Thyroid

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Clinical studies for congenital hypothyroidism Generic or not?

Generic levothyroxine compared with synthroid in young children with congenital hypothyroidism

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Background: The authors wished to determine whether a brand-name drug (Synthroid) resulted in better control of congenital hypothyroidism than generic LT₄.

Methods: This was a 5-year retrospective study conducted at one tertiary care center. Children who were 0–36 months old with congenital hypothyroidism followed up at this center from 2006 to 2011 were treated with either Synthroid exclusively (35 subjects) or generic LT₄ exclusively (27 subjects). The subjects' TSH and free T₄ measurements and how often their LT₄ dose was adjusted were recorded. The TSH variance between the groups was measured as a primary endpoint. Secondary endpoints were the frequency of LT₄ dose changes and the variance in free T₄.

Results: There was no difference in TSH SD in the Synthroid group compared with the generic group (median 3.0 vs. 2.2, $p = 0.27$). Children treated with the generic LT₄ had lower TSH estimated SD (1.35 with 95% confidence interval (CI) (1.194, 1.526)) than the Synthroid group (1.66 with 95% CI (1.536, 1.803)). Similarly, no difference was observed in free T₄ SD between the groups (median 0.29 generic vs. 0.36 Synthroid, $p = 0.11$), but the generic group had lower free T₄ estimated SD than the Synthroid group (0.216 with 95% CI (0.187, 0.249) vs. 0.298 with 95% CI (0.273, 0.326)). Frequency of LT₄ dosing adjustments was similar between the groups, both in total (median 2.0 for generic vs. 3.0 for Synthroid, $p = 0.097$) and when adjusted for number of TSH checks (ratio 0.25 generic vs. 0.31 Synthroid, $p = 0.45$).

Conclusion: In this study of congenital hypothyroidism, generic LT₄ treatment resulted in similar or better control of hypothyroidism compared with Synthroid.

Generic and brand-name L-thyroxine are not bioequivalent for children with severe congenital hypothyroidism

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Background: In the United States, generic substitution of levothyroxine (LT₄) by pharmacists is permitted if the formulations are deemed to be bioequivalent by the Federal Drug Administration. There is however widespread concern beyond the United States that the pharmacokinetic standard used is too insensitive.

Methods: The authors aimed to evaluate the bioequivalence of a brand-name LT₄ (Synthroid) and an AB-rated (AB-rated means that the standard for bioequivalence was met) generic formulation (Sandoz, Princeton, N.J., USA) in children with severe hypothyroidism. This was a prospective randomized crossover study in which patients received 8 weeks of one LT₄ formulation followed by 8 weeks of the other. The setting was an academic medical center. Of 31 children with an initial serum TSH concentration >100 mU/l, 20 had congenital hypothyroidism (CH) (15 of 16 CH patients had severe thyroid dysgenesis or agenesis on imaging) and 11 had autoimmune thyroiditis. The primary endpoint was the serum TSH concentration. Secondary endpoints were the free T₄ and total T₃ concentrations.

Results: The serum TSH concentration was significantly lower after 8 weeks of Synthroid than after generic drug ($p = 0.002$), but thyroid hormone levels did not differ significantly. The difference in TSH was restricted to patients with CH ($p = 0.0005$). Patients with CH required a higher LT₄ dose ($p < 0.0004$) and were younger ($p = 0.003$) but were not resistant to thyroid hormone. The response to generic vs. brand-name preparation remained significant when adjusted for age.

Conclusion: Synthroid and an AB-rated generic LT₄ are not bioequivalent for patients with severe hypothyroidism due to CH. The authors concluded that it would therefore seem prudent not to substitute LT₄ formulations in patients with severe CH, particularly in those <3 years of age.

At first look, the studies seem to reach opposite conclusions, but they indeed assessed different aspects of the question. Years ago the FDA declared LT₄ preparations to be 'new' drugs and required that all existing and future LT₄ products be approved through the new drug application process in order to remain on the US market. However, concerns remained of potentially clinical significance of the accepted differences in a LT₄ concentration of 5% allowed with the most recent pharmacokinetic approach in products designated as bioequivalent by the pharmacokinetic standard used.

The authors of the first study concluded that 'generic LT₄ and Synthroid are at least interchangeable in young children with congenital hypothyroidism'. Such a conclusion is not supported by the data because this retrospective, parallel treatment assessment did not address the interchange of the brand-name product with a rated generics. The only valid conclusion is that contemporary high-quality LT₄ products, both generic and brand name, had similar and fairly consistent clinical outcomes from refill to refill when used to treat congenital hypothyroidism.

In the second study, subjects underwent a 16-week, prospective, randomized, open-label, crossover study of treatment with their usual dose of outpatient LT₄. The study was carefully designed and controlled for many parameters including standardized time of blood sampling, dispensation of medications through a research pharmacy, etc., indeed in a much more robust design than in the first study. At the end of the Synthroid phase of the study, TSH values were significantly lower than after the 8-week generic LT₄ period. This difference in TSH outcomes was most pronounced in the congenital hypothyroidism group. There were no significant differences in FT₄ and TT₃. Of the 16 patients who were receiving the brand-name product Synthroid at study entry, follow-up TSH values at the end of their Synthroid period were not different from those observed at baseline, indicating consistent TSH outcomes. However the study title and the conclusion on bioequivalence is not completely accurate, as it was not a pharmacokinetic study; so the second study showed us that generic and brand-name L-thyroxine are not clinically interchangeable for congenital hypothyroidism.

From a practical point of view we may, as pediatric endocrinologists, alert patients that preparations may be switched at the pharmacy, encourage patients to ask to remain on the same preparation at every pharmacy refill, make sure patients understand the need to have their TSH retested and the potential for dosing readjusted every time their LT₄ preparation is switched.

The battle between generic and brand-name thyroxine is still ongoing! Further research is required to reproduce these results and appropriately assess the ability of pharmacokinetics to predict clinical outcome.

A high prevalence of dual thyroid ectopy in congenital hypothyroidism: evidence for insufficient signaling gradients during embryonic thyroid migration or for the polyclonal nature of the thyroid gland?

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Background: Thyroid ectopy results from the failure of the thyroid precursor cells to migrate from the primordial pharynx to the anterior part of the neck. Most ectopic thyroids are revealed by congenital hypothyroidism and present as a single round mass at the base of the tongue, with no other thyroid tissue. The authors aimed to assess the frequency of dual ectopy, with part of the tissue having partially migrated, which they believed occurs more frequently than previously reported.

Methods: To determine the prevalence of dual ectopy, the authors reviewed the pertechnetate scintigraphies of 81 patients with congenital hypothyroidism from thyroid ectopy diagnosed between 2002 and 2011 at their institution.

Results: The authors found 7 cases (9%) of dual ectopy, representing an incidence ranging from 1:50,000 to 1:70,000.

Conclusion: Almost 1 in 10 cases of congenital hypothyroidism due to thyroid ectopy has dual ectopy.

At least two possible explanations may account for dual thyroid ectopy. First, it is conceivable that dual ectopy arises from insufficient signaling gradients in surrounding tissues, which do not allow the primordial thyroid epithelium to develop properly, and that might lead to partial migration of the caudal part of the anlage (primitive thyroid) but not of the apical component, which would result in dual ectopy, even if the thyroid anlage were initially monoclonal. Second, given that the thyroid arises from a small cluster of thyroid follicular cell precursors, which migrates caudally during the first 7 weeks of gestation, dual thyroid ectopy might be a consequence of a migration defect that probably affects two clusters (clones) of primordial thyroid cells to a different degree, leaving none with the potential to reach the normal location and to develop into a bilobed gland.

From this, the authors suggested that two populations of cells diverged at an early stage of development, which may arise from insufficient signaling gradients in surrounding tissues during early organogenesis or may indirectly support the polyclonal nature of the thyroid. This interesting article shows us that clinical pediatric endocrinology with well-described patients and developmental biology go together well!

Thyroid Function from Birth to Adolescence in Prader-Willi Syndrome

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Background: Prader-Willi syndrome (PWS) is a hypothalamic disorder which raises the question of the possible alterations of the secretion of the pituitary hormones, in addition to the well-known growth hormone deficiency. The authors aimed to describe the response of thyroid-stimulating hormone (TSH) to thyroid-releasing hormone in children and adolescents with PWS, and to compare TSH and total thyroxine (TT₄) concentrations measured on neonatal screening for congenital hypothyroidism in children with PWS and controls.

Methods: All participants had genetically confirmed PWS. The TSH responses to thyroid-releasing hormone, free thyroxine (fT₄), and free triiodothyronine (fT₃) were measured in 21 subjects (14 females and 7 males, mean age 6.4 years). Capillary TT₄ was measured on neonatal screening samples from 23 subjects with PWS (14 females and 9 males), each of whom was matched for birth weight and sex with 4 anonymized controls.

Results: One subject with PWS had tertiary hypothyroidism. In the other 20 subjects, mean TSH level was 1.9 mU/l (range 0.8–4.2) at baseline and 21.8 mU/l (range 10.0–46.7) at 20 min (peak). Mean fT₄

concentration (10.4 pmol/l, range 8.2–13.5) was in the lower one-third of the normal range in 18 subjects, and mean fT_3 concentration (6.1 pmol/l, range 4.8–8.4) was above the median in 13 subjects. In neonates, mean TSH level was 3.1 mU/l (range 0.4–10.0) in subjects with PWS versus 3.3 mU/l (range 0.0–7.0) in controls, and mean TT_4 in subjects with PWS was 111% (range 17–203%) that of controls ($p = \text{n.s.}$).

Conclusion: Thyroid function was normal in the newborn subjects. In older children, frank hypothyroidism was found in only 1 of our 21 subjects.

The authors' finding of lower fT_4 concentration in children and adolescents with PWS is in agreement with most studies. This could prompt some of us to introduce a thyroxine treatment. However, this seems to be unnecessary in almost all the children with PWS as demonstrated here, and thus levothyroxine treatment should not be routinely prescribed to youth with PWS. Indeed the same children had rather normal/high fT_3 concentrations. Increased thyroxine-to-triiodothyronine conversion also could contribute to the relative decrease in fT_4 in the presence of normal fT_3 concentrations. A possible explanation for this effect could be a leptin-mediated upregulation of type 2 deiodinase in response to the low-normal fT_4 levels. In addition, 18 of the 21 subjects of the study were receiving human growth hormone, which also could contribute to lower fT_4 concentrations through increased thyroxine-to-triiodothyronine conversion. Of note, the TRH test is not useful in our daily practice for the diagnosis of hypothyroidism and TRH is no more available widely.

Follow the thyroid after internal and external irradiation!

Twenty-Five Years after Chernobyl: Outcome of Radioiodine Treatment in Children and Adolescents with Very-High-Risk Radiation-Induced Differentiated Thyroid Carcinoma

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Context: After severe nuclear reactor accidents with release of radioactive iodine, elevated thyroid cancer risk in children and adolescents is considered one of the main health consequence for the population exposed.

Methods: The authors studied outcomes after 11.3 years' median follow-up in a selected, very high-risk cohort, 234 Chernobyl-exposed Belarusian children and adolescents with thyroid carcinoma undergoing post-surgical radioiodine therapy (RIT) in Germany. Cumulatively 100 (134) children with (without) distant metastasis received a median 4 (2) RITs and 16.9 (6.6) GBq, corresponding to 368 (141) MBq/kg iodine-131. They evaluated outcomes by the response to therapy and disease status, mortality, and treatment toxicity.

Results: Of 229 patients evaluable for outcome, 147 (64.2%) attained complete remission (negative iodine-131 whole-body scan and TSH-stimulated serum Tg <1 µg/l), 69 (30.1%) showed nearly complete remission (complete response, except stimulated Tg 1–10 µg/l), and 11 (4.8%) had partial remission (Tg >10 µg/l, decrease from baseline in radioiodine uptake intensity in ≥ 1 focus, in tumour volume, or in Tg). Except 2 recurrences (0.9%) after partial remission, no recurrences, progression, or disease-specific mortality were noted. One patient died of lung fibrosis 16 years post-therapy, 2 of apparently thyroid cancer-unrelated causes. The only RIT side effect observed was pulmonary fibrosis in 5/69 patients (7.2%) with disseminated lung metastases undergoing intensive pulmonary surveillance.

Conclusions: Even when radiation-induced differentiated thyroid carcinoma is advanced and initially suboptimally treated, response to subsequent radioiodine therapy and final outcomes are mostly favourable.

The population included in this cohort deserves some comments as they were severely affected and suboptimally treated: virtually all patients had papillary histology. At presentation, just under two-

thirds had local invasion (stage pT₄); almost all had neck lymph node involvement. At final staging following post-RIT whole-body scintigraphy, somewhat under half the cohort had iodine-avid distant metastases, involving the lungs in all cases, plus the bone in 2 cases or the brain in 1. Lung metastases nearly always presented with disseminated 'miliary' spread (n = 54) or mixed diffuse-focal uptake (n = 38) rather than as clearly localized nodules (n = 8). A large majority of patients had undergone radical surgery, i.e. total thyroidectomy and lymph node dissection. Reoperation was necessary in under a quarter of the cohort. Fewer than a third of patients had received prior postoperative therapy besides thyroid hormone. Such therapy included iodine-131, typically in low cumulative activities (median 2.4 GBq), low-dose percutaneous irradiation (total 40 Gy) or single-agent or combination chemotherapy, mostly including bleomycin. Therefore the present observational study involved some of the highest-risk juveniles with Chernobyl radiation-induced differentiated thyroid carcinoma. The authors commented that due to the selection criteria for iodine-131 treatment in Germany, this cohort had frequent local invasion (64.1% pT₄ disease) and distant metastasis (42.7%) and nearly universal nodal involvement (97.0%). This is important to underline this as, if not, the conclusion of the article may appear overoptimistic.

With respect to the Fukushima nuclear incident, due to timely countermeasures (sheltering, evacuation, and a ban on potentially contaminated food and milk), the risk of radiation-induced differentiated thyroid carcinoma in children is much lower than it was after Chernobyl and – if there were any increased pediatric thyroid cancer incidence at all – it is very likely that due to early diagnosis by screening, advanced cases would be avoided.

Malignant and benign thyroid nodules after total body irradiation preceding hematopoietic cell transplantation during childhood

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Background: Thyroid carcinoma after exposure to external radiation during childhood generally follows a linear dose-response relationship, but may plateau at the highest doses, and even slightly decrease at doses of more than 2,000 cGy. Age at irradiation, sex, and duration of follow-up have also been identified as determinants influencing the risk of thyroid tumors. The risk of radiation-induced benign thyroid nodules is also well known.

Methods: The authors aimed to determine the occurrence of thyroid nodules and carcinomas after fractionated total body irradiation (TBI) preceding hematopoietic stem cell transplantation (HSCT) for malignant hematological disease during childhood. They conducted a retrospective university hospital-based observational study. The participants were 76 patients receiving fractionated TBI between 1989 and 2009 as part of the conditioning regimen for HSCT to treat malignant hematological disease, with a median age of 8.2 (5.7–11.4) years, for whom the last ultrasound examination was performed at a median age of 14.2 (11.2–17) years. All patients received 12 Gy fractionated TBI using the protocol (six fractions of 2 Gy for 3 consecutive days). The main outcome measure was cumulative incidence of thyroid nodules detected by ultrasound scans followed by biopsy if necessary.

Results: Thyroid nodules were examined in 21 (28%) patients, 6 (29%) of whom were diagnosed with thyroid carcinoma at the age of 2.2–18.6 years after TBI. The cumulative incidence of nodule occurrence increased with increasing time from diagnosis. The 10-year cumulative incidence of benign and malignant thyroid nodules was 16% (95% confidence interval (CI) 4–27%) and 8% (95% CI 0–16%), respectively. 17 (22%) patients had hypothyroidism (compensated n = 12, in 5 patients it was transient). No significant independent risk factors were identified in the multivariable competing risk model as a function of nodule occurrence.

Conclusion: Short-term and life-long monitoring, with screening for nodules of the thyroid gland using ultrasound scans, is recommended for survivors subjected to TBI for HSCT during childhood.

The results of this study extend our knowledge of the high frequency of thyroid nodules and carcinomas among adolescents and young adult patients who received TBI as part of conditioning regimens for HSCT to treat malignant hematological disease during childhood. The cumulative incidence of nodule occurrence increased with increasing time from diagnosis. This study high-

lights the need for thyroid function investigations and ultrasound scans followed by FNAB of thyroid nodules when required, every 1–3 years, depending on the presence or absence of thyroid dysfunction, size of nodules, and duration of follow-up. This screening should begin at the time of TBI, to provide baseline information, and extend over a follow-up period of at least 30 years, to facilitate the detection of microcarcinomas that have a more favorable prognosis than larger tumors.

Thyroid stem cells: surely more to come, as every year Article of the year in thyroidology

Generation of functional thyroid from embryonic stem cells

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Background: The primary function of the thyroid gland is to metabolize iodide by synthesizing thyroid hormones, which are critical regulators of growth, development and metabolism in almost all tissues. So far, research on thyroid morphogenesis has been missing an efficient stem-cell model system that allows for the in vitro recapitulation of the molecular and morphogenic events regulating thyroid follicular-cell differentiation and subsequent assembly into functional thyroid follicles.

Methods and Results: The authors report that a transient overexpression of the transcription factors NKX2-1 and PAX8 is sufficient to direct mouse embryonic stem-cell differentiation into thyroid follicular cells that organize into three-dimensional follicular structures when treated with thyrotropin. These in vitro derived follicles showed appreciable iodide organification activity. Importantly, when grafted in vivo into athyroid mice, these follicles rescued thyroid hormone plasma levels and promoted subsequent symptomatic recovery.

Conclusion: Mouse embryonic stem cells can be induced to differentiate into thyroid follicular cells in vitro and generate functional thyroid tissue.

Thyroid follicle formation and thyroglobulin expression in multipotent endodermal stem cells

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Background: The thyroid transcription factors-NKx2 homeobox 1 (NKx2-1, formerly called TTF-1) and paired box gene 8 (Pax8) associate biochemically and synergistically in the activation of thyroid functional genes including the sodium/iodide symporter (NIS), thyrotropin (TSH) receptor (TSHR), thyroglobulin (Tg), and thyroid peroxidase (TPO) genes. The aim of this study was to assess the impact of transcriptional induction on thyroid follicular cell (TFC) differentiation from endodermally matured embryonic stem (ES) cells. More specifically, the authors investigated the ability of ectopically expressed Pax8 and NKx2-1 to induce murine ES cells into potential TFCs.

Methods: ES cells were stably transfected with either the Pax8 gene, the NKx2-1 gene, or both genes to study the induction of NIS, TSHR, Tg, and TPO genes as.

Results: The four thyroid-specific genes NIS, TSHR, Tg, and TPO were all significantly activated by expressing both transcription factors within the same ES cell. In contrast, significant but much lower transcriptional activity of the TSHR, Tg, and TPO genes was detected in cells expressing just NKx2-1, and only the NIS and TSHR genes responded to Pax8 alone. No Tg protein expression could be detected prior to their development into endodermal derivatives. After culture with activin A and TSH, those cells transfected with both Pax8 and NKx2-1 demonstrated greatly enhanced expression

of the NIS, TSHR, Tg, and TPO genes, similar to that found in control thyroid cells. Furthermore, these same cells formed three-dimensional neofollicles in vitro and expressed Tg protein.

Conclusion: These findings provide further evidence that co-expression of Pax8 and NKx2-1 in murine ES cells may induce the differentiation of thyroid-specific gene expression within endodermally differentiated ES cells. Moreover, this commits them to form three-dimensional neofollicular structures.

Insight into the complex biological process that leads to the development of thyroid follicular cells making up 95% of the thyroid gland, from the foregut endoderm, has been gained by studying snapshots of the signaling process during embryonic development in wild-type and mutant mice. Although these studies have shown that early steps leading to the formation and growth of the thyroid are independent of TSH signaling, they have failed to reveal all the factors that initiate expression of thyroglobulin (Tg), the signature molecule produced only by the thyroid gland. Pax8, NKx2-1, and Foxe1 are three well-characterized transcription factors that in combination have been specifically shown to regulate thyroid-specific gene expression. None of these transcription factors are expressed exclusively in the thyroid, but their co-expression is unique to the thyroid and appears to form a thyroid-specific gene expression program responsible for initiation and differentiation of thyrocytes. It has been shown that Pax8 and NKx2-1 directly interact and synergistically activate thyroid-specific transcription even in non-thyroid cells and therefore provide a major part of a model system for the differentiation of ES cells into thyroid cells.

These two beautiful articles have differences in the methods. The use by Ma et al. of stable ES cell lines overexpressing both Pax8 and NKx2-1 is in sharp contrast to Antonica et al. who used a transient approach, and will provide opportunities to characterize the detailed molecular events leading to thyroid cell speciation and a predictable supply of thyroid progenitor cells for in vivo studies. These studies show that Pax8 and Nkx2-1 are necessary and sufficient to drive the programming of stem cells in follicular cells that synthesize Tg, Tpo and Nis. These cells are also able to form follicles which produced T₄. However, both studies have been limited to mouse ES cells, and it is uncertain whether such proof of principle defines the exact sequential steps and molecular events in the development of human thyrocytes from human ES cells or induced pluripotent stem cells. But such studies are certainly underway and results are expected anxiously!

Finally and importantly, when transplanted into mice, the ESC-derived cells generated functional thyroid tissue able to rescue thyroid hormone deficits in athyroid animals. The latter finding by Antonica et al. opens a new avenue for application of stem-cell technologies in the treatment of hypothyroidism, an area that has so far received relatively little attention in regenerative medicine. Is that a credible option, in comparison to simple treatment with thyroxine? Time will tell!

Induction of sodium/iodide symporter to treat non-thyroid cancer cells: are we moving forward?

Induction of sodium/iodide symporter (NIS) expression and radioiodine uptake in non-thyroid cancer cells

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Background: To induce the expression of sodium/iodide symporter (NIS) and radioiodine uptake in non-thyroid cancer cells, the authors explored the therapeutic potential of suppressing MAP kinase and PI3K/Akt pathways and histone deacetylase (HDAC) in those cells.

Methods: The authors tested the effects of the MEK inhibitor RDEA119, the Akt inhibitor perifosine, and the HDAC inhibitor SAHA on NIS expression in 13 human cancer cell lines derived from melanoma, hepatic carcinoma, gastric carcinoma, colon carcinoma, breast carcinoma, and brain cancers. They also examined radioiodine uptake and histone acetylation at the NIS promoter in selected cells.

Result: Overall, the three inhibitors induced NIS expression, to various extents, in melanoma and all the epithelial carcinoma-derived cells but not in brain cancer-derived cells. The expression of NIS, at both mRNA and protein levels, was most robust in the melanoma cell M14, hepatic carcinoma cell HepG2, and the gastric carcinoma cell MKN-7 cell. Radioiodine uptake was correspondingly induced, accompanied by robust increase in histone acetylation at the NIS promoter, in these cells when treated with the three inhibitors.

Conclusion: This is the first demonstration that simultaneously suppressing the MAP kinase and PI3K/Akt pathways and HDAC could induce robust NIS expression and radioiodine uptake in certain non-thyroid human cancer cells, providing novel therapeutic implications for adjunct radioiodine treatment of these cancers.

As a transmembrane glycoprotein expressed primarily in follicular epithelial thyroid cells, sodium iodide symporter (NIS) plays a fundamental role in the transfer of iodide from the extracellular space into the thyroid cell for synthesis of thyroid hormones in the thyroid gland. Radioiodine treatment is usually effective in thyroid cancer patients, but it becomes ineffective when thyroid cancer cells have lost the expression of NIS and can no longer take up radioiodine as typically seen in poorly differentiated and undifferentiated thyroid cancers.

The authors were able to induce expression of NIS, to various extents, in some tumor-derived cells, suggesting that this inducible NIS expression may be restricted to skin cancer cells and cancer cells of epithelial cell origin. This is interestingly consistent with the distribution pattern of physiological uptake of radioiodine in the tissues of skin (recovery phase of burn), liver, stomach, colon, and breast (lactating phase) on the body scan of thyroid cancer patients after radioiodine treatment. Remarkably, they also demonstrated radioiodine uptake following the induced expression of NIS, consistent with NIS functionality in these non-thyroid cancer cells. They also added some molecular explanation of the phenomenon by showing that increased histone acetylation at the NIS promoter was an important mechanism involved in the expression of NIS in these non-thyroid cancer cells. As many of these inhibitors have already been proven to be clinically feasible and promising in inhibiting various cancers, their use to also induce NIS expression for adjuvant radioiodine treatment in addition to their direct inhibition of cancer cells may prove to be a novel and effective therapeutic strategy for selected non-thyroid cancers. One of the major goals of these experiments, re-induction of NIS in non-thyroid cells for therapeutic purposes, is to use iodide-131 concentrations effective but not harmful to humans. This is promising approach but we are not yet there.

Genetic and genomic go together well in thyroid research...

The ambiguous role of NKX2-5 mutations in thyroid dysgenesis

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Background: NKX2-5 is a homeodomain-containing transcription factor implied in both heart and thyroid development. Numerous mutations in NKX2-5 have been reported in individuals with congenital heart disease (CHD), but recently a select few have been associated with thyroid dysgenesis, among which is the p.A119S variation.

Methods: The authors sequenced NKX2-5 in 303 sporadic CHD patients and 38 families with at least 2 individuals with CHD.

Results: The p.A119S variation was identified in 2 unrelated patients: 1 was found in the proband of a family with 4 affected individuals with CHD and the other in a sporadic CHD patient. Clinical evaluation of heart and thyroid showed that the mutation did not segregate with CHD in the familial case. Furthermore, none of the 7 mutation carriers had thyroid abnormalities. The functional consequences of the p.A119S variation was tested in a cellular context by performing transactivation assays with

promoters relevant for both heart and thyroid development. There was no difference between wild-type NKX2-5 and p.A119S NKX2-5 in activation of the investigated promoters in the tested cell lines. Additionally, the current literature on the topic was reviewed by the authors: there is no clear evidence for a major pathogenic role of NKX2-5 mutations in thyroid dysgenesis.

Conclusion: The authors demonstrated that p.A119S does not cause CHD or thyroid dysgenesis (TD) and that it is a rare variation that behaves equal to wild-type NKX2-5. Furthermore, the authors suggest that NKX2-5 mutations do not play a major pathogenic role in thyroid dysgenesis.

The pathogenesis of TD is largely unknown; possible roles for environmental, genetic and epigenetic factors have been suggested, and in a minority of humans with TD mutations in *NKX2-1*, *FOXE1*, *PAX8* and *TSH receptor* have been identified. Additionally, in a recent study, mutations in *NKX2-5* were reported in a small proportion of patients with persistent congenital hypothyroidism [1]. However, van Engelen et al. noticed that two mutations (p.A119S – the one they studied in the current article – and p.R25C), present in 3/4 patients, have been reported as a SNP and therefore cast a doubt on their pathogenic role. The role of *Nkx2-5* was clearly demonstrated in the development of thyroid mouse in the homozygous state. The *Nkx2-5* heterozygous embryos show no abnormalities of the thyroid. Altogether, given the wealth of published evidence, NKX2-5 human mutations do not seem to play a major pathogenic role in TD. In the authors' opinion, there is currently not enough evidence to warrant routine genetic testing for NKX2-5 mutations. However, a potential role of NKX2-5 in digenic mechanisms or as a genetic modifier cannot entirely be excluded.

A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function

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Background: Thyroid hormone is essential for normal metabolism and development, and overt abnormalities in thyroid function lead to common endocrine disorders affecting approximately 10% of individuals over their life span. In addition, even mild alterations in thyroid function are associated with weight changes, atrial fibrillation, osteoporosis, and psychiatric disorders.

Methods: To identify novel variants underlying thyroid function, the authors performed a large meta-analysis of genome-wide association studies for serum levels of the highly heritable thyroid function markers TSH and FT₄, in up to 26,420 and 17,520 euthyroid subjects, respectively.

Results: 26 independent associations were found, including several novel loci for TSH and FT₄. Notably, only limited overlap was detected between TSH and FT₄ associated signals, in spite of the feedback regulation of their circulating levels by the hypothalamic-pituitary-thyroid axis. Five of the reported loci show strong gender-specific differences, which offer clues for the known sexual dimorphism in thyroid function and related pathologies.

The authors also tested the association with values outside the normal range, not included in the first part of the study. Importantly, the TSH-associated loci contribute not only to variation within the normal range, but also to TSH values outside the reference range, suggesting that they may be involved in thyroid dysfunction.

Conclusion: Overall, the 20 TSH and the 6 FT₄ associations account, respectively, for 5.64 and 2.30% of total TSH and FT₄ trait variance, respectively. They improve the current knowledge of the regulation of hypothalamic-pituitary-thyroid axis function and the consequences of genetic variation for hypo- or hyperthyroidism.

Within healthy (euthyroid) individuals, TSH and free T₄ (FT₄) levels vary over a narrower range than is seen between individuals, suggesting that each person has a unique HPT axis set-point that lies within the population reference range. Besides environmental factors such as diet, smoking and medication, little is known about the factors that influence this interindividual variation in TSH and FT₄ levels. Lack of genetic loci associated in a reciprocal manner with both TSH and FT₄ is somewhat puzzling, as their presence would be expected from physiology. However, these findings are consistent with other reports. A power calculation showed that this study – in spite of being one of the larger conducted so far on these traits – is underpowered to detect an inverse relationship between TSH and FT₄ variants.

Most of the 16 novel loci implicated in the regulation of TSH are highly represented in the thyroid with the exception of PRDM11, expressed in brain, ABO, in blood, and MIR1179. PDE10A, together with PDE8B and CAPZB, emerged as the strongest currently known genetic determinants of thyroid dysfunction. Both PDE8B and PDE10A are implicated in cAMP degradation in response to TSH stimulation of thyrocytes. In addition, the activity of both PDE10A and CAPZB appear modulated by cAMP. Several detected loci have potential clinical relevance and have been previously implicated both in mendelian endocrine disorders (LHX3 (MIMM#221750), FOXE1 (MIMM#241850), PDE8B (MIMM#614190), NR3C2 (MIMM#177735), INSR (MIMM#609968), GLIS3 (MIMM#610199)) and thyroid cancer (FOXE1 (also implicated in thyroid development), VEGFA, IGFBP5, INSR, NGR, MBIP, FGF7). Furthermore, the TSH-associated variants were found to contribute to TSH levels outside the reference range. Most of the other loci have also biological plausibility.

Overall, the findings add to the developing landscape of the regulation of hypothalamic-pituitary-thyroid axis function and the consequences of genetic variation for hypo- or hyperthyroidism.

The heritability of TSH and FT₄ has been estimated from twin and family studies at about 65 and 40%, respectively, so that some more work is needed obviously to understand the variance that remained unexplained.

Thyroid hormone contributes to cardiovascular function and insulin cell development

Transgenic zebrafish illuminate the dynamics of thyroid morphogenesis and its relationship to cardiovascular development

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Background: Among the various organs derived from foregut endoderm, the thyroid gland is unique in that major morphogenic events such as budding from foregut endoderm, descent into subpharyngeal mesenchyme and growth expansion occur in close proximity to cardiovascular tissues.

Method: The authors generated a novel transgenic zebrafish line in which thyroid-specific expression of a membrane version of a red dye enables live imaging of thyroid development in embryos from budding stage throughout formation of functional thyroid follicles. By using various double transgenic models in which a green dye expression additionally labels cardiovascular structures, a high coordination was revealed between thyroid organogenesis and cardiovascular development.

Results: Early thyroid development was found to proceed in intimate contact with the distal ventricular myocardium. Thyroid budding from the pharyngeal floor is tightly coordinated with the descent of the heart. The role of specific pharyngeal vessels, such as the hypobranchial artery (HA), in guiding late thyroid expansion along the pharyngeal midline, was revealed. An important role of the HA was corroborated by the detailed examination of thyroid development in various zebrafish models showing defective cardiovascular development.

Conclusion: The results from live imaging as well as from 3D reconstruction of thyroid development provided a first dynamic view of late thyroid organogenesis in zebrafish. This is a resource for the design of future studies addressing the molecular mechanisms of these thyroid-vasculature interactions.

A major challenge in developmental biology is to visualize and understand the dynamic nature of the many processes that are involved in the morphogenesis of tissues and organs. Thyroid gland morphogenesis represents one exciting model of endoderm-derived organ development where a complex interplay of intrinsic and extrinsic factors regulates multiple morphogenic events that are involved from thyroid specification in ventral foregut endoderm throughout terminal differentiation of the thyroid gland at a distant site in the anterior neck region. Given their coordinated spatial development, cardiac mesoderm and blood vessels have been considered as prime candidates to influence thyroid development. In mice as in the human embryo, the thyroid primordium is closely apposed to mesodermal tissue forming the heart outflow tract (OFT) and the remodeling aortic arch vasculature appears to guide localization and spatial extension of the growing thyroid primordium during late stages of organogenesis. Abnormal thyroid development has been repeatedly reported in mouse models with defective OFT development.

Zebrafish embryogenesis is one model to dissect basic mechanisms governing cardiovascular and thyroid development in vertebrates. A major advantage of the zebrafish model for such studies is that embryos and larvae can survive for several days without a functional heart and in the absence of blood circulation, facilitating the characterization of possible thyroid phenotypes over an extended developmental period compared to many mouse models with cardiovascular defects.

Therefore a very useful tool has been generated. Experiments are possible in the zebrafish model to obtain knock-down of a specific gene, using morpholino antisense oligonucleotide. The model will await new candidate genes for thyroid dysgenesis to be tested....

Thyroid hormone is required for hypothalamic neurons regulating cardiovascular functions

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Background: Thyroid hormone is well known for its profound direct effects on cardiovascular function and metabolism. While some of the molecular mechanisms underlying the hormone's central control of metabolism have been identified, its actions in the central cardiovascular control have remained enigmatic.

Methods: Mice that are heterozygous for a point mutation in thyroid hormone receptor α_1 , which reduces the affinity to the ligand 10-fold, were studied.

Results: These mice were unable to mount a correct cardiovascular response to stress, activity, or changes in environmental temperature due to a defective autonomous nervous system.

The authors identified a previously unknown population of so-called parvalbuminergic neurons in the anterior hypothalamus that requires thyroid hormone receptor signaling for proper development. Specific stereotaxic ablation of these cells in the mouse resulted in hypertension and temperature-dependent tachycardia, indicating a role in the central autonomic control of blood pressure and heart rate. Moreover, the neurons exhibited intrinsic temperature sensitivity in patch-clamping experiments, providing a new connection between cardiovascular function and core temperature.

Conclusion: Both classes of thyroid hormone receptors are required for the development of a previously unknown population of parvalbuminergic cells in the anterior hypothalamus. Thus, the data identify what is now believed to be a novel hypothalamic cell population potentially important for understanding hypertension and indicate developmental hypothyroidism as a risk factor for cardiovascular disorders. Furthermore, the findings may be beneficial for treatment of the recently identified patients that have a mutation in thyroid hormone receptor α_1 .

Thyroid hormone is a well-known regulator of cardiovascular function and metabolic rate. While most of the cardiovascular and metabolic effects of thyroid hormone have been attributed to direct actions in the corresponding peripheral tissues, such as heart or skeletal muscle and fat, recent studies have demonstrated that the hormone modulates these processes also through the brain: injections of thyroid hormone into different brain regions stimulate energy expenditure, and thyroid hormone signaling is required to establish the metabolic set-point during embryonal development. Similarly, thyroid hormone signaling is needed for the central modulation of heart rate.

With the discovery of the parvalbuminergic neurons, the function of which is controlled through the thyroid hormone receptor, the authors identified a permanent cellular defect in the hypothalamus resulting from developmental hypothyroidism. We may therefore add hypertension to the list of symptoms potentially arising from maternal hypothyroxinemia and/or congenital hypothyroidism. However, this remains to be studied and shown in humans.

Ligand-bound thyroid hormone receptor contributes to reprogramming of pancreatic acinar cells into insulin-producing cells

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Background: The authors recently reported that ligand-bound thyroid hormone receptor- α (TR α) plays a critical role in expansion of the β -cell mass during postnatal development.

Methods: Herein, they used adenovirus vector that expresses TR α driven by the amylase 2 promoter (AdAmy2TR α) to induce the reprogramming of pancreatic acinar cells into insulin-producing cells.

Results: Treatment with L-3,5,3-triiodothyronine increases the association of TR α with the p85 α subunit of PI3K, leading to the phosphorylation and activation of Akt and the expression of Pdx1, Ngn3 and MafA (three major transcription factors driving β -cell development) in purified acinar cells. Analyses performed with a cell lineage tracing system indicated that newly synthesized insulin-producing cells originate from elastase-expressing pancreatic acinar cells. Insulin-containing secretory granules were identified in these cells by electron microscopy. The inhibition of p85 α expression by siRNA or the inhibition of PI3K by LY294002 prevents the expression of Pdx1, Ngn3 and MafA, and the reprogramming to insulin-producing cells. In immunodeficient mice with streptozotocin-induced hyperglycemia, treatment with AdAmy2TR α leads to the reprogramming of pancreatic acinar cells to insulin-producing cells in vivo.

Conclusion: These findings suggest that ligand-bound TR α plays a critical role in β -cell regeneration during postnatal development via activation of PI3K signaling.

The formation of the pancreas and its subsequent differentiation into various types of exocrine and endocrine cells during development are controlled by the activation or repression of a large number of genes. The expression of these genes is regulated by a well-organized cascade of transcription factors. Pancreatic and duodenal homeobox 1 (Pdx1), basic helix-loop-helix factor neurogenin 3 (Ngn3) and MafA are transcription factors that are essential for the transdifferentiation of endocrine cells. Pdx1 controls the growth and development of the pancreatic bud; Ngn3 is required for formation of endocrine progenitors, and MafA and Pdx1 are required for the maturation of β -cells. Cell differentiation type can be reprogrammed by overexpression of selected transcription factors, usually a subset of the transcription factors required for formation of the relevant cell type during normal development. Reprogramming of pancreatic exocrine cells to β -like cells in vivo by introduction of genes for the three transcription factors, Pdx1, Ngn3 and MafA has been described [2]. Other studies have revealed that mature cells have high plasticity in their differentiation capacity [3]. Pancreatic acinar cells can transdifferentiate into endocrine cells. Indeed, under appropriate culture conditions, dedifferentiated acinar cells can be induced to become insulin-expressing cells via Ngn3 expression. This paper shows that overexpression of ligand-bound TR α by adenovirus vector effectively induced the expression of transcription factors that are key molecular factors involved in the development of islets in pancreatic exocrine cells of diabetic mice. These results indicate that gene transfer can induce the reprogramming of pancreatic exocrine cells into insulin-producing cells in vivo. A specific combination of nuclear hormone-dependent transcription factors that reprogram pancreatic exocrine cells into cells that closely resemble β -cells was identified. While many additional factors are also required for β -cell development, further studies are necessary to understand the mechanism of β -cell regeneration.

Assessment of iodine nutrition in populations: past, present, and future

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Background: There has been remarkable progress in the global effort to eliminate iodine deficiency (ID) over the past two decades. In 1993, WHO estimated that 110 countries were affected by goiter and ID. From 2003 to 2011, the number of iodine-deficient countries decreased from 54 to 32 and the number of countries with adequate iodine intake increased from 67 to 105. Currently, 71% of the global population has access to iodized salt, up from 20% in 1990. Because many countries have eliminated ID or are approaching that goal, their emphasis is now shifting to sustaining these achievements. But salt iodization programs are fragile and require a long-term commitment from governments.

Methods: Iodine status has been historically assessed by palpation of the thyroid and reported as goiter rates. Goiter is a functional biomarker that can be applied to both individuals and populations, but it is subjective. Iodine status is now assessed using an objective biomarker of exposure, i.e. urinary iodine concentrations (UICs) in spot samples and comparison of the median UIC to UIC cut-offs to categorize population status.

Results: The use of urinary iodine concentrations in spot samples has improved standardization, but inappropriate use of the crude proportion of UICs below the cut-off level of 100 µg/l to estimate the number of iodine-deficient children has led to an overestimation of the prevalence of iodine deficiency. A new approach is proposed in the current review, in which UIC data are extrapolated to iodine intakes, adjusted for intraindividual variation, and then interpreted using the estimated average requirement cut-point model.

Conclusion: This new approach may allow national programs to define the prevalence of iodine deficiency in the population and to quantify the necessary increase in iodine intakes to ensure sufficiency. In addition, thyroglobulin can be measured on dried blood spots to provide an additional sensitive functional biomarker of iodine status.

Iodine intakes in the United States, the United Kingdom, Australia, and New Zealand have fallen in recent years, mainly due to reluctance of the food industry to use iodized salt. Ensuring sustainability in countries with successful programs requires regular surveillance and occasional adjustments to the iodine content in salt. On the other hand, as salt iodization spreads across the globe, over-iodized salt has contributed to excess iodine intakes in a number of countries and regions, and both iodine deficiency and excess can damage health. Now more than ever, accurate and reliable methods to monitor iodine status are needed.

The recommendations cover worldwide neonatal screening, approaches to assess the cause (including genotyping) and the severity of the disorder, the immediate initiation of appropriate l-thyroxine supplementation and the frequent monitoring to ensure dose adjustments to keep thyroid hormone levels in the target ranges, a trial off treatment in patients suspected for transient CH, regular assessments of developmental and neurosensory functions, consulting health professionals as appropriate and education about CH [4]. The interested reader is referred to a full-text review in Léger et al. [4]. Don't miss it, we will comment on it next year!

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