Functional analysis of a hypomorphic allele shows MMP14 catalytic activity is the prime determinant of the Winchester syndrome phenotype

Cindy Lee, Hor Jun Ying, Keith Goh
Supervisor: Prof Maurice van Steensel

A MISDIAGNOSIS; A NEW DISCOVERY

2007 - two Dutch brothers initially thought to have Borrone syndrome have instead an intrinsically milder phenotype without SH3PXD2 mutation. A **novel** homozygous missense mutation in **MMP14**, substitution **p.Arg111His (R111H)**, was identified.

MMP14 encodes membrane-bound metalloproteinase, which requires cleavage of N-terminal pro-domain sequence for activation. MMP14 mutations result in **Winchester Syndrome (WS)**, characterised by recessive skeletal dysplasia with osteolysis, encompassing multicentric osteolysis and arthropathy.

AIMS OF STUDY

Effects of R111H mutation on MMP14 intracellular processing and function, compared to known mutations

To connect loss of MMP14 activity and clinical manifestations of WS, using knockout (KO) zebrafish model

METHODS

ASSESSING MMP14 ACTIVITY IN VITRO

Immunoblot
Pro-MMP14 is tagged with HA and EGFP. Presence of EGFP alone indicates removal of HA and, hence, activation of MMP14

Immuno-fluorescence microscopy
Visualise EGFP tagged-MMP14 trafficking to cell membrane

Gelatin degradation
Test MMP14 catalytic activity

Gelatin zymography
Visualise cleavage of pro-MMP2 to MMP2 by MMP14

Fig 1. Known MMP14 mutations and target sites. N-terminal HA-tag and C-terminal EGFP-tag.

ZEBRAFISH MODEL

1. **CRISPR-Cas9** was used to knock out MMP14a/b in zebrafish
2. **Microcomputed Tomography** to visualize skeletal changes in KO zebrafish
3. **Histology** of zebrafish bone to visualise bone ossifications

MMP14 INTRACELLULAR PROCESSING

MMP14 Post-translational Processing

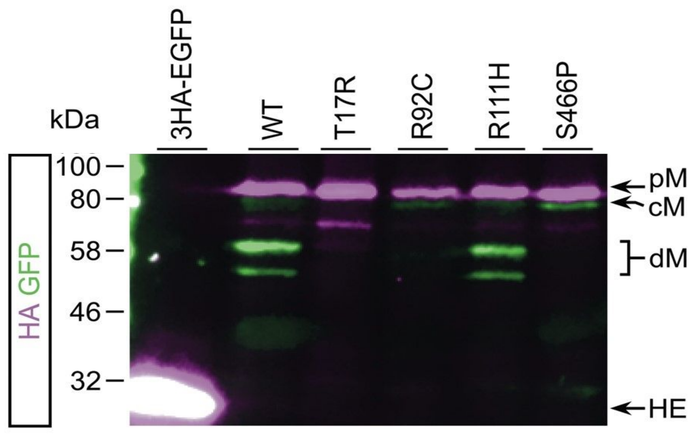

Fig 2. Western blot with anti-HA and anti-GFP

EGFP signal present for WT and R111H, indicating cleavage of HA-tagged pro-domain and successful activation of MMP14 metalloproteinase

MMP14 Localisation

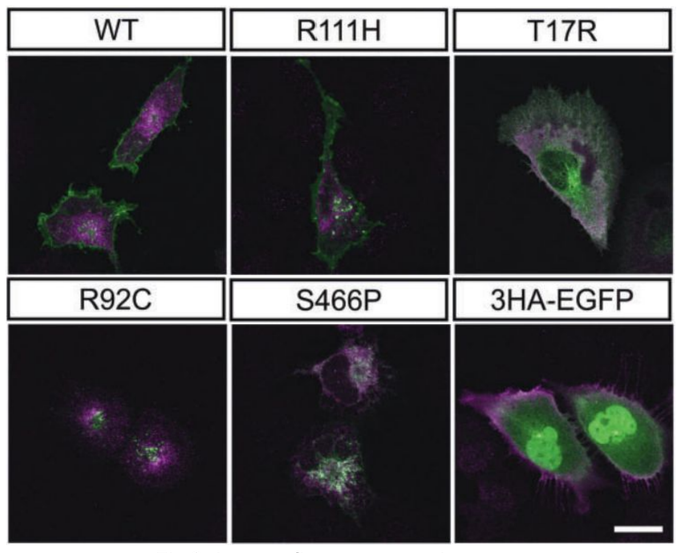

Fig 3. Immunofluorescence microscopy

Presence of EGFP signal at the cell membrane for R111H, indicating normal intracellular trafficking of MMP14 encoded metalloproteinase

MMP14 CATALYTIC ACTIVITY

Pro-MMP2 Cleavage

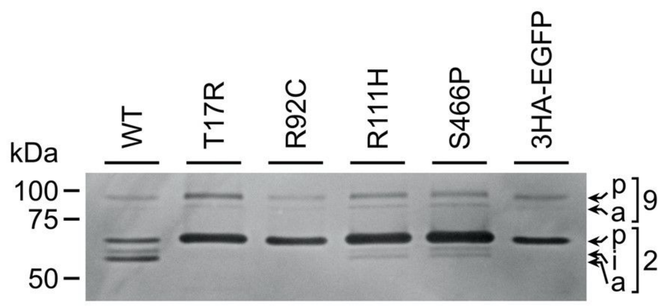

Fig 4. Gelatin zymography

MMP14-WT activates pro-MMP2 (p2) to its intermediate (i2) and active (a2) forms. Pro-MMP2 cleavage activity is partially retained in R111H and S466P

Gelatin Degradation

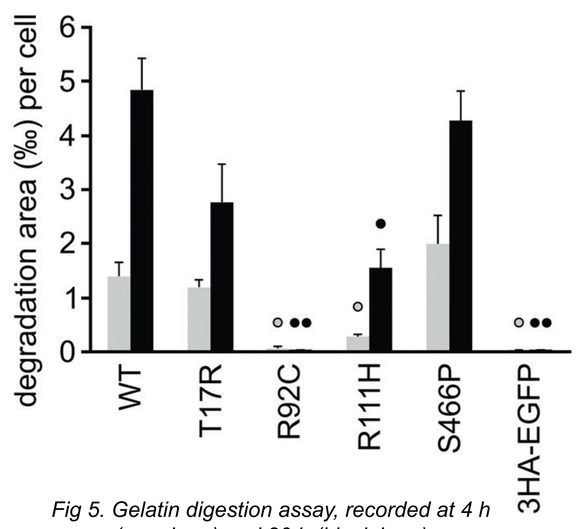

Fig 5. Gelatin digestion assay, recorded at 4 h (grey bars) and 20 h (black bars)

R92C mutant abolishes gelatin degradation, R111H mutant retains some activity, T17R and S466P mutants do not impair gelatin degradation

MMP14A/B KO ZEBRAFISH MODEL

MMP14a/b KO Zebrafish Phenotype

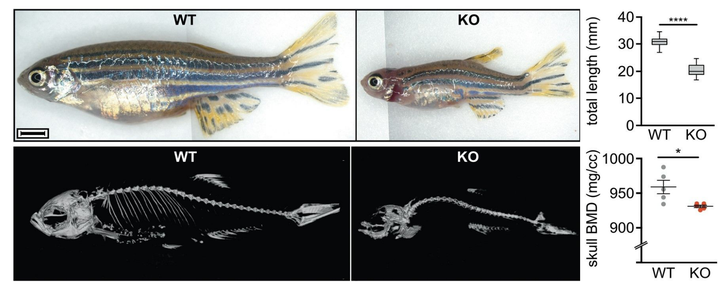

Fig 6. Microcomputed tomography

- Small head showing dorsal hyperextension
- Weberian-prehemal hyperkyphosis (angle formed by lines connecting Weberian and prehemal vertebral bodies)
- Decreased total body length
- Reduced skull bone mineral density

MMP14-dependent Collagen Remodeling

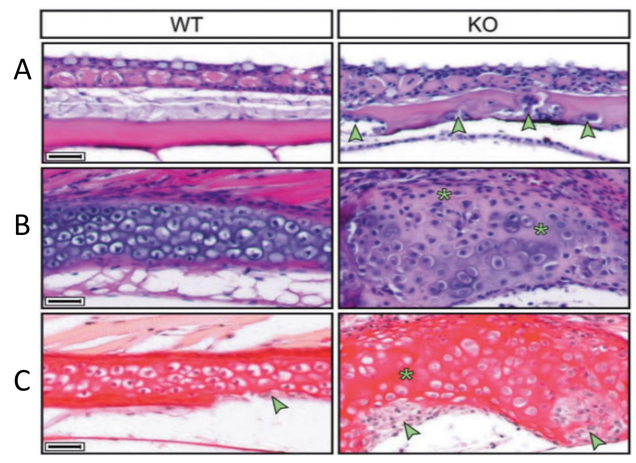

Fig 7. Histology [A] frontal bone - irregularly thickened with cell clusters (arrowheads), [B] supraoccipital bone - cell-free area in cartilage core, devoid of proteoglycans (asterisks), [C] supraoccipital bone - collagen-poor peripheral bone matrix with large cell-rich areas (arrowheads)

Loss of MMP14a/b results in impaired membranous and endochondral ossification

DISCUSSION

- **Pro-MMP2 cleavage by MMP14 may be the most relevant activity to the WS phenotype:** Although T17R impaired gelatin degradation to a lesser extent than R111h, it completely abrogated pro-MMP2 cleavage *(Fig 8.)*. With T17R having a worse phenotype and supportive evidence from other studies that highlighted the loss of MMP2 resulting in WS phenotypes, pro-MMP2 cleavage may be the most relevant activity of MMP14 to the WS phenotype[1, 2]. Therefore, as pro-MMP2 is only partially impaired in R111H as compared to the other mutation, it explains the milder phenotype observed in the 2 Dutch brothers.
- **Novel animal model:** MMP14a/b KO zebrafish encapsulating the essential aspects (eg. condition worsening with age and disruption of skeletal remodeling) of WS, it is a promising animal model for investigating WS.
- **Wrong treatment?:** Current treatment of WS with bisphosphonates, which targets osteoclast, may not be ideal as the main pathophysiology of WS is the disruption of ossification due to disorganization of cartilage *(Fig 7.)*. This is supported by the worsening phenotype with age which corresponds to the later stages of skeletal development. Hence, a treatment targeting the disorganization of collagen may be a better option.

MMP14	Intracellular Processing		Catalytic Activity	
	MMP14 processing	MMP14 localisation	Pro-MMP2 cleavage	Gelatin degradation
WT	●	●	●	●
R111H	●	●	●	●
T17R	●	●*	●	●
R92C	●	●	●	●
S466P	●	●	●	●

Fig 8. Green: Unaltered, Amber: Impaired, Red: Severely impaired/absent

Limitations

- Does not encapsulate the full catalytic activity of MMP14 which includes other extracellular matrix proteins (eg. α-1 integrin) and membrane proteins (eg. CD44)[3].
- K/O Zebrafish was used instead of specific MMP14 mutation. Unlike humans, zebrafish contains MMP14a and MMP14b, but previous observed studies on MMP14b have been conflicting. Therefore, the study KO both MMP14a and MMP14b.
- Cause of early death in zebrafish and human is different. Spinal cord impingement was the main cause in zebrafish, while heart failure is the main cause in humans.

References

1. Zankl,A.,Bonafe,L.,Calcaterra,V.,DiRocco,M.and Superti-Furga, A. (2005) Winchester syndrome caused by a homozygous mutation affecting the active site of matrix metalloproteinase 2. Clin. Genet., 67, 261–266.
2. Rouzier, C., Vanatka, R., Bannwarth, S., Philip, N., Coussement, A., Paquis-Flucklinger, V. and Lambert, J.C. (2006) A novel homozygous MMP2 mutation in a family with Winchester syndrome. Clin. Genet., 69, 271–276.
3. Andrieu, C., Montigny, A., Bibonne, A., Despin-Guitard, E., Alfandari, D., & Théveneau, E. (2020, April 1). MMP14 is required for delamination of chick neural crest cells independently of its catalytic activity. Development. Retrieved December 16, 2021, from https://doi.org/10.1242/dev.183954

To watch the presentation, please click on the video below: