VU VRIJE UNIVERSITEIT AMSTERDAM

# VU Research Portal

## Hypomyelination with tooth and bone involvement: New insights

Cayami, F.K.

2018

***document version***
Publisher's PDF, also known as Version of record

**Link to publication in VU Research Portal**

***citation for published version (APA)***
Cayami, F. K. (2018). *Hypomyelination with tooth and bone involvement: New insights: Brain MR imaging and in vitro models*. [PhD-Thesis - Research and graduation internal, Vrije Universiteit Amsterdam].

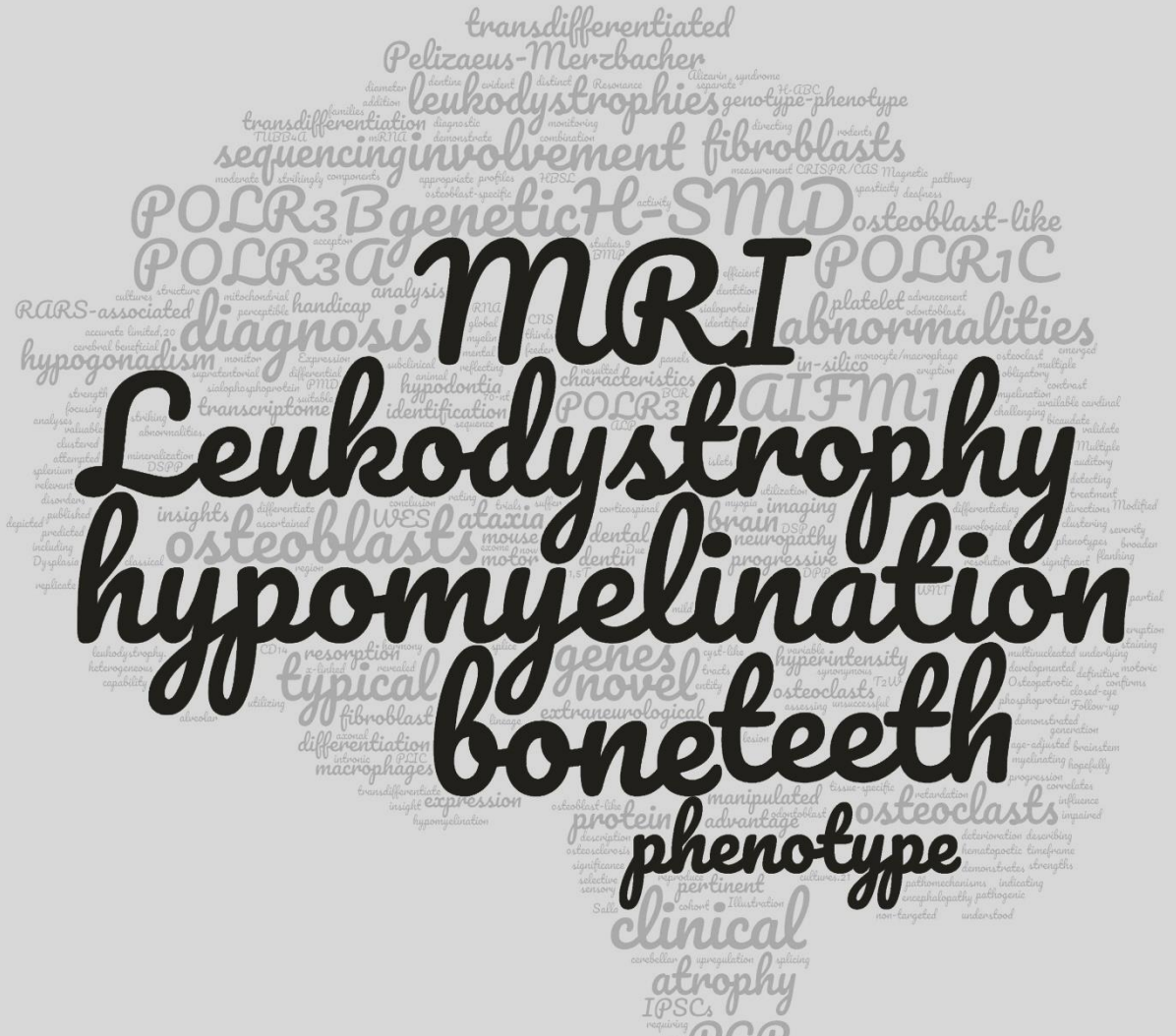
**General rights**
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

**Take down policy**
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

**E-mail address:**
**vuresearchportal.ub@vu.nl**

Download date: 13. Oct. 2024




# CHAPTER 4

# DISCUSSION

# &

# SUMMARY

## Genetic Heterogeneity in Hypomyelinating Leukodystrophies

Hypomyelinating leukodystrophies are a heterogeneous group of diseases with a broad differential diagnosis. With non-specific neurological signs such as developmental delay, ataxia, pyramidal signs and progressive motor deterioration, it may be challenging to achieve a definitive final diagnosis. MRI not only provides important tools to separate hypomyelination from other leukodystrophies, it can also direct the genetic diagnosis of hypomyelination and can be utilized beyond its main function as a diagnostic tool.



Since the description of *POLR3A*, *POLR3B* and *POLR1C* as the responsible genes only some years ago,[1–5] 4H leukodystrophy emerged as the second most common hypomyelinating leukodystrophy.[6] However, **Chapter 2.1** describes that the mutations in *POLR3A* and *POLR3B* are rarely found in patients with unclassified hypomyelination. As *POLR1C* was not known as a gene for 4H leukodystrophy at that time, we might have underestimated the presence of *POLR1C* mutations in this cohort. Follow-up with whole exome sequencing (WES) revealed no *POLR1C* mutations and resulted in another diagnosis in 12 of the 22 patients: three patients with *TUBB4A* mutations leading to hypomyelination with atrophy of basal ganglia and cerebellum (H-ABC) without basal ganglia changes, one with hypomyelination of early myelinating structure (HEMS), one with hypomyelination with brain stem and spinal cord involvement and leg spasticity (HBSL), two with Salla disease, three with *RARS*-associated hypomyelination, one with mutations in *GJB1* and one with mutations in a novel hypomyelinating leukodystrophy gene (*TMEM106B*).[7]

Recent articles confirm the clinical heterogeneity of hypomyelination.[8–13] Application of WES in 26 infants classified as having hypomyelinating leukodystrophy resulted in diagnosis of 35% of patients after 8 patients had been screened out by chromosomal analysis, CGH array and targeted sequencing of certain genes (*PLP1*, *GJC2*, *MBP*, *TREX1*).[12] However, based on available MR images of the patients, the inclusion criteria were applied incorrectly, resulting in an erroneous diagnosis of hypomyelination of

some patients. Similar to our cohort, two patients had *TUBB4A* mutations (one of them with typical MRI abnormalities) and only one patient had *POLR3B* mutations.[12] Clinically, the patient with *POLR3B* mutations in our cohort had severe myopia and hypogonadism, features which could have led to the diagnosis of 4H leukodystrophy on clinical grounds already. In spite of these shortcomings, this study reinforces our impression that, even after application of whole-exome sequencing (WES), mutations in POLR3-related genes remain rare in unclassified hypomyelination with neither typical clinical nor MRI characteristics, underlining the important role of thorough MRI analysis and clinical assessment.



## MRI in hypomyelinating leukodystrophies: Recent insights

Clinical severity in 4H leukodystrophy is strikingly variable, ranging from subclinical ataxia to severe motoric handicap, which makes monitoring clinical progression difficult. MRI is not only valuable for diagnosis but also for assessing severity of white matter abnormalities;[14] therefore we wondered whether this would also apply to hypomyelinating leukodystrophies. **Chapter 2.2** provides a novel rating scale for 4H leukodystrophy, reflecting both hypomyelination and atrophy. The scale (for both hypomyelination and atrophy) correlates well with motor handicap, making it indeed valuable for clinical use. The main advantage of our 4H leukodystrophy scoring system compared to other scoring systems for leukodystrophies[15–17] is the more accurate measurement of cerebral atrophy by using an age-adjusted bicaudate ratio (BCR) and brainstem diameter. Another advantage is that the scoring system was designed for conventional T1W and T2W MR images, usually available from standard MR imaging, making it possible to use it also for retrospective studies.

Advanced MRI provides additional modalities to assess myelin such as proton magnetic resonance spectroscopy (MRS), myelin water fraction (MWF) from quantitative T1 and T2, magnetization transfer imaging (MTI) or diffusion tensor imaging (DTI) with all their advantages and limitations.[18,19] Currently, MWF and radial diffusivity (RD) from DTI are

the most promising parameters to evaluate myelin. MWF has strong correlation with myelin based on histological assessment irrespective of inflammation or water content changes[20,21] while RD is the most sensitive DTI parameters and correlates well with motor handicap for several hypomyelinating leukodystrophy.[22] In the future, MRI analysis combining basic and advanced MRI will also be beneficial as monitoring tool for clinical trials.

The advancement of MRI techniques provides, among others, higher resolution, especially with high field strengths. **Chapter 2.3** demonstrates findings at 3T MRI in 4H leukodystrophy, revealing novel MRI characteristics not perceptible with lower field strength imaging. Multiple myelin islets, closed-eye sign, a small cyst-like lesion of the splenium and hypomyelination of the spinal cord were well visible on T2W MR images acquired at 3T whereas they were barely visible on 1,5T MR images. Especially the presence of myelin islets is interesting, as it suggests that, even in the hypomyelinated 4H brain, some cells have kept their myelinating potential, which might be exploited in treatment approaches.



## Phenotypic variability in 4H leukodystrophy

In contrast to the largest study to date, describing 105 patients with 4H leukodystrophy who all had diffuse hypomyelination,[23] our study in **Chapter 2.4** reveals that diffuse hypomyelination is not obligatory for POLR3 leukodystrophy. Eight patients neither of whom had typical hypomyelination were diagnosed with mutations in *POLR3A* or *POLR3B*. Five patients did have partial hypomyelination whereas three had normal myelination. There were two pertinent MRI findings: selective involvement of corticospinal tracts particularly evident at the level of PLIC as T2 hyperintensity, present only in *POLR3A* patients, and moderate to severe cerebellar atrophy with non-specific T2 hyperintensity of the supratentorial white matter in both *POLR3A* and *POLR3B* patients. In the meantime, we identified four more patients with only cerebellar atrophy on MRI in the Netherlands, all with biallelic mutations in *POLR3B*.

This makes mutations in POLR3-related genes an important differential diagnosis also for disorders from the spectrum of spinocerebellar ataxias.

The broadening clinical spectrum with possibly a specific phenotype-genotype relation in patients with POLR3-genes mutations is also illustrated by recent studies.[24–30] Biallelic truncating mutations in *POLR3A* were associated with a severe phenotype, a rare neonatal progeroid syndrome called Wiedemann-Rautenstrauch syndrome (WRS), although we wonder whether the intronic mutation leading to false splicing, c.1909+18G>A; p.(Y637Cfs*23), really leads to complete absence of POLR3A or rather allows expression of a low amount of wildtype protein.[24,25] Compound heterozygous mutations in *POLR3A*, specifically C.1909+22G>A in one of the alleles, causing activation of a new cryptic splice site and consequently reduction of total *POLR3A* mRNA level in cells, were ascertained in adolescent-onset progressive spastic ataxia without hypomyelination.[26] Interestingly, dental abnormalities were frequent (65%) in these patients.[26] Similar to our findings in patients without hypomyelination (**Chapter 2.4**), five of the patients were tested directly for *POLR3A* and *POLR3B* mutations because of the presence of extraneurological features, namely hypodontia and short stature. Likewise, heterozygous *POLR3B* mutations were identified in patients with isolated hypogonadotropic hypogonadism (IHH) without neurological or dental anomalies.[27] Polymicrogyria and cataract were described in two siblings with *POLR3B* mutations.[28] *POLR3B* mutations were also identified in 2 patients with an overlapping phenotype of 4H leukodystrophy with cerebellar atrophy (described as cerebellar hypoplasia) with endosteal sclerosis (CHES), which is a variant of 4H syndrome.[29] Recently, heterozygous missense mutations of *POLR3A* and/or *POLR3C* were found in four patients with severe varicella zoster infection, suggesting a role in immune response for POLR3.[30]

Mutations in *POLR1C* (and also *POLR1D*) had earlier been identified in patients with Treacher-Collins syndrome (TCS), characterized by mandibular dysostosis, facial bone hypoplasia and cleft palate caused by abnormal growth of structures derived from the

first and second branchial arch.[31,32] Mutations in *POLR1A* were implied in another dysmorphy syndrome, acrofacial dysostosis.[33] *POLR1C*, *POLR1D* and *POLR1A* all encode subunits of RNA polymerase 1 (POLR1); the proteins encoded by *POLR1C* and *POLR1D* are also shared by POLR3.[34] *POLR1C* mutations leading to TCS presumably impair POLR1 function whereas *POLR1C* mutations leading to 4H leukodystrophy only impair assembly and nuclear import of POLR3[5] while loss of function mutations in both *POLR1C* and *POLR1D* disturb the neural crest cell (NCC)-derived structures development in zebra fish. Interestingly, homozygous mutations of *POLR1A* (and in another gene, *OSBPL11*, the significance of which is unknown) were recently identified in two brothers with ataxia, psychomotor retardation and leukodystrophy with cerebellar atrophy,[35] further complicating our understanding of these entities.



Even with these broadening phenotypes, targeted genetic testing for POLR3 genes for 4H leukodystrophy remains recommended for patients with typical MRI features, also without additional extraneurological abnormalities, and also for patients without hypomyelination, but with neurological signs (especially ataxia) in combination with dental abnormalities, hypogonadotropic hypogonadism, high myopia or short stature. On the other hand, in hypomyelination without typical MRI features and other extraneurological features of 4H leukodystrophy, non-targeted genetic testing such as WES or gene panels for leukodystrophies is recommended.

## *In vitro* Model for Hypomyelinating Leukodystrophies

Certain hypomyelinating leukodystrophies have typical involvement of other organs, which may provide clues for diagnosis as demonstrated by 4H leukodystrophy. The unique combination of brain abnormalities with hypodontia and hypogonadotropic hypogonadism led to identification of a group of patients with similar phenotype and allowed the identification of the underlying genetic abnormalities.[1,4,36]

A similar approach was applied in detecting the genetic cause of another hypomyelinating leukodystrophy in **Chapter 3.1**. We identified the largest patient number to date of a disease previously only described in one family.[37] Because of the pertinent bone abnormalities, we called this entity "Hypomyelination with Spondylometaphyseal Dysplasia (H-SMD)" and ascertained mutations in the responsible gene, *AIFM1*. At the same time, two families with a similar phenotype were associated to *AIFM1* based on *in-silico* analysis without further functional studies.[38] Mutations in *AIFM1* were previously associated with very different phenotypes ranging from axonal sensory neuropathy with deafness and mental retardation (Cowchock syndrome)[39,40] and pure auditory neuropathy spectrum disorders[41] to a severe x-linked mitochondrial encephalopathy.[42,43] As depicted in figure 1, the mutations that we found in H-SMD were point mutations clustered together within a 70-nt region flanking the exon 7 acceptor splice site while mutations leading to other diseases were spread throughout the gene. The H-SMD mutations we found were missense, synonymous and intronic mutations, which, based on *in-silico*

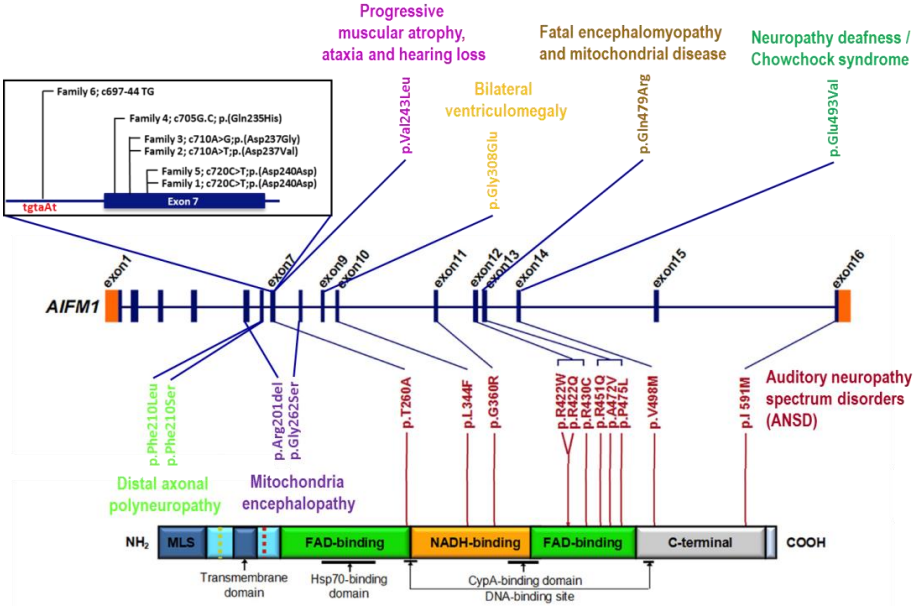



**Figure 1.** Illustration of the *AIFM1* gene with known diseases with their mutations and novel mutations related to H-SMD (black box). Modified from Zong, L. et al.[41]

analysis, were not predicted pathogenic (in the case of the 3 missense mutations) neither did they influence splicing.

With our *in vitro* model using osteoblasts derived from patients' fibroblasts in order to reproduce one of the affected tissues (bone), we were able to show that AIFM1 protein was significantly lower in patient-derived osteoblasts. There was also tissue-specific expression (in osteoblasts, but not in fibroblasts), which may explain why only specific organs are affected. Still, we have not yet understood the tight genotype-phenotype relationship in this disease giving rise to such a varying spectrum of disorders. It will be interesting to investigate the expression of the AIFM1 protein in other cell types in affected tissues such as oligodendrocytes. In conclusion, **Chapter 3.1** describes a novel hypomyelinating leukodystrophy with the associated gene as well as the significance of suitable *in vitro* models to validate mutations with regard to tissue-specific expression of genes.



The similarities of bone and tooth tissue inspired us to investigate the possibility of involvement of tooth-related cells such as osteoblasts. We attempted to transdifferentiate fibroblast cell lines from patients with 4H leukodystrophy to odontoblasts. Unfortunately, we could not detect odontoblast markers such as dentine phosphoprotein (DPP), dentin sialoprotein (DSP) and dentin sialophosphoprotein (DSPP) in transdifferentiated cells.

We also hypothesized that osteoclasts might play an important role in the dentition abnormalities of 4H patients. Osteoclasts, which are derived from the monocyte/macrophage lineage of hematopoietic stem cells, are multinucleated giant cells working in harmony with osteoblasts in bone growth and remodeling.[44] Osteoclasts are essential in alveolar bone resorption to allow tooth eruption.[45,46] Osteopetrotic rodents, with reduced bone resorption caused by fewer osteoclasts, often suffer from failure of tooth eruption.[47,48] Due to these insights and the fact that a few 4H leukodystrophy patients have a mild form of osteosclerosis, we wondered whether impaired osteoclast function could explain the dental phenotype. However, to

date, protocols for differentiation of osteoclasts from human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) are limited,[49] and we were unsuccessful to replicate both published protocols.[50,51] This might be due to our use of feeder-free cultures while both protocols used mouse fibroblasts as feeder cells to maintain the stem cells. As described previously, the variability of the feeder cells might affect the pluripotency and differentiation capability of the stem cells mainly iPSCs.[52] Furthermore, we tried different experimental approaches to differentiate macrophages from recent protocols, which use feeder-free iPSCs cultures.[53] We were able to create CD14 positive macrophages (Figure 2) from ESCs, but were unable to differentiate these to osteoclasts.



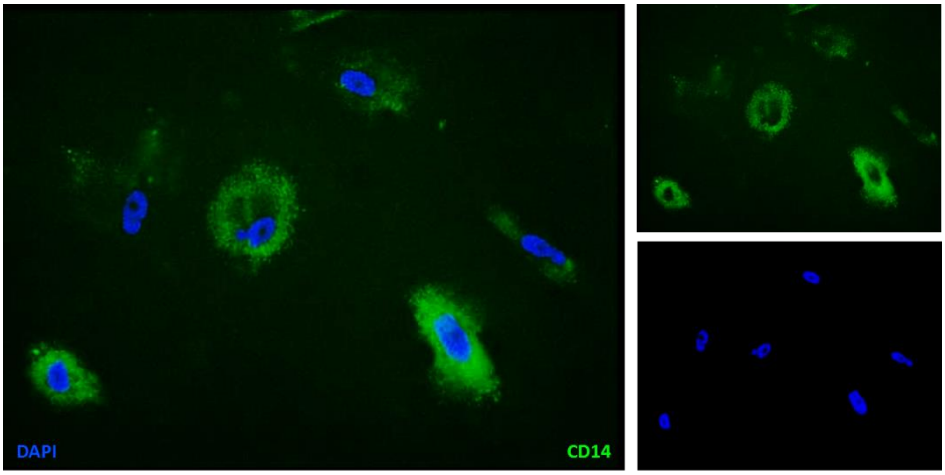



**Figure 2.** Immunofluoroscence staining of differentiated macrophages from human iPSCs with positive staining of CD14+ (green) and counterstain with DAPI (blue).

The importance of an appropriate *in vitro* model with osteoblast-like cells to study the bone phenotype in H-SMD was described in **Chapter 3.2.** We used a special approach, direct transdifferentiation from skin-derived fibroblasts to osteoblast-like cells as one of main cells in bone tissue. Transdifferentiation techniques were introduced in 1987 by conversion of fibroblasts to myoblasts.[54] With increasing recognition of determinants of cell fate differentiation, induction with lineage-specific transcription factors instead of pluripotency transcription factors produced fast and highly efficient

cell-specific models. Hitherto, transdifferentiation from fibroblasts to other cell types such as hepatocytes[55], corneal limb epithelial cells[56], neurons[57] and cardiomyocytes[58,59] have been successfully performed.

RNA-seq performed in our transdifferentiated cells also showed significant upregulation of IGF, WNT and BMP pathway components, specifically of *IGF1*, *IGF2*, *WNT2*, *WNT11* and *BMP4*. Although it was already known that these three pathways are known to be upregulated in osteogenic differentiation,[60] our result narrows down specific components important for the transdifferentiation. This finding offers the possibility to target specific pathways to obtain pure populations of one type of transdifferentiated cells, without genetic alteration, using a non-gene integrating approach, cell membrane permeable proteins or small molecule compounds[61,62] and may even be used for *in vivo* transdifferentiation in the future to aid tissue regeneration purposes.



Another way to generate specific cell types from patient skin-derived fibroblasts is via iPSCs. Yamanaka introduced the use of pluripotent factors to revert adult cells back to pluripotency state which facilitated the generation of suitable cell types[63] that can be used for studies on disease pathogenesis or for *in vitro* personalized drug screening to find new and specific treatments.[64] However, several limitations in this approach make transdifferentiation an interesting alternative in certain situations. iPSCs require genetic reprogramming through viral transfections of the genome, which raises the question about safety and stability of the genetic properties while the transdifferentiation approach maintains the original genetic profile of the cell.[65] In addition, our transdifferentiation model is a highly efficient system, which needs a relatively short timeframe in comparison to iPSCs, which require dedifferentiation to stem cell stage before differentiation to the relevant tissue. Thus, transdifferentiation can be a time efficient and less resource-demanding alternative to iPSCs for both functional analysis and personalized drug screening.

iPSCs, and transdifferentiated cells as their alternative, can also be used *in vivo* for cell-replacement therapy. Cell-replacement therapy, involving transplantation of certain cell types, requires xenogenic-free culture protocols, which can be resolved by using transdifferentiation techniques, to minimize potential adverse effects. Another concern of iPSC transplantation is their potentially higher risk of neoplasm (including teratoma) formation.[66] On the other hand, *in vivo* transplantation of transdifferentiated cells, performed in rats and mice was shown to be safe from tumor formation.[67,68] Still, further studies are needed to ascertain the safety in humans.

In conclusion, transdifferentiation of dermal human fibroblasts to osteoblasts provides a suitable *in vitro* model to investigate genetic diseases with bone involvement such as H-SMD (**Chapter 3.1**) or fibrodysplasia ossificans progressiva (FOP)[69] or primary bone diseases such as osteoporosis imperfecta (OI). In the future, this method might be applied not only for exploring possible treatments, but also for cellreplacement therapy.

## Future directions

Our studies provided additional information on MRI in 4H leukodystrophy, the most common hypomyelinating leukodystrophy after Pelizaeus-Merzbacher disease. An MRI scoring system with integrating quantitative MRI parameters will provide a useful tool to monitor future therapeutic studies. In addition, we now have identified patients with POLR3-related disorders without hypomyelination at imaging. The broad application of next generation sequencing techniques will, without doubt, further broaden the spectrum of this disorder and hopefully also give a better insight into possible genotype-phenotype relationships. The application of more advanced MR imaging will also provide more detailed characteristics in hypomyelinating leukodystrophies.



*In vitro* models using manipulated human cells to mimic the affected tissues will become more and more important for genetic disorders, also including hypomyelinating leukodystrophies. Given the unique involvements in tooth and bone are very striking in 4H leukodystrophy and H-SMD, future functional studies should also focus on these tissues. We expect to gain essential insights into the pathomechanisms of these disorders by not only focusing on the CNS. *In vitro* models may also be manipulated with new techniques such as CRISPR-Cas9 to generate different affected cell types with specific mutations, which will be invaluable when mouse models fail to demonstrate the phenotype present in humans as is the case for 4H leukodystrophy.[70] All the approaches either *in vitro* model or gene editing techniques will hopefully bring choices of personalized treatment for the patients.

## References


1. Bernard G, Chouery E, Putorti ML, et al. Mutations of POLR3A encoding a catalytic subunit of RNA polymerase Pol III cause a recessive hypomyelinating leukodystrophy. Am J Hum Genet 2011;89:415-423
2. Daoud H, Tétreault M, Gibson W, et al. Mutations in POLR3A and POLR3B are a major cause of hypomyelinating leukodystrophies with or without dental abnormalities and/or hypogonadotropic hypogonadism. J Med Genet 2013;50:194-197
3. Shimojima K, Shimada S, Tamasaki A, et al. Novel compound heterozygous mutations of POLR3A revealed by whole-exome sequencing in a patient with hypomyelination. Brain Dev May 2013
4. Saitsu H, Osaka H, Sasaki M, et al. Mutations in POLR3A and POLR3B encoding RNA Polymerase III subunits cause an autosomal-recessive hypomyelinating leukoencephalopathy. Am J Hum Genet 2011;89:644-651
5. Thiffault I, Wolf NI, Forget D, et al. Recessive mutations in POLR1C cause a leukodystrophy by impairing biogenesis of RNA polymerase III. Nat Commun 2015;6:7623
6. Cayami FK, La Piana R, van Spaendonk RML, et al. POLR3A and POLR3B Mutations in Unclassified Hypomyelination. Neuropediatrics 2015;46:221-228
7. Simons C, Dyment D, Bent SJ, et al. A recurrent de novo mutation in TMEM106B causes hypomyelinating leukodystrophy. Brain November 2017
8. Nafisinia M, Sobreira N, Riley L, et al. Mutations in RARS cause a hypomyelination disorder akin to Pelizaeus–Merzbacher disease. Eur J Hum Genet 2017;25:1134-1141
9. Nakayama T, Wu J, Galvin-Parton P, et al. Deficient activity of alanyl-tRNA synthetase underlies an autosomal recessive syndrome of progressive microcephaly, hypomyelination, and epileptic encephalopathy. Hum Mutat 2017;38:1348-1354
10. Dorboz I, Aiello C, Simons C, et al. Biallelic mutations in the homeodomain of NKX6-2 underlie a severe hypomyelinating leukodystrophy. Brain 2017;140:2550-2556
11. Chelban V, Patel N, Vandrovcova J, et al. Mutations in NKX6-2 Cause Progressive Spastic Ataxia and Hypomyelination. Am J Hum Genet 2017;100:969-977
12. Arai-Ichinoi N, Uematsu M, Sato R, et al. Genetic heterogeneity in 26 infants with a hypomyelinating leukodystrophy. Hum Genet 2016;135:89-98
13. Shahrour MA, Ashhab M, Edvardson S, Gur M, Abu-Libdeh B, Elpeleg O. Hypomyelinating leukodystrophy associated with a deleterious mutation in the ATRN gene. Neurogenetics 2017;18:135-139
14. Bakshi R, Neema M, Healy BC, et al. Predicting clinical progression in multiple sclerosis with the magnetic resonance disease severity scale. Arch Neurol 2008;65:1449-1453
15. Eichler F, Grodd W, Grant E, et al. Metachromatic Leukodystrophy: A Scoring System for Brain MR Imaging Observations. Am J Neuroradiol 2009;30:1893-1897

16. Loes DJ, Peters C, Krivit W, et al. Globoid cell leukodystrophy: distinguishing early-onset from late-onset disease using a brain MR imaging scoring method. AJNR Am J Neuroradiol 1999;20:316-323

17. Loes DJ, Hite S, Moser H, et al. Adrenoleukodystrophy: a scoring method for brain MR observations. AJNR Am J Neuroradiol 1994;15:1761-1766

18. Pouwels PJW, Vanderver A, Bernard G, et al. Hypomyelinating leukodystrophies: Translational research progress and prospects. Ann Neurol 2014;76:5-19

19. Barkovich AJ, Deon S. Hypomyelinating disorders: An MRI approach. Neurobiol Dis 2016;87:50-58

20. Gareau PJ, Rutt BK, Karlik SJ, Mitchell JR. Magnetization transfer and multicomponent T2 relaxation measurements with histopathologic correlation in an experimental model of MS. J Magn Reson Imaging 2000;11:586-595

21. MacKay A, Laule C, Vavasour I, Bjarnason T, Kolind S, Mädler B. Insights into brain microstructure from the T2 distribution. Magn Reson Imaging 2006;24:515-525

22. Steenweg ME, Wolf NI, van Wieringen WN, Barkhof F, van der Knaap MS, Pouwels PJW. Quantitative MRI in hypomyelinating disorders. Neurology 2016;87:752-758

23. Piana R La, Tonduti D, Dressman HG, et al. Brain Magnetic Resonance Imaging (MRI) Pattern Recognition in Pol III-Related Leukodystrophies. J Child Neurol 2014;29:214-220

24. Jay AM, Conway RL, Thiffault I, et al. Neonatal progeriod syndrome associated with biallelic truncating variants in POLR3A. Am J Med Genet A 2016;170:3343-3346

25. Paolacci S, Bertola D, Franco J, et al. Wiedemann-Rautenstrauch syndrome: A phenotype analysis. Am J Med Genet A 2017;173:1763-1772

26. Minnerop M, Kurzwelly D, Wagner H, et al. Hypomorphic mutations in POLR3A are a frequent cause of sporadic and recessive spastic ataxia. Brain 2017;140:1561-1578

27. Richards MR, Plummer L, Chan Y-M, et al. Phenotypic spectrum of POLR3B mutations: isolated hypogonadotropic hypogonadism without neurological or dental anomalies. J Med Genet 2017;54:19-25

28. Jurkiewicz E, Dunin-Wąsowicz D, Gieruszczak-Białek D, et al. Recessive Mutations in POLR3B Encoding RNA Polymerase III Subunit Causing Diffuse Hypomyelination in Patients with 4H Leukodystrophy with Polymicrogyria and Cataracts. Clin Neuroradiol 2017;27:213-220

29. Ghoumid J, Petit F, Boute-Benejean O, et al. Cerebellar hypoplasia with endosteal sclerosis is a POLR3-related disorder. Eur J Hum Genet 2017;25:1011-1014

30. Ogunjimi B, Zhang S-Y, Sørensen KB, et al. Inborn errors in RNA polymerase III underlie severe varicella zoster virus infections. J Clin Invest 2017;127:3543-3556

31. Dauwerse JG, Dixon J, Seland S, et al. Mutations in genes encoding subunits of RNA polymerases I and III cause Treacher Collins syndrome. Nat Genet 2011;43:20-22

32. Schaefer E, Collet C, Genevieve D, et al. Autosomal recessive POLR1D mutation with decrease of TCOF1 mRNA is responsible for Treacher Collins syndrome. Genet Med 2014;16:720-724

33. Weaver KN, Watt KEN, Hufnagel RB, et al. Acrofacial Dysostosis, Cincinnati Type, a Mandibulofacial Dysostosis Syndrome with Limb Anomalies, Is Caused by POLR1A Dysfunction. Am J Hum Genet 2015;96:765-774

34. Goodfellow SJ, Zomerdijk JCBM. Basic mechanisms in RNA polymerase I transcription of the ribosomal RNA genes. Subcell Biochem 2013;61:211-236

35. Kara B, Köroğlu Ç, Peltonen K, et al. Severe neurodegenerative disease in brothers with homozygous mutation in POLR1A. Eur J Hum Genet 2017;25:315-323

36. Tétreault M, Choquet K, Orcesi S, et al. Recessive mutations in POLR3B, encoding the second largest subunit of Pol III, cause a rare hypomyelinating leukodystrophy. Am J Hum Genet 2011;89:652-655

37. Neubauer BA, Stefanova I, Hübner CA, et al. A new type of leukoencephalopathy with metaphyseal chondrodysplasia maps to Xq25-q27. Neurology 2006;67:587-591

38. Mierzewska H, Rydzanicz M, Biegański T, et al. Spondyloepimetaphyseal dysplasia with neurodegeneration associated with AIFM1 mutation - a novel phenotype of the mitochondrial disease. Clin Genet 2017;91:30-37

39. Cowchock FS, Duckett SW, Streletz LJ, et al. X-linked motor-sensory neuropathy type-II with deafness and mental retardation: A new disorder. Am J Med Genet 1985;20:307-315

40. Rinaldi C, Grunseich C, Sevrioukova IF, et al. Cowchock Syndrome Is Associated with a Mutation in Apoptosis-Inducing Factor. Am J Hum Genet 2012;91:1095-1102

41. Zong L, Guan J, Ealy M, et al. Mutations in apoptosis-inducing factor cause X-linked recessive auditory neuropathy spectrum disorder. J Med Genet 2015;52:523-531

42. Ghezzi D, Sevrioukova I, Invernizzi F, et al. Severe X-linked mitochondrial encephalomyopathy associated with a mutation in apoptosis-inducing factor. Am J Hum Genet 2010;86:639-649

43. Ardissone A, Piscosquito G, Legati A, et al. A slowly progressive mitochondrial encephalomyopathy widens the spectrum of AIFM1 disorders. Neurology 2015;84:2193-2195

44. Asagiri M, Takayanagi H. The molecular understanding of osteoclast differentiation. Bone 2007;40:251-264

45. Wise GE. Cellular and molecular basis of tooth eruption. Orthod Craniofac Res 2009;12:67-73

46. Wise GE, Frazier-Bowers S, D'Souza RN. Cellular, Molecular, and Genetic Determinants of Tooth Eruption. Crit Rev Oral Biol Med 2002;13:323-334

47. Marks SC. Pathogenesis of osteopetrosis in theia rat: Reduced bone resorption due to reduced osteoclast function. Am J Anat 1973;138:165-189

48. Cotton WR, Gaines JF. Unerupted dentition secondary to congenital osteopetrosis in the Osborne-Mendel rat. Proc Soc Exp Biol Med 1974;146:554-561

49. Chen I-P. The Use of Patient-Specific Induced Pluripotent Stem Cells (iPSCs) to Identify Osteoclast Defects in Rare Genetic Bone Disorders. J Clin Med 2014;3:1490-1510

50. Jeon OH, Panicker LM, Lu Q, Chae JJ, Feldman RA, Elisseeff JH. Human iPSC-derived osteoblasts and osteoclasts together promote bone regeneration in 3D biomaterials. Sci Rep 2016;6:26761

51. Grigoriadis AE, Kennedy M, Bozec A, et al. Directed differentiation of hematopoietic precursors and functional osteoclasts from human ES and iPS cells. Blood 2010;115:2769-2776

52. Pruksananonda K, Rungsiwiwut R. Moving toward Xeno-free Culture of Human Pluripotent Stem Cells. In: *Pluripotent Stem Cells - From the Bench to the Clinic*. InTech; 2016.

53. Lachmann N, Ackermann M, Frenzel E, et al. Large-Scale Hematopoietic Differentiation of Human Induced Pluripotent Stem Cells Provides Granulocytes or Macrophages for Cell Replacement Therapies. Stem Cell Reports 2015;4:282-296

54. Davis RL, Weintraub H, Lassar AB. Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell 1987;51:987-1000

55. Huang P, Zhang L, Gao Y, et al. Direct Reprogramming of Human Fibroblasts to Functional and Expandable Hepatocytes. Cell Stem Cell 2014;14:370-384

56. Cieślar-Pobuda A, Rafat M, Knoflach V, et al. Human induced pluripotent stem cell differentiation and direct transdifferentiation into corneal epithelial-like cells. Oncotarget 2016;7:42314-42329

57. Xu Z, Chu X, Jiang H, Schilling H, Chen S, Feng J. Induced dopaminergic neurons: A new promise for Parkinson's disease. Redox Biol 2017;11:606-612

58. Chen Y, Yang Z, Zhao Z-A, Shen Z. Direct reprogramming of fibroblasts into cardiomyocytes. Stem Cell Res Ther 2017;8:118

59. Cao N, Huang Y, Zheng J, et al. Conversion of human fibroblasts into functional cardiomyocytes by small molecules. Science 2016;352:1216-1220

60. Kim JH, Liu X, Wang J, et al. Wnt signaling in bone formation and its therapeutic potential for bone diseases. Ther Adv Musculoskelet Dis 2013;5:13-31

61. Xie X, Fu Y, Liu J. Chemical reprogramming and transdifferentiation. Curr Opin Genet Dev 2017;46:104-113

62. Qin H, Zhao A, Fu X. Small molecules for reprogramming and transdifferentiation. Cell Mol Life Sci 2017;74:3553-3575

63. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-676

64. Hunsberger JG, Efthymiou AG, Malik N, et al. Induced Pluripotent Stem Cell Models to Enable In Vitro Models for Screening in the Central Nervous System. Stem Cells Dev 2015;24:1852-1864

65. Prasad A, Manivannan J, Loong DTB, Chua SM, Gharibani PM, All AH. A review of induced pluripotent stem cell, direct conversion by trans-differentiation, direct reprogramming and oligodendrocyte differentiation. Regen Med 2016;11:181-191

66. Cieślar-Pobuda A, Knoflach V, Ringh M V., et al. Transdifferentiation and

reprogramming: Overview of the processes, their similarities and differences. Biochim Biophys Acta - Mol Cell Res 2017;1864:1359-1369

67. Chen Z, Niu M, Sun M, et al. Transdifferentiation of human male germline stem cells to hepatocytes in vivo via the transplantation under renal capsules. Oncotarget 2017;8:14576-14592

68. Yao H, Gao M, Ma J, et al. Transdifferentiation-Induced Neural Stem Cells Promote Recovery of Middle Cerebral Artery Stroke Rats. Ai J, ed. PLoS One 2015;10:e0137211

69. Micha D, Voermans E, Eekhoff MEW, et al. Inhibition of TGFβ signaling decreases osteogenic differentiation of fibrodysplasia ossificans progressiva fibroblasts in a novel in vitro model of the disease. Bone 2016;84:169-180

70. Choquet K, Yang S, Moir RD, et al. Absence of neurological abnormalities in mice homozygous for the Polr3a G672E hypomyelinating leukodystrophy mutation. Mol Brain 2017;10:13

## Summary

Hypomyelinating leukodystrophies are a group of diseases with large variability in their genetic background, less so in their clinical and radiological features. MRI pattern recognition and advances in genetic testing are now able to classify most hypomyelinating leukodystrophies. However, the phenotypic differences can be considerable even in a single hypomyelinating leukodystrophy, such as 4H syndrome. This thesis explores the radiological and genetic differences of hypomyelinating leukodystrophies, particularly 4H leukodystrophy, as described in **Chapter 2**, and describes a novel intriguing hypomyelinating leukodystrophy with unique involvement of bone tissue (hypomyelination with spondylometaphyseal dysplasia, H-SMD) in **Chapter 3**. It also validates an *in vitro* model of direct transdifferentiation of fibroblasts to osteoblast-like cells to investigate bone involvement.



### MRI in hypomyelination leukodystrophies

**Chapter 2.1** describes the importance of pattern recognition in 4H leukodystrophy. Without typical 4H leukodystrophy MRI findings, direct genetic testing of POLR3 genes is not recommended. Instead, alternative diagnoses of other hypomyelinating leukodystrophies and utilization of whole exome sequencing (WES) should be considered. **Chapter 2.2** explores the utilization of MRI not only as diagnostic tool, but also as a tool correlating to clinical disease severity. This MRI scoring system, based on the degree of hypomyelination and atrophy in 4H leukodystrophy, can be simply applied for future studies such as to monitor diseases progression in clinical trials or be adapted as biomarker for other hypomyelinating leukodystrophies.

**Chapter 2.3** provides new MRI features of 4H leukodystrophy which are clearly visible on 3T MRI: myelin islets, closed eye sign and cyst-like lesion in the splenium. On the other hand, diffuse hypomyelination is no longer an obligatory MRI feature for 4H leukodystrophy as described in **Chapter 2.4**. Six patients with *POLR3A* mutations and two patients with *POLR3B* mutations all had either partial hypomyelination or

adequate myelination, but two distinct patterns: specific involvement of corticospinal tracts in four out of six patients with *POLR3A* mutations and cerebellar atrophy in absence of diffuse hypomyelination in patients with either *POLR3A* or *POLR3B* mutations. Other classical clinical criteria – hypodontia and hypogonadotropic hypogonadism – may still suggest the correct diagnosis, even when the cardinal MRI features are lacking.

## Bone involvement in hypomyelinating leukodystrophies

**Chapter 3.1** explores the non-neurological involvement in a unique hypomyelination leukodystrophy, H-SMD. Diffuse hypomyelination accompanied by bone abnormalities, spondylometaphyseal dysplasia, in a group of 12 patients led to identification of mutations in or near exon 7 of the *AIFM1* gene, within a region of 70 base pairs. When analyzing WES data, these mutations were initially overlooked as some of them were intronic or synonymous, and also because *AIFM1* mutations were previously associated with other distinct clinical presentations without bone abnormalities. By using an *in vitro* model, which mimics the involved tissue, the effect of the mutations, namely a reduced expression of *AIFM1* on mRNA and protein level only in osteoblasts without affecting the fibroblasts, could be confirmed although the specific mechanism still needs to be elucidated.



An *in vitro* model applied for H-SMD was validated in **Chapter 3.2**. Highly efficient platelet lysate-based transdifferentiation of skin-derived fibroblasts to osteoblasts-like cells was characterized on functional, protein and mRNA level. Positive staining of mineralization assays, positive immunofluorescence staining of osteoblast-specific proteins and significantly increased mRNA expression of osteoblast-specific markers confirmed the properties of transdifferentiated osteoblasts. RNA-seq supported the successful transdifferentiation by clustering transdifferentiated cells separately from fibroblasts and showed significant upregulation of two important pathways in bone differentiation involving WNT and BMP.