ESE Research Roadmap/EndoCompass Chapter by Thyroid Working Group

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Introduction

The thyroid is a bilobed gland located in close proximity to the trachea. The endocrine function of the thyroid depends on the follicular cells (the thyrocytes) and the parafollicular cells (C-cells). The thyrocytes are polarized cells that form follicles surrounding a colloid niche. These highly-specialized follicular units enable biosynthesis and MCT8 transporter-mediated export of thyroid hormones (TH), 3,5,3'5'-tetraiodothyronine (thyroxine, T4) and 3,5,3'-triiodothyronine (T3) (PMID: 34981746). In the circulation, TH are rapidly bound by plasma proteins, so less than 0.5% of TH circulates in a free form (FT4, FT3). Whilst 100% of T4 is synthesized in the thyroid, most of T3 (80%) is generated in extrathyroidal tissues due to the activity of iodothyronine deiodinases (DIOs) that catalyse T4 deiodination. TH enter and leave target cells through plasma membrane transporters (e.g. MCT8). Due to DIOs and plasma membrane transporters, intracellular TH concentrations can be independent of their serum/plasma levels (PMID: 36206932). Parafollicular C cells are distributed throughout the thyroid. They synthesize and secrete calcitonin, thereby regulating calcium homeostasis.

TH affect all human tissues, regulating key developmental and metabolic processes. Both TH excess (thyrotoxicosis) and deficiency (hypothyroidism) may adversely affect quality of life (QoL) as well as causing significant paediatric and adult morbidities, including marked foetal and childhood developmental impairment (predominantly hypothyroidism), and severe adult and childhood cardiovascular and metabolic dysfunction. A successful pan-EU implementation of neonatal screening for congenital hypothyroidism has prevented severe motor and intellectual disabilities in many children in recent decades.

Thyroid diseases affect 200 million patients worldwide (PMID: 24622353). They pose a heavy burden on patients, their families, societies, health systems and EU economies. Hence, development of efficient diagnostic and therapeutic strategies for thyroid dysfunction is crucial for European public health.

Subtopic 1. Thyroid neoplastic disorders

1. Thyroid neoplastic disorders: Epidemiology, societal impact, research state of the art

Palpable nodules affect ~5% of the population, while up to 68% patients have nodules detectable by ultrasound techniques. Thyroid Imaging Reporting and Data System (TI-RADS) based on ultrasound imaging, and Bethesda cytological microscopy examination scores support therapeutic decisions (PMID: 36714145). Although most nodules are benign and have favourable prognosis, both approaches are insufficient to identify aggressive tumours leading to clinically persistent/metastatic disease. In this regard, the use of molecular markers is coming to the fore in pre-operative diagnosis.

Thyroid cancer (TC) affects both sexes. It is the fifth most commonly diagnosed cancer in women worldwide, and the second most common in women older than 50 years. Its incidence including early onset TC is increasing probably mainly due to detection of subclinical disease through imaging and ultrasonography scrutiny (PMID: 35271818, PMID: 37168378).

In 2020, TC incidence rates were 10.1 per 100,000 women and 3.1 per 100,000 men, with mortality 0.5 per 100,000 women and 0.3 per 100,000 men, reaching > 87,000 diagnoses in Europe. Differentiated thyroid carcinoma (DTC), the most common TC type, includes papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, and rare oncocytic carcinoma of the thyroid (OCA). The other, rare TC types include anaplastic thyroid carcinoma (ATC) and medullary thyroid carcinoma (MTC), the last one representing a biologically distinct TC type originating from the C cells with features closer to neuroendocrine tumors [ref: https://www.liebertpub.com/doi/full/10.1089/thy.2014.0335] and with

hereditary inheritance in about 25% of cases . At the time of diagnosis, 10-20% of MTC patients and more than 40% of ATC patients have distant metastatic disease. In almost 40% of these patients metastases emerge during the follow-up (PMID: 31549998, PMID: 35521769).
In children, TC is a rare disease; however also the incidence of, mainly papillary, paediatric TC is rising (PMID: 34680253). There are some major differences between adult and paediatric differentiated TC regarding clinical, molecular, and pathological characteristics. Compared to adults, paediatric patients more often present with advanced disease at diagnosis, with more frequent lymph node involvement, distant metastasis, and multifocal disease (PMID: 36228315). Interestingly, despite this presentation in more advanced stage, paediatric DTC has an excellent prognosis.
Thyroid carcinomas are driven by various genetic changes, knowledge of which benefits the pre-operative and post-operative diagnosis and prognosis of the disease. In adults, the most common TC pathogenic gene variants are *BRAF*, *RAS*, and *RET* mutations that affect MAPK (mitogen-activated protein kinase) and PI3K/mTOR/Akt (phosphatidylinositol-3 kinase/mammalian target of rapamycin/protein-kinase B) signalling pathways. The rare (~10%) coexistence of *BRAF* V600E mutation and *TERT* (telomerase reverse transcriptase) promoter variants defines a very small, high-risk patient group (PMID: 24617711). The knowledge of TC molecular background facilitated introduction of tyrosine kinase inhibitors (TKIs). Although great progress has been made recently in the development of targeted TKIs, new therapies for specific genetic changes still need to be introduced. In paediatric DTC, the most common genetic alterations are *RET/PTC* and *NTRK* fusions (PMID: 34036219, PMID: 32495721). Alterations of epigenetic modifications including DNA hypermethylation, miRNA, lncRNAs (long non-coding RNAs) etc. play crucial roles in malignant transformation and have already provided therapeutic and diagnostic opportunities in other cancers (PMID: 16951157, PMID: 32955829, PMID: 35369397). However, their role in TC is largely underexplored.
The apparent and widespread TC overdiagnosis poses a heavy burden on patients' QoL by exposing them to unnecessary diagnostics and/or treatments. It also increases the financial costs of health-care systems, thereby limiting the resources that are necessary for patients that indeed are affected by malignant disease.

2. Thyroid neoplastic disorders: Future research priorities

The key future research areas in thyroid neoplasms include:

- identification of TC causes; in particular the causes of the increasing TC incidence
- development of personalized medicine-oriented strategies regarding: i) malignant nodules; ii) aggressive tumours or metastatic disease; iii) novel treatment options and combating drug resistance.
- in patients with chronic forms of TC (e.g., metastatic MTC) web-based patient decision aids providing tailored support over time and addressing specific needs, QOL, realistic expectations, and impact on patient' partner and family should be developed.
- developing multi-disciplinary expert programs for transition of care from childhood to adult care in patients with hereditary forms of TC

Specific aims, to achieve these goals include:

2.1. Building the base for research:

a. Establishing biobanking procedures for TC and normal thyroid tissues (including liquid biopsies); it is important to focus strongly on rare types of TC cancer and metastatic nodes and tumours;
b. Creation of a pan-EU database (involving adult and paediatric cohorts) comprehensively describing individual TC tissue phenotypes, including clinical data and detailed molecular characterization using omics methods.

2.2. Identifying the causes of the increased TC incidence.

The knowledge of the key gene mutations that drive carcinogenic process in the thyroid

does not give information on the ultimate causes of these molecular alterations (apart from the small subgroup of TC directly caused by radiation exposure or genetic predispositions to the hereditary TC). The incidence of TC is growing while the reasons for this increase are unknown. Some debatable causes include obesity, lifestyle, environmental factors, EDCs, or chemicals with mechanisms of action different from EDCs; however detailed studies are needed to identify the exact triggers of carcinogenic process in the thyroid. Functional studies involving in vitro/ex vivo/in vivo models and large-scale omics technologies are needed to verify:

2.2.1 *Autoimmunity.*

The previously suggested causative associations between AITD and increased risk of TC and other cancers (PMID: 35903279, PMID: 24084773, PMID: 28443243) should be evaluated by large-scale clinical trials combined with molecular analyses.

2.2.2. *Obesity*

The role of obesity/fat tissue in TC development and progression, including the role of immunomodulatory and pro-inflammatory functions of fat tissue

2.2.3. *Environmental pollution*

The role of environmental polluting factors should be also explored, including dose-dependent studies of ex vivo, in utero and in vivo exposures, with special focus on epigenetic modifications as mediators of environmental pollution effects.

2.3. Application of cutting-edge methods to understand TC biology. Single-cell/spatial omics, long read Seq, and large-scale epigenetics methods should be involved to analyze TC, particularly in aggressive and rare TC types (ATC, MTC, OCA). It requires establishing a pan-EU multicenter expert group for rare TC molecular characterization using multi omics methods. The project should combine biobanking, wet lab, and bioinformatic analysis including artificial intelligence (AI), which should be validated in various European centers. The studies should be aimed at delineation of the impact of molecular TC alterations on cellular processes. In particular, the research should be targeted on signalling pathways, metabolic alterations, non-coding RNAs (including small non coding RNAs (sncRNAs) and lncRNAs), cancer immunology, and microenvironment) using experimental in vitro/ex vivo/in vivo models to define novel drug targets and combat drug resistance.

2.4. Application of artificial intelligence in:

a. nodule classification/diagnosis to reduce unnecessary FNAB and facilitate TC diagnosis;
b. "reverse AI"-based analysis of the imaging data, to predict tumour molecular background based on image features, thereby facilitating cost-effective decisions on the appropriate treatment.

2.5. Identification of new prognostic biomarkers and their translation into clinics by the introduction of routine pre-operative/post-operative testing across the EU, based on uniform optimal diagnostic and therapeutic strategies specified for children, adults, and the elderly.

2.6. The issue of possible overdiagnosis should be thoroughly evaluated. The impact on mental health and QoL for patients with thyroid nodules requires evaluation due to their high rate and challenges in determining tumour aggressiveness.

2.7. Introduction and validation of randomized, multicenter studies on the use of thermal ablation (TA) in the treatment of autonomously functioning thyroid nodules (AFTN) and TC.

2.8. Paediatric DTC: introduction of collaborative prospective studies on the role of radio-iodine following thyroidectomy.

3. Thyroid neoplastic disorders: anticipated impact of future research

Despite multiple studies on the molecular TC background, the ultimate causes of the increasing TC incidence are still unknown. Discovery of the exact stimuli that trigger molecular alterations in TC will help to establish procedures and guidelines that will help to avoid cancerogenic exposures across the EU. This will also help to introduce closer monitoring of individuals being at risk of TC development. Establishing biobanking procedures and a pan-EU database for TC (in particular for rare TC subtypes), as well as application of cutting-edge methods (omics, AI) and detailed modelling of the TC cellular processes will facilitate research to increase knowledge about TC pathogenesis leading to the identification of diagnostic, prognostic, and predictive biomarkers, development of efficient novel drugs and increased effectiveness of the existing therapies. Introduction of routine pre-operative/post-operative testing across the EU, inclusion of AI-based predictive methods and establishing TA utility in treatment of AFTN and TC will facilitate cost-effective decisions on the appropriate treatment. Elucidation of the impact on QoL of patients with thyroid nodules will help to introduce proper procedures preventing deterioration of mental health and high societal and public costs.

Because paediatric DTC has such excellent survival, research must mainly be aimed at decreasing the adverse effects of treatment while maintaining the numbers cured. For this aim, studies should focus on determining which child needs higher-intensity treatment versus those in whom lower-intensity treatment will suffice. Comparable to the current "trend" in adult TC, children may currently be overtreated, and low-risk patients may not need adjuvant radio-active iodine (RAI) for survival. For this, future studies must aim to develop a dynamic prediction model for tumour behaviour based on genetic, pathological and imaging findings. To develop such a model, we need a large multicenter prospective study, randomized and correcting for other determinants such as thyroidectomy and prophylactic central lymph node dissection. To conduct a prospective collaborative study within Europe, with long enough follow-up time, may be a very challenging aim, however. An alternative way to gather more evidence on outcomes in relation to given treatment in paediatric DTC with sufficient patient numbers, may be to combine current European and American cohorts to create a large cohort of patients. By collaborating overseas large patient cohorts would be created. With such a dynamic prediction model, children would have the same survival rates but with improved outcomes, and reduced adverse effects of (over)treatment.

Subtopic 2. Thyroid non-neoplastic disorders

1. Thyroid non-neoplastic disorders: Epidemiology, societal impact, research state of the art

Non-neoplastic thyroid disorders (NNTDs) are defined by improper TH actions (due either to impaired extrathyroidal activation and intracellular availability of TH or impaired TH receptor activation) and/or abnormal TH concentrations in serum/plasma or tissues e.g. due to excess or deficient TH synthesis. Their causes include genetic, autoimmune and environmental factors (e.g. micronutrients deficiency, endocrine disruptors). NNTDs arise from dysfunctions of the thyroid gland (DTG) and disorders of TH

signalling (DTHS), and can be manifested as hypothyroidism or thyrotoxicosis which have important developmental or metabolic consequences.

Micronutrients (e.g. iodine, selenium, iron) are essential for TH biosynthesis and metabolism. Their proper supply is crucial for adequate thyroid function and TH homeostasis. Moderate to severe iodine deficiency (ID) in pregnant women results in mild to severe foetal brain impairment (cretinism). Iodine fortification in many countries helps preventing moderate-severe ID. Mild ID still remains worldwide during pregnancy, with >70% European countries having insufficient iodine intake during pregnancy (PMID: 32460688).

1.1. Dysfunctions of the thyroid gland (DTG)

DTG include (but are not limited to) congenital hypothyroidism (CH) and autoimmune thyroid diseases (AITD), comprising Graves' Disease (GD) and Hashimoto's Thyroiditis. Acquired DTG are common, related to the country's iodine status and patients' age and sex with an estimated prevalence of 3.8% in Europe (PMID: 24423323). GD prevails in iodine sufficiency (21/100 000 individuals/year incidence), while autonomous thyroid hyperfunction is more common in ID (PMID: 21908653; 29569622). Autoimmune hyperthyroidism has a prevalence of 0.2-1.3% and autoimmune hypothyroidism of 1-2% which increases with age and is 8-10 times more prevalent in women (PMID: 29569622). Primary congenital hypothyroidism detected by newborn screening (NBS) has an incidence of between 1:2000 and 1:3000 and central congenital hypothyroidism has an incidence of between 1:16000 and 1:30000, depending on the screening strategy (PMID: 33272083). DTG diagnostic is based on TSH and FT4 testing, sometimes combined with FT3 evaluation. NBS for CH facilitates early treatment, preventing neurocognitive defects.

Levothyroxine (LT4) monotherapy is a standard treatment for primary hypothyroidism but ~15%-20% of biochemically euthyroid patients on standard therapy with LT4 report impaired QoL (fatigue, depressed mood) (PMID: 35042968) and are offered different treatments (e.g. increased LT4 dosage, liothyronine alone or associated with LT4, desiccated thyroid extracts) (PMID: 33777817, PMID: 37071856, PMID: 37285954). GD treatments include anti-thyroid drugs (carbimazole, methimazole, propylthiouracil), total thyroidectomy and RAI and new immune-modulating treatments. Approximately 40% of GD patients develop Graves' orbitopathy (GO)/thyroid eye disease (TED) that affects QoL and requires specific therapy (PMID: 20181974; PMID: 32691849). Active, moderate-to-severe TED is usually treated with glucocorticoids. Alternative TED therapies (*i.e*, teprotumumab, tocilizumab, rituximab) were recently proposed (PMID: 31889140).

AITD is associated with microbiome changes. Animal studies indicated that gut microbiome is altered in GD/TED, antibiotics ameliorated but human GD/TED faecal transplantation exacerbated induced disease (PMID: 29801507, PMID: 33593429). In human GD patients, microbiota may affect the functioning of the immune system (PMID: 32785703), while specific bacteria species correlate with the presence of thyroid autoantibodies (PMID: 36683389). It is currently unclear whether modulating the microbiota is useful in human AITD treatment.

1.2. Disorders of thyroid hormone signalling (DTHS)

DTHS include TH transport defects (e.g. MCT8 mutations causing Allan-Herndon-Dudley syndrome), resistance to TH alpha/beta due to mutations in the respective TH receptors (THRA/THRB), and deiodination defects (e.g. selenoprotein deficiencies due to SBP2 and $tRNA^{[Ser]Sec}$ mutations). DTHS are rare (1/10,000 individuals), but the associated multisystem manifestations may be devastating, posing a heavy burden on patients and society (PMID: 35999191). DTHS mechanistic understanding, therapeutic and diagnostic options are limited due to the lack of systematic collection of clinical and functional data.

1.3. Interconnections between DTG and non-communicable diseases

TH status affects fertility and the functioning of the reproduction hormone system (both in females and males). There is a global decrease of fertility (at least in countries of higher socio-economic status / 'global north') of which causes are largely unclear. European countries display among the highest global prevalences of lifetime infertility (ISBN 978-92-4-006831-5). Studies suggest the AITD may adversely affect birth outcomes (PMID: 23015651, PMID: 36059162). However, it is unclear to what extent the high prevalence of DTG are responsible for the increasing problem of infertility in the EU.

Obesity affects DTG in children and adults (PMID: 30874808, PMID: 35173456, PMID: 35484151), while DTG impair adipose tissue functioning (PMID: 24152690, PMID: 27941950, PMID: 32375048), increasing the risk of dyslipidaemia/obesity comorbidities. The European population is heavily affected by obesity epidemics (ISBN: 978-92-890-5773-8), which may have a large impact on functioning of the thyroid gland. Studies in paediatric cohorts suggest that obesity may lead to misdiagnosis of thyroid disease (PMID: 30874808). Thus, neglecting the role of fat tissue in DTG management may lead to misdiagnosis and inadequate treatment. Furthermore, TH affect the functioning of cancer cells and TH receptors were shown to act as tumor suppressors (PMID: 30814976). However, studies on DTG-cancer associations provided conflicting results (PMID: 30814976) and it needs to be clarified if DTG affects cancer risk and should be used as a prognostic factor in cancer monitoring.

2. Thyroid non-neoplastic disorders: future research priorities

2.1. Research priorities in Dysfunctions of thyroid gland

i. Iodine deficiency:

- Observations suggest that mild ID during pregnancy is associated with lower offspring IQ (PMID: 23706508, PMID: 23633204) or Attention Deficit Hyperactivity Disorder (ADHD) (PMID: 33267727). However, **adequate randomised controlled trials (RCTs) during pregnancy with follow-up of children's cognition are lacking**. The link between ID and the risk of developing neuropsychiatric disorders is also unclear (PMID: 36678261). Human trials should be accompanied by studies using iPSC (induced pluripotent stem cells)-based human models, and animal models to explore in utero effects of the mild ID on brain structure, neuronal signalling, neuron-glia interactions. Spatial omics studies should be involved to analyse 3D molecular changes. Spatial focus should be given on the role of epigenetics and non-coding RNAs as mediators of the effects of mild ID.

ii. DTG diagnostics:

- High inter-individual, but limited intra-individual variation in TSH/TH concentrations leads to wide population-based reference intervals (RIs) (PMID: 11889165). Optimal individual RIs might be much narrower and genetically-determined (PMID: 11889165; 15001606; 32271924), and may also vary depending on assay, age, pregnancy or LT4 treatment (PMID: 35861700; 32183257; 36762702). **Future studies should define optimal RIs for thyroid function tests in specific populations/individuals (pregnancy, elderly, LT4 treated patients), based on clinical/genetic profiles.** RIs for newly identified TH biomarkers are also required.

- Serum TSH/FT4 concentrations do not always reflect TH function in peripheral tissues (PMID 16887882). **Tissue-specific biomarkers/omics profiles and their clinical application, may support personalized NNTDs treatment.**

iii. Congenital hypothyroidism:

- TSH-only screening detects primary, but misses central CH and some DTHS (MCT8 deficiency, THRA/THRB mutations). Early central CH detection by NBS may be associated with better neurocognitive outcomes. **Central CH incidence and the benefits of its early NBS detection need to be clarified** in pan-European, multicentre studies on children cognition and QoL, along with the analyses of the NBS-based testing cost-effectiveness.
- Genetic NBS was suggested as an alternative to the metabolite-/hormone-based NBS (PMID: 33272083). However, only ~20% of CH cases have genetic explanations. **The causes of CH, especially thyroid/pituitary maldevelopment, require clarification.**
- Detailed human, animal, and in vitro studies are needed to improve understanding of the developmental biology and physiology of human thyroid (including at single cell level), its perturbations and the determinants of goitrogenesis.
- Compared with mild disease, severe primary CH requires considerably higher serum FT4 for TSH normalizations. **Questions remain around the cause(s) of this phenomenon, and whether the serum TSH or the FT4 concentration needs to be normalized**.
- Lowering of NBS TSH thresholds increased the number of newborns with mild/subclinical CH and even transient CH. **Questions concern the benefits of treatment of this group of children with respect to growth and long-term neurocognitive outcomes and metabolic effects, e.g. cardiovascular disease**.

iv. AITD

- The mechanisms behind the increasing AITD incidence should be explored; including the role of environmental polluting factors (the research should involve dose-dependence studies performed in vitro/ex vivo/in utero/in vivo models, with special focus on large-scale epigenetic studies to verify the role of epigenetic modifications as mediators of the impact of environmental pollution). The impact of the analyzed compounds on thyroid cells, thyroid gland microenvironment and immune system should be analyzed by cutting-edge technologies such as spatial and single cell transcriptomics, proteomics and metabolomics.
- The causal associations between microbiome and AITD (including paediatric GD) require elucidation. Future human microbiome studies should apply standardized protocols (doi.org/10.1186/s40168-021-01048-3), preferably using shotgun sequencing, and include reference reagents (doi.org/10.1186/s40168-020-00856-3) to enable comparison of data from disease cohorts in different countries and facilitate meaningful meta-analyses. Regarding QoL of AITD patients: the impact of the microbiome-brain axis should be also explored by means of in vitro and in vivo AITD models; these studies also focus on identification of pre- and probiotics supplements that may help to alleviate persisting AITD symptoms despite normal TH concentrations.
- **New protocols of LT4/liothyronine combinations are needed to restore euthyroidism in target tissues and prevent cardiovascular and bone harmful effects**. Furthermore, QoL of treated GD patients requires evaluation along with studies aimed at identifying the means to prevent excess weight gain following restoration of euthyroidism.

- Immunological remission in paediatric GD may be improved by prolonged anti-thyroid drug treatment and immune-modulating therapy (rituximab, Ki70 monoclonal antibody, other immune-modulating drugs) (PMID: 34981748, PMID: 34687316**). Patients' QoL after such treatments when compared with thyroidectomy/RAI (that required life- long LT4 supplementation) require evaluation.**
- Future **GD/TED** research **should reveal the etiopathology, diagnostic/prognostic/treatment response biomarkers, and novel drugs targeting immune dysregulation and persistent mental syndromes, along with RCTs** for optimal therapies.

2.2. Research priorities in Disorders of thyroid hormone signalling:

- **Improving understanding of the genetic and acquired etiologies of DTHS** pathologies by evaluating and phenotyping affected humans with appropriate parallel ex vivo/in vitro experiments
- **Elucidating the cellular, molecular and physiological role of TH metabolism**, in particular to verify biologically/thyromimetic activity of T4 and TH metabolites other than T3. The studies should involve careful analysis of the physiological effects at the level of cell, tissue and organism as a whole, with detailed analysis of the possible molecular effects on cellular signaling pathways. This should be done using careful evaluation of ex vivo/in vitro and in vivo models with cutting edge large scale multi-omics methodology as well as validations with the help of the traditional methods of molecular biology.
- **Cell/tissue-specific DTHS consequences and their mechanisms require analyses using human-relevant models** (e.g. patient-derived cells, setting up *in vitro* co-cultures or organoid models and using induced pluripotent stem cells) to develop diagnostic and therapeutic strategies, including identification of biomarkers of tissue/cell-specific TH signalling alterations in the context of DTHS genetic variants. The ultimate goal should be identification of the mechanisms by which TH contribute to the intercellular/intertissue interaction and communication to maintain organism homeostasis.
- **Pan-EU projects systemically collecting real-world, standardized data** (including genetics, diagnosis and clinical outcomes) are needed to fully characterize the short and long-term health DTHS consequences.
- **Therapeutic options should be investigated using multi-centre trials supported by ex vivo/in vitro cellular studies** designed appropriately for rare disorders**.**

3.3. Research priorities in Interconnections between DTG and non-communicable diseases

- Interdisciplinary clinical and basic/molecular studies are needed to reveal the associations between DTG and:
  i) obesity;
  ii) cancer,
  iii) fertility disorders
  and translate them into clinical practice.
  Recent studies provided hints for the causative associations between AITD and cancer, including TC (PMID: 35903279, PMID: 24084773, PMID: 28443243). Specific questions related to DTG-associated fertility disorders include: i) studies to investigate the mechanisms underpinning associations of thyroid autoimmunity with adverse pregnancy outcomes and whether autoimmunity can be modulated to improve outcomes; ii) management of pregnancy in patients with rare thyroid disease or

conditions (e.g. central hypothyroidism) in which TSH is not an appropriate biomarker of thyroid hormone status; iii) relative roles of maternal T4 and T3 in foetal physiology and placental TH transport; iv) long term outcome data of babies born to mothers with Graves' disease or thyrotoxicosis. v) large-scale prospective studies on causal links between subclinical hypothyroidism and infertility as well as the efficacy of the assisted reproductive techniques (ART); vi) clarification of the influence of hypothyroidism and AITD on male fertility and ART; regarding the role of AITD in infertility treatments, the significance of thyroglobulin antibodies requires evaluation (PMID: 36059162).

The research should include in vitro/ex vivo/in vivo DTG models, involving both sexes (DTG phenotypes are sex-specific (PMID: 30814976)) and omics technologies (e.g. single-cell RNAseq, spatial omics, multicolor flow cytometry, multicolor immunofluorescence analysis). Subcellular effects of TH alterations need to be explored by cutting edge methods such as super-resolution confocal microscopy to complement the molecular data. Paracrine and endocrine interactions between fat tissue and the thyroid as well as the immune system should be explored to find how the secretory functions of the adipocytes affect the signaling pathways and the functioning of thyrocytes, thyroid gland microenvironment and the immune cells. Conversely, the role of the hypo- and hyperthyroidism in cancer development should be mechanistically explored to reveal the impact of TH/TSH changes on signalling pathways and the functioning of target tissues such as breast gland.

3. Thyroid non-neoplastic disorders: anticipated impact of future research

Due to their prevalence, DTG considerably influence public health. The proposed research directions will significantly improve health of European populations:

a. Defining the mild ID/selenium deficiency effects, primary and central CH etiopathology and central CH screening will help to better understand these disorders, avoid their consequences on neurocognitive and metabolic outcomes. Intelligence/IQ largely influence educational success, occupational status, use of health services, lifestyle and recreational choices or crime. A lower average IQ increases the percentage of intellectually disabled children, limiting their potential and increasing healthcare and societal costs. To introduce nutrient supplementation on the population level, treatment studies are essential due to iodine's narrow therapeutic window. This research will help to harmonise international practice during pregnancy when levothyroxine therapy is necessary in women with TSH greater than 2.5 mU/L, greater than 4 mU/L or 10 mU/L in order to reduce the consequences of primary hypothyroidism during gestation on Intelligence and IQ of the neonates and infants.
b. Defining reliable TSH/TH RIs and tissue-specific biomarkers of TH action will support personalized DTG treatments, preventing application of inefficient/non-optimal therapies. This will result in high cost-effectiveness since LT4 is one of the most commonly prescribed drugs worldwide (PMID: 36412275)
c. Alternative treatment regimens for hypothyroidism will improve patients QoL while remaining euthyroid. New levothyroxine formulations are needed in order to treat patients with abnormal absorption of actual or present thyroid hormone formulations.
d. Novel GD/TED diagnostic/treatment options will improve patients QoL and provide cost effectiveness by avoiding cardiovascular and bone-affecting comorbidities
e. Robust AITD-linked microbiome data will define life-style changes to reduce disease risk

f. New DTHS data will (i) improve disease awareness, permitting timely diagnosis and limiting devastating outcomes, (ii) yield appropriate diagnostic tools, (iii) generate new therapies with the potential for usage across other DTG, (iv) inform guidelines. DTHS are rare but gene variants in similar pathways with milder impact may be more frequent in the general population and modulate common NNTDs' outcomes.
g. Delineation of DTG associations with non-communicable diseases will: i) help to discover efficient and cost-effective obesity treatments; ii) clarify whether DTG patients should be monitored for cancer risks

Abbreviations

ADHD: Attention Deficit Hyperactivity Disorder

AFTN: autonomously functioning thyroid nodules

AI: artificial intelligence

AITD: autoimmune thyroid disease

ATC: anaplastic thyroid carcinoma

CH: congenital hypothyroidism

DTC: Differentiated thyroid carcinoma

DTG: dysfunctions of the thyroid gland

DTHS: disorders of thyroid hormone signalling

EDCs: Endocrine-disrupting chemicals

FNAB: Fine-needle aspiration biopsy

FT3: free T3

FT4: free T4

GD: Graves' Disease

GO: Graves' orbitopathy

ID: iodine deficiency

LT4: Levothyroxine

MTC: medullary thyroid carcinoma

NBS: Newborn Screening

NNTDs: Non-neoplastic thyroid disorders

OCA: oncocytic carcinoma of the thyroid

PTC: papillary thyroid carcinoma

QoL: quality of life

RAI: radioactive iodine

RIs: reference intervals

T3: 3,5,3’-triiodothyronine

T4: 3,5,3’,5‘-tetraiodothyronine, thyroxine

TA: thermal ablation

TC: Thyroid cancer

TED: thyroid eye disease

TH: thyroid hormone(s)

TI-RADS: Thyroid Imaging Reporting and Data System

TKIs: tyrosine kinase inhibitors