Functional and morphological phenotypes of the mouse thyroid gland associated with thyroid-specific Mct8 deficiency

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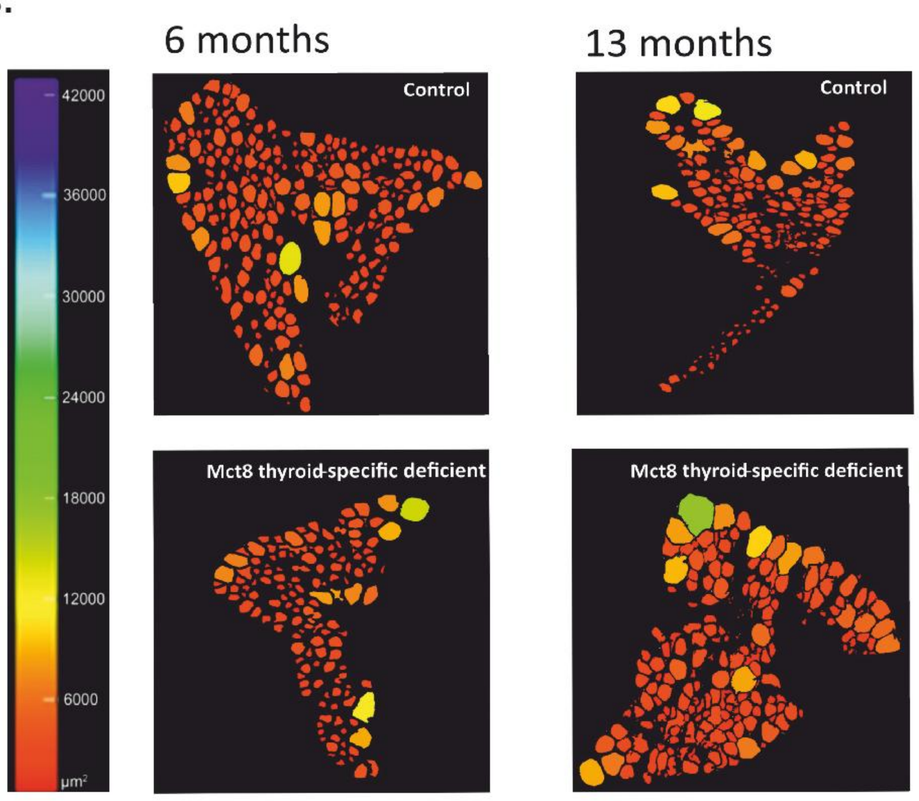
Introduction: Mct8 is a thyroid hormone-specific transporter located at the basolateral plasma membrane domain of thyrocytes[1]. To investigate the significance of Mct8 to the thyroid gland, while excluding peripheral effects observed in global knockout models, we used a Cre-LoxP thyroid-specific Mct8-deficient mouse model. Phenotypes of this model were investigated with respect to morphology of the gland, as well as its functional role in the processing of thyroglobulin to active thyroid hormone, T3.

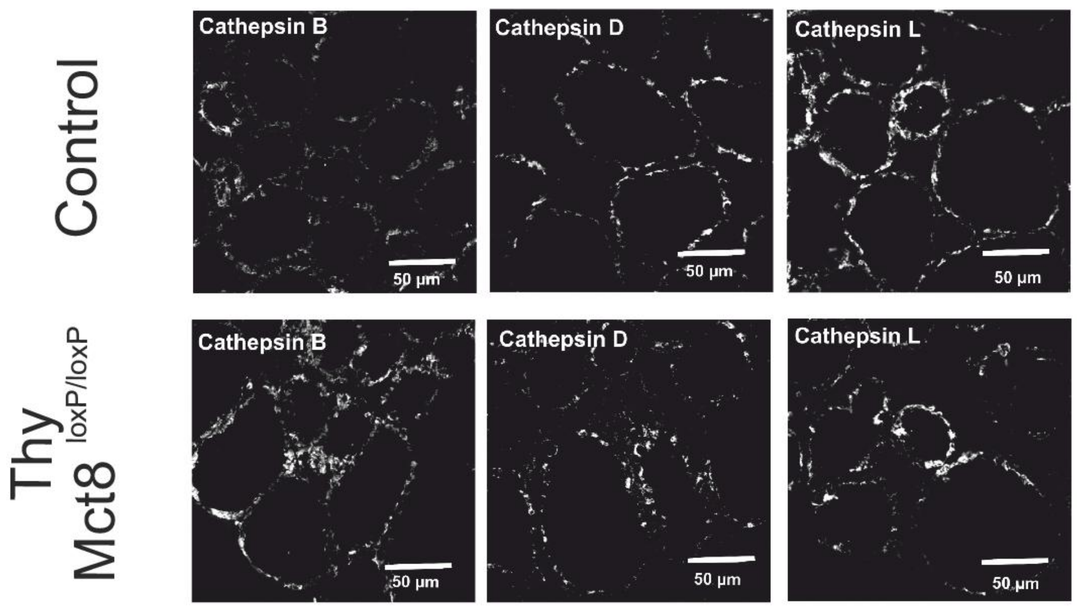
Methods: Morphology of the thyroid follicles and thyrocytes were investigated using a computer-based cell biology toolbox. Indirect immunofluorescence of tissue cryosections was used to analyse thyroglobulin status, along with expression and localisation of thyroglobulin-processing cathepsins.

Results:

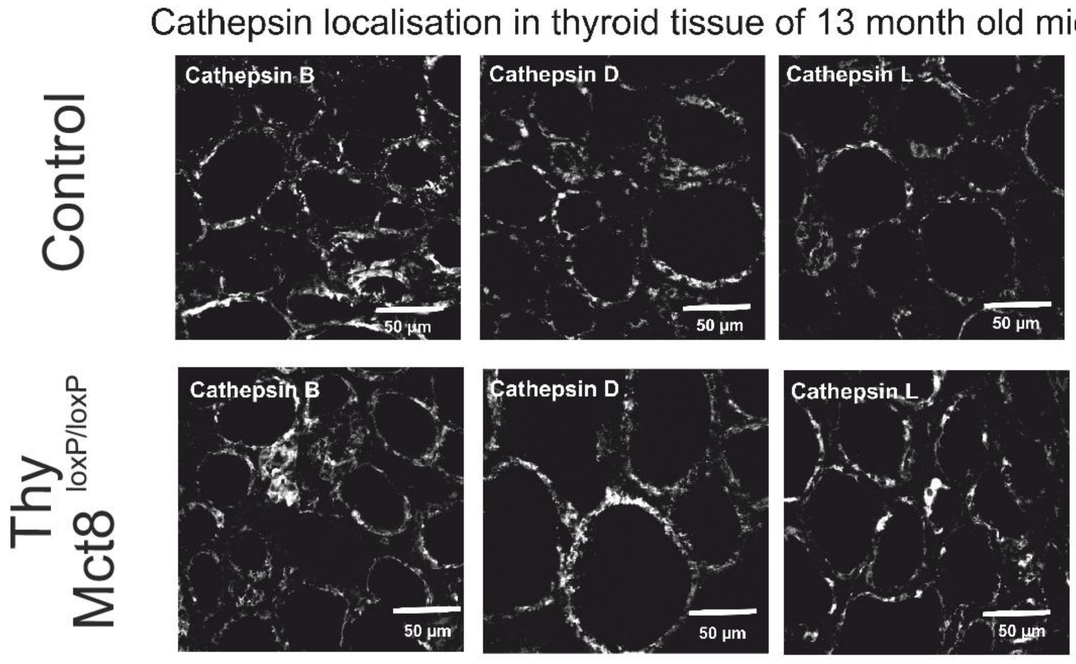


Thyroid morphology: Average lumen size, indicative of thyroglobulin storage volume, of the thyroid gland did not deviate with respect to Mct8 thyroid-specific deficiency. However, initial analysis suggests there is a high cell death rate in the Mct8 deficient model as well as an increase in epithelial extension into the follicle lumen, indicative of increased thyrocyte activity.

Cathepsin localisation in thyroid tissue of 6 month old mice



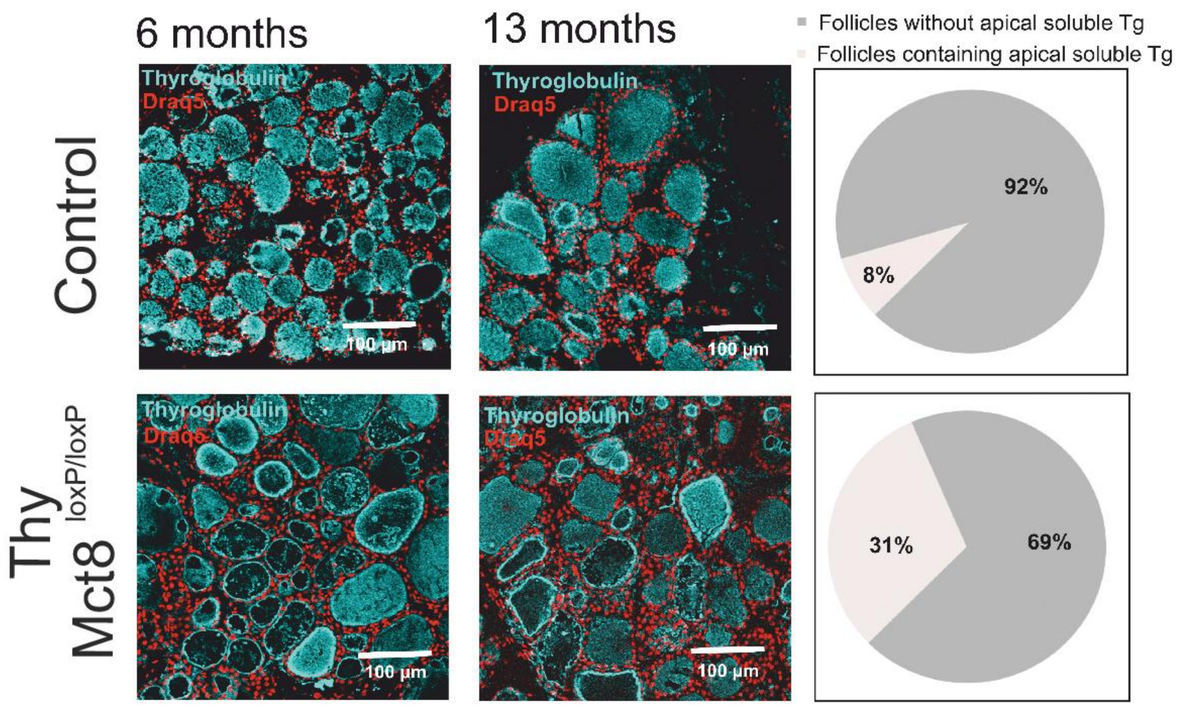
Cathepsin localisation in thyroid tissue of 13 month old mice



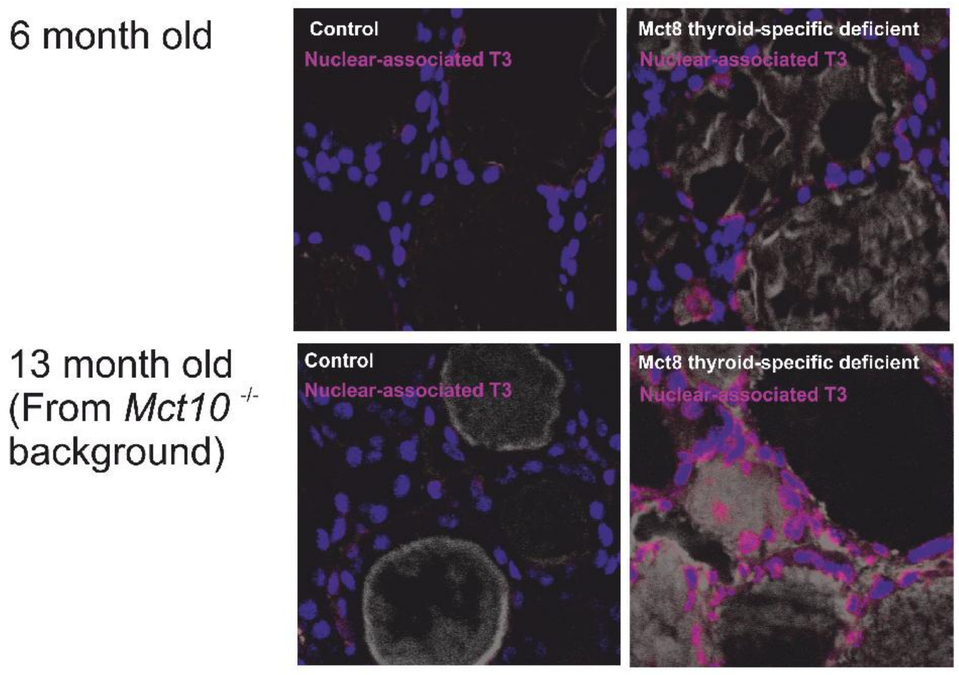
Cathepsin expression:

Cathepsin expression and localisation: Altered localisation and increased protein levels of cathepsins were observed in Mct8 thyroid-specific deficient animals over the control.

Thyroglobulin degradation status:



State of thyroglobulin: The more intense staining of thyroglobulin at the apical plasma membrane of Mct8 thyroid-specific deficient thyrocytes is indicative of greater solubility of thyroglobulin which may be due to enhanced amounts and more prominent localisation of thyroglobulin-processing proteases at the apical plasma membrane in thyroid Mct8 deficiency.



T3 at the nucleus: Enhanced localisation of active thyroid hormone T3 at the nucleus and in the cytoplasm was observed in the Mct8 thyroid-specific deficient model. This could be indicative of a thyrotoxic state. Greater T3 localisation to the nucleus was observed in the older animals with additional *Mct10* $^{-/-}$.

Conclusion: In the case of Mct8 thyroid-specific deficient animals, an altered thyroglobulin staining pattern is observed indicative of greater thyroglobulin-processing protease activity. Despite greater thyroglobulin utilisation, average follicle size does not vary significantly. This may be due to the looser compaction of thyroglobulin keeping follicle size relatively constant despite a lower protein level of thyroglobulin in the lumen. An increase in epithelial extension of the thyrocyte monolayer and changes in cathepsin expression, and localisation of cathepsins D and L are observed. Cathepsins B and L are known to process thyroglobulin in the follicle lumen and cathepsin D contributes to thyroid hormone utilisation[2]. This enhanced thyroglobulin processing occurs despite thyroid hormone release from the thyrocyte being impaired, resulting in an intracellular thyrotoxic state. This indicates a functional/regulatory link between thyroid hormone transporter Mct8 with the thyroid hormone liberating proteases.

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2. Friedrichs, B., Tepel, C., Reinheckel, T., Deussing, J., von Figura, K., Herzog, V., Peters, C., Saftig, P., and Brix, K. (2003). Thyroid functions of mouse cathepsins B, K, and L. Journal of Clinical Investigation *111*, 1733-1745