Is thyroid status a common denominator of age-related disease?

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CHAPTER 10

General discussion and future perspectives

GENERAL DISCUSSION

In this thesis, we aimed to investigate the potential causality of associations between circulating concentrations of thyroid parameters and markers of age-related diseases. We conducted multi-cohort studies, Mendelian randomization and a combination of those two techniques in an attempt to approach a causal estimate in the absence of sufficiently large randomized trials.

The research described in this thesis was not performed in isolation, but within a larger framework of diverse disciplines and fields of research. Fundamental research into physiology and the pathophysiological mechanisms of diseases is essential for gaining deeper understanding of disease etiologies. Studies involving human participants are often not suitable for this type of research. Within the THYRAGE project, several novel techniques were developed with cultured cells and with model organisms such as mice and tadpoles.[1, 2] These advanced techniques have contributed greatly to the knowledge of thyroid hormone action at the cellular level. In the brain and skeleton, the presence of specific thyroid hormone transporters was shown to be an essential regulator of maintenance and repair.[3, 4] Moreover, the balanced expression of deiodinases (enzymes activating and deactivating thyroid hormones) proved crucial for growth of skeletal muscle and of malignant tumors.[5, 6] However, the most striking common finding in all of these different tissues has been the importance of spatiotemporal fine-tuning of thyroid hormone levels dependent on the current needs of tissues and even at single-cell level.[7] Thus, thyroid hormones do appear to play a causal role in tissue maintenance and repair, although the accuracy of timing and tuning might be more important than the overall thyroid status. We hypothesized that loss of tissue maintenance and repair contributes to the aging process and to the development of age-related disease, which was further elaborated in **Chapter 2**. By extension, we hypothesized that thyroid hormones might also be causally involved in aging and age-related diseases.

Translation of fundamental research to human subjects is complex. Classical observational studies can be performed to assess whether similar relationships are apparent. Since the most universal characteristic of aging is increasing mortality[8], we first explored the association between thyroid parameters and mortality. As described in **Chapter 3**, we observed a lower mortality in nonagenarians with relatively more active thyroid hormone (fT3) and relatively less inactive thyroid hormone (fT4) in the circulation. As this observation was irrespective of familial longevity, we concluded that those parameters might be a universal characteristic of longevity. Interestingly, circulating TSH levels were not associated with mortality in this population. These observations were in line with another study in a younger cohort with a mean age of 65 years; here higher

fT4 was also consistently associated with higher mortality while associations with TSH were inconsistent.[9]

In addition, we were interested whether thyroid status was associated with age-related diseases. Within the thyroid studies collaboration several large multi-cohort studies have been performed using individual participant data; these studies have indicated associations between thyroid dysfunction and coronary heart disease, heart failure, fractures and anemia.[10-13] We replicated the association between thyroid dysfunction and anemia based on clinical diagnosis registration in **Chapter 6**. As the literature for the relationship between thyroid status and cognitive decline had been inconclusive[14-17], we performed a multicohort study using individual participant data described in **Chapter 4.** In this study we did not find evidence for an association between a single measurement of thyroid dysfunction and cognitive function, future cognitive decline or developing dementia. These negative findings were in line with another multi-cohort study among individuals of 80 years or older, in which no association was observed between subclinical or overt thyroid dysfunction and cognitive function and various other functional outcomes.[18] Overall, the classic observational studies so far did show some associations between thyroid status and age-related diseases though causality cannot be ascertained with these designs.

In addition to classical observational studies, Mendelian randomization can be applied as an alternative approach to investigate potential causal associations. In Mendelian randomization, genetic variation is used as a natural experiment to allocate traits randomly, presumably independent of other characteristics.[19] This independence of traits is an assumption based on Mendel's second law, though in practice it has been difficult to prove with more complex traits. Nevertheless, the types of bias encountered are generally different from classical observational studies, which at least offers a complementary source of evidence. In **Chapter 5**, we assessed whether genetically determined variation in TSH was associated with bone mineral density as an intermediate outcome for osteoporosis. We did not observe an association, which could be interpreted as no relationship between long-term slightly lower TSH within the reference range and bone strength. A more recent study using Mendelian randomization also did not find an association between genetically determined hyperthyroidism and bone mineral density, though it is important to realize that individuals who develop hyperthyroidism are usually treated successfully to euthyroidism.[20] Another disease we explored with Mendelian randomization was anemia in **Chapter 6**, for which we did not find any association with genetically determined variation in circulating levels of TSH or fT4. We did however find some indications that intracellular regulation of thyroid hormone levels may play a role in developing anemia. For diabetes mellitus, several Mendelian randomization studies have

been conducted to assess potential causal effects of thyroid status, though with mixed results.[21-23] An added level of complexity is the interplay of both thyroid function and diabetes mellitus with obesity. A recent Mendelian randomization study provided some evidence that obesity leads to an increase in circulating TSH and fT3 but not fT4, though no associations were observed vice versa.[24] In **Chapter 7**, we attempted to disentangle the association of thyroid status, body mass index and diabetes mellitus using Mendelian randomization. Overall we did not observe an association of genetically determined thyroid status and diabetes mellitus, though higher TSH might be protective for diabetes mellitus only in individuals with an intrinsically lower risk of high body mass. In **Chapter 8** and **9** we investigated the association between genetically determined thyroid status and risk of coronary artery disease. Upon first exploration in **Chapter 8**, no association was found between genetically determined TSH or fT4 and coronary artery disease. However, this first study had certain important limitations; the variation explained in TSH and fT4 by the genetic instruments was only modest and the study population for the outcome was of mixed ancestry. Therefore we revisited the same hypothesis, but with stronger genetic instruments in a larger and more homogeneous population in **Chapter 9**. Despite the more rigorous design, we only found a slight increase in risk of coronary artery disease with increase in TSH. These findings are in line with other Mendelian randomization which also demonstrated a negligible increase in risk of coronary artery disease.[22, 25, 26] With all the negative study outcomes, one might wonder whether the technique of Mendelian randomization is actually valid for assessing the role of thyroid status. As a kind of positive control, we assessed in **Chapter 9** the association between genetically determined thyroid status and a metabolomic profile including a vast array of lipid particles. Here, we observed associations between higher TSH and higher levels of very low-density lipoprotein (VLDL) subclasses and components, triglycerides, and triglyceride content of lipoproteins which were similar across multiple methods including Mendelian randomization. Multiple previous Mendelian randomization studies all identified positive associations between TSH and low-density lipoprotein (LDL) cholesterol and total cholesterol, while no associations for high-density lipoprotein (HDL) cholesterol or circulating triglycerides were observed.[22, 23, 27, 28] Although these results do not fully match ours, it is important to note that our study used a Nuclear Magnetic Resonance panel which gives different insights from traditional lipid panels as used in the previous studies. Regardless, these studies do emphasize that robust and biologically plausible associations can be obtained from Mendelian randomization studies. Interestingly, two recent independent studies did reveal that higher genetically determined TSH might decrease the risk of developing dementia.[29, 30] Although the risk estimates were small and the data used in these studies were partly overlapping, the congruence is striking. These findings do shed a new light on our null finding in **Chapter 4**. It could be that the Mendelian

randomization assesses the role of long term exposure to slightly higher TSH in relation to developing dementia, which might be more influential and possibly more relevant than the snapshot value for thyroid status used in our study. On the other hand, the Mendelian randomization studies might be biased by horizontal pleiotropy. The genetic variants associated with thyroid status have been mapped to various functional pathways, including growth factors and transporters but a considerable share has a yet unknown function.[31] Especially the variants involved in transcription and growth factors might have effects on the brain independent of thyroid status, while the genetic variants of unknown function also cannot be ruled out for having thyroid hormone independent effects on the brain. Lastly, these two studies could also report mere chance findings. Of course, all these considerations are also applicable to the research described in this thesis, which is why multiple branches of research are required before rigorous conclusions can be drawn regarding causality.

Definitive answers regarding causality of thyroid status in age-related disease were expected to be provided by the TRUST and IEMO 80-plus thyroid trial; two parallel multicenter randomized placebo-controlled trials of levothyroxine treatment for subclinical hypothyroidism in older adults.[32, 33] Both TRUST, which included individuals of 65 years or older in Switzerland, Ireland, the United Kingdom and the Netherlands, and the IEMO 80-plus thyroid trial, which included individuals of 80 years or older in Switzerland and the Netherlands, did not find evidence for benefit or harm of levothyroxine treatment compared to placebo for subclinical hypothyroidism.[34, 35] Also among participants with higher burden of hypothyroidism symptoms, treatment with levothyroxine did not bring about any measurable changes when compared to placebo.[36] In line with these null-findings, the more in-depth sub-studies also did not find any difference between the participants treated with levothyroxine and those treated with placebo regarding cardiovascular outcomes, depression or bone health.[37-39] Hence, it seems that minor changes in the hypothalamic-pituitary-thyroid axis do not cause any major changes in hypothyroidism complaints nor in risk of common diseases among older adults.

Future perspectives

Translation from fundamental research to human subjects is challenging, but translation from research to clinical practice is yet another leap. The most straightforward would be that if treatment with levothyroxine is equivalent to placebo for subclinical hypothyroidism in older adults, treatment should not be initiated. The advice not to treat subclinical hypothyroidism except for a few special circumstances was indeed published shortly after the trial results.[40] In response to the trial results, many patients wondered whether they could discontinue their levothyroxine treatment which had been initiated for subclinical

hypothyroidism. Discontinuation is different from not initiating treatment, though it seems logical that an ineffective treatment could be discontinued safely. Therefore, further research is required to investigate whether and how discontinuation can be performed safely.

In addition, we might need to take a further step back and reconsider our screening methodologies. Currently, in screening of thyroid function TSH levels are measured first, followed by fT4 levels if the TSH level is outside the reference range. The vast majority of individuals with TSH levels outside the reference range have fT4 levels within the reference range, thus resulting in many diagnoses of subclinical hypothyroidism. If we decide not to treat subclinical hypothyroidism, we might not need to test for it either. Alternatively, targeted screening might need to change focus to fT4 levels with subsequent TSH level measurement if fT4 levels are outside of the reference range, which would theoretically only diagnose individuals with overt thyroid dysfunction for whom treatment indication is unequivocal. Further research is needed to assess whether this screening method would be effective and to investigate how many cases of clinically relevant thyroid dysfunction would be missed. Moreover, cost-effectiveness might also need to be addressed since the price of fT4 testing is higher than that for TSH testing in most clinical chemical laboratories in the Netherlands.

On the other hand, for the individuals who do need treatment for hypothyroidism there might also still be room for improvement. The current treatment entails suppletion of T4, with the assumption that deiodination in the peripheral tissues will compensate to produce sufficient T3. However, full compensation to physiological balance is often not achieved.[41] A considerable proportion of patients are dissatisfied with their treatment.[42] Theoretically, these could be patients who suffer from insufficient T3 production despite normalization of TSH and fT4. Multiple randomized clinical trials failed to prove benefit of adding T3 to levothyroxine treatment.[42] Nevertheless, patient organizations in the United Kingdom vocally protested when T3 prescriptions were withdrawn on NHS advice due to lack of evidence and high economic cost. Although the trials were negative, it is important to mention that most were underpowered and might not have included patients who were dissatisfied with levothyroxine treatment.[42] Economic analysis of this lack of evidence has resulted in an advice that a rigorous trial is economically worthwhile, despite all previous efforts.[43] Hence, a properly powered trial with patients selected for having persistent complaints while being optimally treated with levothyroxine might still benefit the field.

In the more distant future, research that is currently further removed from the clinic may also contribute to patient care. For instance, thyroid status might be used as a marker to aid predictions for individual patients. Especially in geriatric

medicine, weighing potential benefits and burden of treatment for an individual patient is becoming more common in daily practice. However, estimating an individual patient's prognosis remains a difficult task. Various tools have been developed that are aiming to help these predictions, such as the APOP screener for older adults in the emergency department.[44] There are several other environments and situations where individualized vulnerability estimates are desirable, most importantly around major treatment decisions. Thyroid status might add to individual patients' characterization in this context. However, its added value to other estimators of frailty is yet to be established.

Lastly, in the long term, we might not just want to tailor medical treatment to the individual but also to individual cell types or even cells in certain states. As the fundamental research in model organisms has shown, cells have differential needs when going through different states. With the continuous progress in the delivery of medication in the body through techniques such as specialized peptides and mRNA vaccines, new treatment opportunities arise. Although speculating, in the distant future thyroid hormone antagonists aimed at neural progenitor cells might aid remyelination after flare ups of multiple sclerosis or thyroid hormone agonists aimed specifically at cancer cells might inhibit tumor growth.

In conclusion, targeting thyroid status to alter the aging process is most likely an illusion, nevertheless multiple clinically relevant questions around thyroid function in older individuals remain to be answered.

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