasculopathies
Autosomal recessive

*ARSA* (ASA defect, sulfatide storage), *PSAP* (sphingolipid activator protein saposin B)

Three subtypes: late infantile, juvenile, adult

Ethnic and private mutations; reduced or abolished enzyme activity

Some degree of genotype-phenotype correlation (e.g., homozygous null = late infantile)
Lysosomal disorders:
enzymatic defect → storage material → cell death
Metachromatic leukodystrophy (MLD)
Myelin stain Macrophages full of storage
Metachromatic leukodystrophy: hematopoietic stem cell transplantation
Metachromatic leukodystrophy and hematopoietic stem cell transplantation: supposed mechanism of action
Metachromatic leukodystrophy and hematopoietic stem cell transplantation: clinical evolution
Metachromatic leukodystrophy

no transplantation

after transplantation
Donor cells reach the brain of transplanted MLD patients, carry the enzyme and digest the storage material

Wolf et al., 2020
ASA Immunopositivity in the frontal white matter

Control

Non Transplanted

Transplanted

Wolf et al., 2020
NO enzymatic cross correction
from donor macrophages to resident (neural) cells

ASA / LN3
ASA / OLIG2
ASA / GFAP

Wolf et al., 2020
Transplantation promotes survival of the oligodendrocytes

Wolf et al., 2020
Transplantation is associated with remyelination
(“normalization” of white matter signal changes on MRI)

\[ \text{G-Ratio} = \text{The ratio of the inner axonal diameter to the total outer diameter} \]

Wolf et al., 2020
Transplantation modulates microglia activation status

IBA-1 / CD45

Number of CD45+ IBA-1+ cells per mm²

Control - HSCT + HSCT

CD40 CD40 CD163 CD163

CD40 / MR

Positive cells per mm²

CD40+ MR+ CD40/MR+

Wolf et al., 2020
Transplantation skews the macrophages towards an anti-inflammatory phenotype that supports remyelination.
A new classification of leukodystrophies

Myelin disorders
Astrocytopathies
Leuko-axonopathies
Leuko-microgliopathies
Leukovasculopathies
DISEASE

↑ and ↓ of specific proteins

LOSS OF PHYSIOLOGIC FUNCTIONS

- chronic glial scarring
- lack of normal gliotic repair

GAIN OF PATHOLOGIC FUNCTIONS

- impact on the ECM
- inhibition of oligodendrocyte maturation
- lack of myelin
- impact on axons
- impact on myelin deposition and maintenance

lack of normal gliotic repair
Vanishing White Matter

- Mutations in $E_{IF}2B1-5$, encoding the 5 eIF2B subunits
- eIF2B: initiation of translation of all mRNAs
  regulation of general mRNA translation rate
- Disease mechanisms? Altered expression of specific proteins?
GFAP

loss of all white matter structures
proliferation of oligodendrocytes
lack of reactive gliosis
no selective myelin loss
Reactive gliosis

(a) Mild to moderate astrogliosis

Healthy → Mild - Moderate Insult → Hypertrophy + Molecular & Functional Changes → Potential for resolution

(b) Severe astrogliosis with scar formation

Healthy → Severe Insult → Persisting Mature Glial Scar → Proliferated astrocytes + Other cell types

Bordering along regions of overt tissue damage & inflammation due to:
- Trauma
- Ischemia
- Cytotoxicity
- Infection
- Autoimmune inflammation

Axons → Glial Scar Barrier → Inflammatory Cells

Sofroniew, Trends in Neuroscience 2009
VWM white matter astrocytes proliferate, remain immature and lack mature function (e.g. astrogliotic scar tissue formation)

Bugiani et al., 2010, 2011
VWM white matter astrocytes remain immature

Bugiani et al., 2010, 2011
VWM white matter: lack of myelin, but too many oligodendrocytes
Oligodendrocytes proliferate and are increased in number, but they remain immature and lack of mature myelination function.
Deficient maturation of macroglial cells in VWM white matter driven by astrocytic dysfunction
Highly elevated high molecular weight hyaluronan in VWM white matter

- Produced by astrocytes
- Known to inhibit oligodendrocyte precursor maturation
- Its level correlates with the severity of white matter abnormality
- Hyaluronan synthase2 (HAS2) increased in immature (CD44+) white matter astrocytes

ECM hyaluronan

Bugiani M, et al., 2013
Does the VWM defect impact oligodendrocytes and astrocytes at the same time or is one causing the dysfunction of the other?

Studies in cocultures, using VWM mouse cells

Dooves S & Bugiani M, et al., 2016
VWM astrocytes have a negative impact on both WT and VWM oligodendrocytes, but VWM oligodendrocytes display normal myelin production with WT astrocytes.

So, VWM OPCs do not have an intrinsic problem.
VWM mice and patients: the retinal eye pathology

Wild-type

VWM mice

Fig. 3 Cartoon of the retina to show where the major components of the ERG originate.

Dooves S & Bugiani M, et al., 2016
VWM female patients:
the pathophysiology of ovarian dystrophy

Bugiani et al, 2018
A new classification of leukodystrophies

Myelin disorders
Astrocytopathies
Leuko-axonopathies
Leuko-microgliopathies
Leukovasculopathies
Hypomyelination with Atrophy of Basal ganglia and Cerebellum (H-ABC)

- Mutations in TUBB4A
- Defect in β-tubulin, affecting microtubules
- Probably affecting axonal transport

Axonal spheroids, lack of myelin, lack of oligodendrocytes, mild gliosis.

Axonal dysfunction, secondary lack of myelin deposition.
<table>
<thead>
<tr>
<th>Disease description</th>
<th>Whispering Dysphonia (DYT4)</th>
<th>H-ABC</th>
<th>Isolated Hypomyelination</th>
<th>Isolated Hypomyelination</th>
<th>Early infantile encephalopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic acid change</td>
<td>c.4C&gt;G</td>
<td>c.745G&gt;A</td>
<td>c.763G&gt;A</td>
<td>c.845G&gt;C</td>
<td>c.1242C&gt;G</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Imaging features</th>
<th>No structural Abnormalities</th>
<th>Hypomyelination and atrophy of the basal ganglia, cerebellum, and corpus callosum</th>
<th>Hypomyelination and atrophy of cerebellum</th>
<th>Hypomyelination and atrophy of the cerebellum</th>
<th>Severe hypomyelination, normal basal ganglia, severe atrophy of cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical features</td>
<td>Dysphonia, gait affected, and dystonia</td>
<td>Ataxia, dystonia and intellectual disability</td>
<td>Spastic quadriaparesis, ataxia</td>
<td>Spastic paraparesis, intellectual disability</td>
<td>Severe intellectual disability, motor deterioration, epilepsy, early death</td>
</tr>
</tbody>
</table>

Curiel, ... Bugiani, 2017
H-ABC: two distinct neuropathological phenotypes

Curiel, ... Bugiani, 2017
H-ABC: the neuronal phenotype

Curiel, ... Bugiani, 2017
H-ABC: the oligodendrocytic phenotype

Curiel, ... Bugiani, 2017
H-ABC, the oligodendrocytic phenotype (hypomyelination only) is like the Taiep rat
Conclusions

• The definition of leukodystrophies had to be revised
• Genetic disorders in which *any* white matter structural component is primarily affected

Importance of a new definition

• Better understanding of the complexity of the brain white matter
• When treating patients with leukodystrophies, we need to repair more than myelin alone
The intrinsic repair potential of leukodystrophies

- No oligos
- No myelin
- Axonal influence

- Many oligos (progenitors)
- No myelin

- Many oligos
- Little myelin

- Normal oligos
- Normal myelin
- Astrocytic influence

- Repair
Amsterdam Leukodystrophy Centre

Thanks to
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