Thyroid

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In the last 12 months significant advances have been achieved in central areas of thyroid research – clinical outcome studies have increased our knowledge on the short- and long-term effects of congenital hypothyroidism as well as maternal thyroid disorders during pregnancy on the infant. Basic research has shed more light on the molecular mechanisms of the hypothalamic-pituitary-thyroid axis and thyroid hormone transporter and receptor function in development and disease. Further, the Fukushima accident in Japan prompted us to review some of the data generated after Chernobyl. This chapter aims at giving a representative overview of the key publications in thyroidology.

Important for clinical practice

Maternal thyroid function during early pregnancy and cognitive functioning in early childhood: the Generation R Study

Henrichs J, Bongers-Schokking JJ, Schenk JJ, Ghassabian A, Schmidt HG, Visser TJ, Hooijkaas H, de Muinck Keizer-Schrama SM, Hofman A, Jaddoe VV, Visser W, Steegers EA, Verhulst FC, de Rijke YB, Tiemeier H
The Generation R Study, Erasmus Medical University Center, Rotterdam, The Netherlands.

J Clin Endocrinol Metab 2010;95:4227–34.

Background: Thyroid hormones are known to be essential for neurodevelopment from early pregnancy onwards. Transfer of T₄ from the mother to the conceptus has even been demonstrated before placenta-tion. Population-based data on the association between maternal thyroid function in early pregnancy and children's cognitive development are sparse. Therefore the objective of the authors was to study associations of maternal hypothyroxinemia and of early pregnancy maternal TSH and free T₄ (FT₄) levels across the entire range with cognitive functioning in early childhood.

Methods: They investigated a population-based cohort in the Netherlands. 3,659 children and their mothers were included in the study. In pregnant women with normal TSH levels around 13 weeks' gestation, mild and severe maternal hypothyroxinemia were defined as FT₄ concentrations below the 10th and 5th percentile, respectively. Children's expressive vocabulary at 18 months was reported by mothers using the MacArthur Communicative Development Inventory. At 30 months, mothers completed the Language Development Survey and the Parent Report of Children's Abilities measuring verbal and non-verbal cognitive functioning.

Results: Maternal TSH was not related to the cognitive outcomes. An increase in maternal FT₄ predicted a lower risk of expressive language delay at 30 months only. However, both mild and severe maternal hypothyroxinemia was associated with a higher risk of expressive language delay across all ages (odds ratio (OR) = 1.44; 95% confidence interval (CI) = 1.09–1.91; p = 0.010 and OR = 1.80; 95% CI = 1.24–2.61; p = 0.002, respectively). Severe maternal hypothyroxinemia also predicted a higher risk of non-verbal cognitive delay (OR = 2.03; 95% CI = 1.22–3.39; p = 0.007).

Conclusion: Hypothyroxinemia of the mothers in early pregnancy is a risk factor for cognitive delay in early childhood.

Previous smaller studies have suggested that maternal hypothyroxinemia can negatively affect cognitive function of the children [1–3]. However the scale of the present study is much larger than any

other before. This gives a power not only to test the consequences of low T_4 on the offspring cognition but also to correlate the whole range of FT_4 during early pregnancy with cognition in early childhood. One can be 'picky' regarding the methodology, using questionnaires, with no direct access to the children, but the large scale of the study is certainly the reason for this. The question is how this should be applied to pregnancy in general. A first difficulty is the lack of trimester-specific range of FT_4 and TSH for pregnant women. The authors overcome this problem in their cohort by defining FT_4 levels below the 10th percentile as hypothyroxinemia. A second difficulty is the paucity of data providing evidence for beneficial outcome of infants of hypothyroxinemic mothers after iodine or T_4 supplementation. Therefore, intervention studies with iodine or thyroxine supplementation are needed to further justify general screening for hypothyroxinemia in early gestation. For the interested reader, further papers from this mother/child cohort from the Netherlands were published this year [4, 5].

Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence

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BMJ 2011;342:d2616.

Background: The authors wished (1) to evaluate the association between thyroid autoantibodies and miscarriage and preterm birth in women with normal thyroid function, and (2) to assess the effect of treatment with levothyroxine on pregnancy outcomes in the group of women having thyroid antibodies.

Methods: A meta-analysis was performed from Medline, Embase, Cochrane Library, and SCISEARCH (inception-2011) without any language restrictions. The studies that evaluated the association between thyroid autoantibodies and pregnancy outcomes were selected by two reviewers. Odds ratios from individual studies were pooled separately for cohort and case-control studies.

Results: Thirty articles with 31 studies (19 cohort and 12 case-control) involving 12,126 women assessed the association between thyroid autoantibodies and miscarriage. Five studies with 12,566 women evaluated the association with preterm birth. Twenty-eight of 31 studies evaluating miscarriage showed a positive association between thyroid autoantibodies and miscarriage. More than a tripling in the odds of miscarriage with the presence of thyroid autoantibodies was observed in the meta-analysis of the cohort studies (odds ratio 3.90, 95% confidence interval 2.48–6.12). For case-control studies the odds ratio for miscarriage was also statistically significant (1.80; 1.25–2.60). There was a significant doubling in the odds of preterm birth with the presence of thyroid autoantibodies (2.07; 1.17–3.68). Two randomized studies evaluated the effect of treatment with levothyroxine on miscarriage. Both showed a fall in miscarriage rates, and meta-analysis showed a significant 52% relative risk reduction in miscarriages with levothyroxine (relative risk 0.48, 0.25–0.92). A 69% relative risk reduction on the rate of preterm birth was noted in one study which reported on the effect of levothyroxine.

Conclusion: The presence of maternal thyroid autoantibodies is strongly associated with miscarriage and preterm delivery. There is evidence that treatment with levothyroxine can attenuate the risks.

The authors remind us that miscarriage affects up to 1 in 5 women who conceive, making it the commonest complication of pregnancy. Preterm birth occurs in 6–10% of pregnancies. Given the high prevalence of thyroid autoantibodies in women of the reproductive age group, the observed increased risk for miscarriage and preterm birth in these women is clinically highly relevant at the individual and population level.

The mechanism behind these findings is still speculative. Pregnancy is an inflammatory process involving a shift in the regulation of cytokine networks within the local placental-decidual environment. Dysregulation of local inflammatory processes can be associated with miscarriage and premature delivery. The presence of thyroid autoantibodies can reflect a generalized activation of the immune system and specifically a dysregulated activity of the immune system at the fetal-maternal interface. Furthermore, the presence of thyroid autoantibodies might be a marker of underlying subtle alteration in thyroid reserve. An argument for this hypothesis is that treatment with thyroxine improved the outcome. However, there have been only two small randomized trials involving a total of 187 women to assess the protective effect of L- T_4 for miscarriage or preterm birth. Therefore, and in anal-

ogy with the publication on hypothyroxinemia in early pregnancy, a large placebo-controlled randomized trial is needed before general screening of thyroid autoantibodies can be advocated.

Congenital hypothyroidism with a delayed thyroid-stimulating hormone elevation in very premature infants: incidence and growth and developmental outcomes

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J Pediatr 2011;158:538–42.

Background: The authors address the question of the detection of congenital hypothyroidism (CH) in babies with delayed TSH elevation after birth. Their hypothesis was that very low birth weight (VLBW) and extremely low birth weight (ELBW) infants have an increased incidence of CH with a delayed thyroid-stimulating hormone (TSH) elevation. They further evaluated the outcomes of these infants in comparison to control infants.

Methods: Retrospective analysis of newborn thyroid screening data for 92,800 live births in Rhode Island to identify CH with a delayed TSH elevation. Developmental, growth, and endocrine outcomes of the index cases were assessed at 18 months of corrected age.

Results: CH with a delayed TSH elevation occurred in 1 in 58 ELBW, 1 in 95 VLBW, and 1 in 30,329 infants weighing $\geq 1,500$ g ($p < 0.0001$). The incidence of head circumference < 10 th percentile was higher in VLBW infants with CH associated with a delayed TSH elevation ($p < 0.05$), and the mean head circumferences, weights, lengths, and developmental scores were similar to matched control infants.

Conclusion: The incidence of CH with a delayed TSH elevation was higher in ELBW and VLBW infants compared with infants weighing $\geq 1,500$ g. The outcomes of these infants were comparable with matched control infants at 18 months.

Delayed TSH rise is a non-classical screening result suggesting thyroid dysfunction and is particularly frequent in premature infants. It is only detected in newborn screening programs with a routine or physician-based second TSH-based screening test 2–6 weeks after birth in preterm infants with $< 1,500$ g birth weight. The presented study was realized within the context of the Rhode Island Newborn Screening program, measuring both thyroxine and TSH at 48 h of age with repeated specimens collected in infants $< 1,500$ g birth weight at 2, 6, and 10 weeks of age or until they reach 1,500 g.

This study confirmed previous data showing highest incidence of congenital hypothyroidism with delayed TSH rise in extremely low birth weight infants ($< 1,000$ g; 1:58) versus very low birth weight infants (1,000–1,499 g; 1:295) and neonates with a birth weight $> 1,500$ g (1:30,329). Further, the authors showed for the first time that most untreated cases of congenital hypothyroidism with delayed TSH rise were transient, however the underlying mechanisms remained unclear. Finally, although clearly limited by sample size, the authors found no differences in psychomotor development of affected patients at 18 months of age compared to matched controls. However, 3 infants with congenital hypothyroidism and delayed TSH rise had a head circumference < 10 th percentile versus none in the controls. From this set of data, it remains unclear whether to treat these infants or not. As stated in the accompanying editorial, 'it may rather be prudent to treat those until more data on the neurocognitive abilities of those children are available' [6].

Subtle health impairment and socioeducational attainment in young adult patients with congenital hypothyroidism diagnosed by neonatal screening: a longitudinal population-based cohort study

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J Clin Endocrinol Metab 2011;96:1771–82.

Background: Improved neurodevelopmental outcome has been documented with screening programs resulting in the early treatment of patients with congenital hypothyroidism (CH). However, knowledge about long-term health in those patients is scarce. The aim of the authors was to assess health status, and socioeconomic attainment, for a population-based registry of young adult patients.

Methods: The authors included 1,748 eligible patients diagnosed during the first decade after the introduction of the national neonatal screening program in France. Questionnaires were used and completed by 1,202 of the selected patients with a median age of 23.4 years. The comparison group included 5,817 subjects from the last French Decennial Health Survey. Measures of health status included medical conditions, hearing and visual status, sociodemographic characteristics, and quality of life.

Results: Patients with CH were significantly more likely than their peers to report associated chronic diseases (5.7 vs. 2.9%), hearing impairment (9.5 vs. 2.5%), visual problems (55.4 vs. 47.9%), and being overweight with a body mass index of at least 25 kg/m² (22.8 vs. 15.7%). Importantly, fewer patients attained the highest socioeconomic category (14.6 vs. 23.1%) and were in full-time employment (39.9 vs. 44.8%). They were more likely to still be living with their parents. A lower health-related quality of life than their healthy peers, particularly for mental dimensions, was documented. The main determinants of educational achievement and health-related quality of life scores were: (1) CH severity at diagnosis, (2) treatment adequacy, and (3) the presence of other chronic health conditions.

Conclusion: These findings highlight the need for careful monitoring of neurosensory functioning, weight, and long-term treatment adequacy throughout childhood and adulthood.

This unique population-based, large-scale study of young adults affected by congenital hypothyroidism provides important information on long-term health and socioeconomic status. The general health outcomes reveal important differences in comparison with a control group, with increased risk for both hypothyroidism-associated and -independent comorbidities. Of special concern is the fourfold increased rate of patients with hearing impairment. Interestingly, graduating from high school was associated with athyreosis, but not with older age or L-T₄ dose at the start of treatment. Later on, adequacy of long-term L-T₄ substitution was a major determinant for graduating from high school. About 20% of the subjects had serum TSH concentrations above the upper limit of the reference range at the last available examination, suggesting non-optimal follow-up or poor compliance with treatment in these young adults. However, from a public health standpoint, these impairments had little impact because most patients were well integrated into society, in education, or with at least some employment. The major limitation of the study is that the analyzed population was diagnosed and treated according to historical guidelines and therefore might not be representative for infants diagnosed today. Nevertheless, there remains room for improvement of monitoring of affected children, adolescents and young adults and care during transition from pediatric to adult follow-up.

Thyroid cancer risk in Belarus among children and adolescents exposed to radioiodine after the Chernobyl accident

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Background: Previous studies showed an increased risk of thyroid cancer among children and adolescents exposed to radioactive iodines released after the Chernobyl (Chernobyl) accident. However, the effects of factors influencing dose response, such as screening, iodine deficiency, and age at exposure, need to be clarified.

Methods: The authors screened 11,970 individuals in Belarus aged 18 years or younger at the time of the accident who had estimated ^{131}I thyroid doses based on individual thyroid activity measurements and dosimetric data from questionnaires. They obtained fine needle biopsy when needed and surgery was performed so that pathology established the final diagnosis. By statistical modeling the excess odds ratio per gray (EOR/Gy) for thyroid cancer was calculated.

Results: For thyroid doses <5 Gy, the dose-response was linear ($n = 85$; EOR/Gy = 2.15, 95% confidence interval: 0.81–5.47), but at higher doses the excess risk fell. The EOR/Gy varied significantly in several situations: (1) being increased among those with prior or screening-detected diffuse goiter, (2) being larger for men than women, and (3) with a non-significant trend for persons exposed before age 5 than those exposed between 5 and 18 years.

Conclusion: 10–15 years after the Chernobyl accident, thyroid cancer risk was significantly increased among individuals exposed to fallout as children or adolescents, but the risk appeared to be lower than in other Chernobyl studies and studies of childhood external irradiation.

Twenty-five years after the Chernobyl accident and months after the Fukushima accident in March this year, studies on thyroid carcinoma prevalence among children exposed to radioactive iodine have regained wide interest. Parallel studies in Ukraine and Belarus and sequential risk analyses in the same population are providing an increasing knowledge for more precise risk estimates depending on exposed ^{131}I doses and dose-response modifying factors.

The strength of the Belarus thyroid cancer screening program study are (1) the individual estimates of ^{131}I thyroid dose based on thyroid radioactivity measurements performed within 2 months after the accident as the key baseline parameter, (2) a standardized screening protocol in all included patients by physician blinded for the individual initial ^{131}I thyroid dose, and (3) a high compliance of the patients with FNA and surgery in case of suspected malignancy centralized in two hospitals. However, this extensive study underlies a possible non-participation bias: only 32.6% of the eligible and initially analyzed population could be screened. About 50% were not traceable, while about 17% did not participate.

The mean thyroid dose as well as the excess odds ratio/Gray was lower in the present Belarus study group compared to the Ukraine population (0.56 vs. 0.78 Gy; EOR/Gy 3.16 vs. 7.35) [7]. In both studies those younger at the time of exposure tended to have higher risks per unit of dose, and half of the prescreening diagnosed cases were 0–4 years old at exposure (23 of total 48 prescreening cases), however not reaching statistically significant difference. The calculated risks were somewhat lower than those reported from the study of the survivors of atomic bombings in Japan [8].

Future analyses by the authors of incident thyroid cancers identified during additional screening cycles will further shed light on the efficacy of thyroid cancer screening, the importance of age at exposure and the observed time trend effects.

Mutation of thyroid hormone receptor- β in mice predisposes to the development of mammary tumors

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Oncogene 2011.

Background: Several findings suggest that thyroid hormone receptor- β (TR β) mutations could increase the risk of mammary tumor development. The authors explored the role of TR β mutants in vivo in breast tumor development and progression, using an animal model.

Methods: First, a knock-in mouse model harboring an inactivating mutation in the gene encoding TR β (Thrb(PV) mouse) was analyzed in the heterozygous and homozygous state. Second, the impact of the ThrbPV mutation in a mouse model of mammary tumors (the Pten(+/-) mouse) was investigated. Finally, underlying mechanisms of ThrbPV-associated tumorigenesis were investigated in vitro.

Results: The TRhrbPV mutation was associated with mammary hyperplasia in 36%, however only in the homozygote state. Further, mammary hyperplasia was observed in 60% of Thrb(PV/+)Pten(+/-) and 77% of Thrb(PV/PV)Pten(+/-) mice versus 33% of Thrb(+/-)Pten(+/-) mice. The Thrb(PV) mutation increased the activity of signal transducer and activator of transcription (STAT5) to increase cell proliferation and the expression of the STAT5 target gene in the mammary gland. Cell-based studies with a breast cancer cell line showed that thyroid hormone (T₃) repressed STAT5 signaling in TR β -expressing cells through decreasing STAT5-mediated transcription activity and target gene expression, whereas sustained STAT5 signaling was observed in TR β PV-expressing cells.

Conclusion: A specific TR β mutation promoted the development of mammary hyperplasia via aberrant activation of STAT5, thereby conferring a genetic ground for tumorigenesis.

Based on the hypothesis that TR β may act as a tumor suppressor, the authors analyzed the development and occurrence of mammary tumors in different murine mouse strains harboring an inactivating mutation in the TR β gene in a heterozygous or homozygous state with or without crossing them with PTEN+/- mice with high susceptibility to mammary tumors. The authors demonstrated for the first time that inactivating mutations in TR β confer an increased risk for breast tumor development. On the molecular level, TR β inactivation led to increased STAT5 signaling and increased cell proliferation. These results are in accordance with previous in vitro data demonstrating increased invasiveness and metastatic potential in a hypothyroid environment, linking thyroid hormone status to cancer development [9]. These provocative murine data add new arguments for this controversial association. However, whether they can be transferred to human mammary tumor development remains to be proven.

New genetic studies

Transcriptome, methylome and genomic variations analysis of ectopic thyroid glands

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PLoS One 2010;5:e13420.

Background: Congenital hypothyroidism due to thyroid dysgenesis (TD) is predominantly a sporadic disease. Incomplete migration resulting in ectopic thyroid tissue is the most common cause (60–80% of TD). Germinal mutations in the thyroid-related transcription factors NKX2.1, FOXE1, PAX-8, and NKX2.5 have been identified in less than 10% of patients with sporadic TD. Monozygotic twins have a high discordance rate of 92% for TD, suggesting that somatic events, genetic or epigenetic, play an important role in the etiology of TD.

Methods: The authors wished to assess the role of somatic genetic or epigenetic processes in TD, using ectopic thyroid tissues that were removed for medical reasons. They analyzed gene expression, genome-wide methylation, and structural genome variations in normal versus ectopic thyroid tissues.

Results: 1,011 genes were more than twofold induced or repressed and of interest for the authors. Expression array was validated by quantitative real-time RT-PCR for 100 genes. 19 genes were exclusively associated with thyroid ectopy after correction for thyroid activity state. Some of these 19 genes are involved in embryonic development and in the Wnt pathway. None of the thyroid-related transcription factors (FOXE1, HHEX, NKX2.1, NKX2.5) showed decreased expression, whereas PAX8 expression was associated with thyroid activation state. Further, the authors show that the expression profile was independent of promoter and CpG island methylation and of structural genome variations.

Conclusion: This study is the first large-scale genetic and integrative molecular analysis of ectopic thyroid tissue. A differential gene expression compared to that of normal thyroids was demonstrated in ectopic thyroids. However, a molecular basis could not be defined to unravel the so far elusive cause of defective thyroid migration during embryogenesis.

This study is unique by its approach to study directly the very rarely available ectopic thyroid tissue by the combinatory use of gene expression, DNA methylation and genomic variation analysis. This integrative molecular analysis is further strengthened by double control of gene expression results versus normal and versus hyperfunctioning thyroid tissues, to exclude effects of different activity state of the thyrocytes at thyroidectomy.

The major result of this study is the identification of 19 genes showing convergent induction or repression dependent on the localization of the thyroid. This small set of genes was enriched in well-known pathways involved in migration and cellular movement (i.e. Wnt pathway) supporting the biological plausibility of the results. However, in contrast to cancer, the transcriptome of ectopic thyroid tissues was not modified by somatic genomic variations or DNA methylation. The major limitation of the study, as already stated by the authors, is that the transcriptome of the ectopic thyroid tissues might rather be the consequence than the cause of thyroid dysgenesis and would not necessarily identify genes that might only be expressed during a developmentally regulated short time window.

A coherent organization of differentiation proteins is required to maintain an appropriate thyroid function in the Pendred thyroid

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J Clin Endocrinol Metab 2010;95:4021–30.

Background: Mutations in the gene coding for pendrin, an apical Cl^-/T^- exchanger, are responsible for Pendred syndrome. The authors investigated the thyroid of a patient with Pendred syndrome to better understand the intrathyroidal compensatory mechanisms when pendrin is lacking.

Methods: The expression of proteins involved in the cellular machinery of the thyroid follicular cells was analyzed.

Results: Within the entire thyroid gland three morphological zones were identified: nearly normal follicles with iodine-rich thyroglobulin in the colloid, small follicles without iodine-rich thyroglobulin in lumina, and destroyed follicles. In thyrocytes within nearly normal follicles, dual oxidase (Duox) and thyroid peroxidase (TPO) were localized at the apical pole, oxidative stress markers (OS) and cell apoptosis were absent, but expression of the chloride/proton exchanger (ClC-5), which has been previously shown to be potentially implicated in the apical transport of iodide and is mainly expressed at the apical pole of thyrocytes in normal thyroids was strongly increased. In small follicles without thyroglobulin, Duox and TPO were aberrantly present and increased in the cytosol and associated with high OS, apoptosis, cell proliferation, but moderate ClC-5 expression.

Conclusion: These observations suggest that lack of Pendrin results in a spectrum of morphological and functional changes occurring successively resulting finally in thyrocyte destruction.

This extensive histological study of thyroid tissue of a patient with Pendred syndrome gives new insights into the pathophysiological mechanisms of thyroid disease in Pendred syndrome. They

describe in detail the consequences of impaired apical iodide transport due to Pendrin mutation and identified three sequential stages of morphological and functional alterations: A first stage of still intact hormonogenesis due to compensated apical iodide transport associated with increased CLC-5 expression, a second stage of intracytoplasmatic hormonogenesis accompanied by increased oxidative stress and apoptosis ultimately resulting in cell destruction. This slowly progressive pathophysiological mechanism is in accordance with the usually late onset of congenital hypothyroidism and goiterogenesis in patients with Pendred syndrome. Further, the two last publications highlight the interest of the direct study of the human thyroid tissue in congenital hypothyroidism. These difficult to realize analyzes significantly increase our knowledge on genetic or cellular mechanisms of thyroid dysgenesis and dyshormonogenesis.

Genome-wide association study identifies four genetic loci associated with thyroid volume and goiter risk

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Am J Hum Genet 2011;88:664–73.

Background: Goiter is a frequent condition, especially in iodine-deficient areas. Sibling studies from Denmark, a region with previously mild to moderate iodine deficiency, demonstrated a higher correlation for thyroid volume in monozygotic twins as compared to dizygotic twins, suggesting that genetic factors account for approximately 61–78% of the interindividual variation of the thyroid volume.

Methods: The authors realized a genome-wide association study with goiter in a cohort consisting of 3,620 adult participants of the Study of Health in Pomerania, a region of Germany. The study was replicated in a second large cohort of 1,290 adult subjects.

Results: Four genetic loci were associated with thyroid volume in this genome-wide approach. Two independent loci were located upstream of and within the CAPZB gene, which codes for the β -subunit of the barbed-end F-actin-binding protein. This protein modulates actin polymerization, a process crucial in the colloid engulfment during thyroglobulin mobilization in the thyroid. The third locus marked FGF7 (fibroblast growth factor 7). Members of the fibroblast growth factor family have been discussed as putative signal molecules involved in the regulation of thyroid development. Therefore, the authors suggested that the causative sequence variant underlying the observed association causes an enhanced FGF7 signal, thus mediating a more pronounced proliferation of thyroid cells in risk-allele carriers, resulting in thyroid hyperplasia and increased thyroid volume. The fourth locus represents a ‘gene desert’ on chromosome 16q23, located directly downstream of the predicted coding sequence LOC440389. Experimental proof of the formerly predicted mature mRNA, however, demonstrates that LOC440389 indeed represents a real gene, expressed in the thyroid gland.

Conclusion: This study increases the knowledge about genetic factors and physiological mechanisms influencing thyroid volume.

Familial clustering of goiter development in iodide-deficient regions has raised the question of multifactorial etiology combining environmental and individual genetic factors. Earlier reports in family and twin studies suggested considerable influence of a hereditary familial component. This large genome-wide association study in two independent adult cohorts of areas of comparable iodine supply identified 4 loci associated with thyroid volume and goiter risk independently of increased familial risk. It remains to be shown whether these loci are also associated with early goiter genesis in childhood or adolescence in iodine-deficient regions.

Psychomotor development of children from an iodine-deficient region

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J Pediatr 2011.

Background: The impact of low thyroxine during pregnancy on offspring development has been studied but the impact of low iodine status has been more rarely evaluated. The authors aimed to assess the psychomotor development of the progeny of women from a moderately iodine-deficient area for whom thyroid function and urinary iodine content during pregnancy was measured.

Methods: The development of 86 children was assessed by the Bayley Scale of Infant Development at 12, 18, and 24 months.

Results: Maternal serum-free thyroxine (FT₄) levels in the first trimester of pregnancy were the major determinant of psychomotor development at 18 and 24 months. Children born from mothers with FT₄ levels <25th percentile (<10 pg/ml) had an OR of 2.1 for mild-to-severe delay. Furthermore, alterations in behavior were already observed at 12 months and were related to subsequent changes in development. Neonatal thyroid status did not influence development. The progeny of mothers with hypothyroxinemia or iodine deficiency had a common developmental phenotype: decreased Psychomotor Development Index (PDI) by 18 months that at 24 months persisted and presented with mental impairment.

Conclusion: This study highlights the need to implement active measures of iodine supplementation periconceptionally and during pregnancy because the negative effects on development and behavior might be prevented through preemptive action.

Breast-milk iodine concentration declines over the first 6 months postpartum in iodine-deficient women

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Am J Clin Nutr 2010;92:849–56.

Background: As shown before, thyroid and iodine status have been investigated in pregnant women. However, little is known about the iodine status of lactating mothers and their infants during the first 6 months postpartum. If deficient, the amount of supplemental iodine required to improve status is also unknown. The objective of the authors was to determine maternal and infant iodine status and the breast-milk iodine concentration (BMIC) over the first 6 months of breastfeeding.

Methods: They conducted a randomized, double-blind, placebo-controlled supplementation trial in lactating women who received placebo (n = 56), 75 µg iodine/day (n = 27), or 150 µg iodine/day (n = 26) after their infants' birth until 24 weeks postpartum. Maternal and infant urine samples, breast milk and serum samples were collected and analyzed.

Results: Iodine deficiency was defined as UIC <100 µg/l. Over 24 weeks, the median urinary iodine concentration (UIC) of unsupplemented women and their infants ranged from 20 to 41 µg/l and 34 to 49 µg/l, respectively. Mean maternal UIC was 2.1–2.4 times higher in supplemented than in unsupplemented women (p < 0.001) but did not differ significantly between the two supplemented groups. BMIC was 1.3 and 1.7 times higher in women supplemented with 75 µg iodine/day (p = 0.030) and 150 µg iodine/day (p < 0.001), respectively, than in unsupplemented women. BMIC in the placebo group decreased by 40% over 24 weeks (p < 0.001). Thyrotropin and free thyroxine did not differ significantly between groups.

Conclusions: Daily iodine supplementation increased BMIC and prevented the decline of BMIC in these women over the first 6 months compared with women who received a placebo. Nevertheless, the concentration of iodine in breast milk was still well below the cutoff of 100–200 µg/l recommended by the WHO and would be unable to meet the iodine requirements of these young infants.

The first study is the third publication of the same cohort of pregnant mothers and their infants from an iodine-deficient region. This study reports the so far most complete longitudinal documentation of iodine and thyroid status of pregnant mothers in all three trimesters as baseline and the psychomotor development of the children at 12, 18 and 24 months. First, the study confirms the data presented in the paper of Heinrichs et al. in this chapter on the association of hypothyroxinemia during the first trimester and delayed psychomotor development. Second, it extends our knowledge on the influence of iodine deficiency: urinary iodine in the first as well as the third trimester was significantly related to psychomotor development at 24 months. The authors proposed prescription of iodine supplementation preconceptionally, during pregnancy and during lactation as a preventive measure for developmental delay.

The second publication goes in line with the proposals of the first paper. It represents the first longitudinal study examining the iodine status of lactating mothers and their breastfed infants during a 6-month randomized, double-blind, placebo-controlled supplementation trial with two different doses of iodine in iodine-deficient mothers postpartum. The supplementation doses were however lower than the WHO recommendations for exclusively lactating mothers in iodine-deficient regions (75 and 150 µg vs. 250 µg iodine intake per day). The urinary and breast-milk iodine content was increased by low or high supplementation dose of iodine compared to placebo, but the used doses were insufficient to normalize iodine status in the mother or the infant in an iodine-deficient area supporting the WHO recommendation of 250 µg iodine intake per day. Further dose-finding studies are needed in iodine-deficient regions.

Iodine deficiency in northern Paris area: impact on fetal thyroid mensuration

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Background: The authors studied the prevalence and impact on fetal thyroid development of iodine deficiency in pregnant women in the northern part of the Paris conurbation, an area with a low-income and migrant population. More specifically, the authors asked the question of the reliability to diagnose iodine deficiency by a proxy, i.e. the size of the fetal thyroid during the first trimester of pregnancy.

Methods: 110 patients underwent several determinations of urinary iodine excretion (UIE) and of serum FT₄, FT₃, and TSH. Fetal thyroid gland size was assessed using ultrasonography.

Results: The authors found evidence of widespread iodine deficiency (mean UIE 49.8 µg/l (SD 2.11)). Iodine deficiency did not correlate significantly with maternal thyroid parameters. Both the ratio of fetal thyroid gland diameter over fetal head circumference and UIE were available for 90 women at 22 GA. In these patients, the fetal thyroid gland diameter/head circumference ratio showed a significant negative correlation with UIE ($\rho = 20.25$; $p = 0.02$). The fetal thyroid size at 22 GA however could not be used on an individual basis to predict the iodine status in those women.

Conclusion: Iodine deficiency during pregnancy is still a problem in the geographical area of Paris and affects the fetal thyroid gland.

Altogether these last three articles point to the importance of T₄, but also iodine supply in pregnant and lactating women, to allow optimal neuromotor and cognitive development in their offspring. They also point to the unmet needs for universal screening of pregnant women for thyroid as well as iodine status.

Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance

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Background: Many nutritional and hormonal signals regulating energy balance are integrated in the hypothalamus. However, it is unclear whether thyroid hormones have an effect on the central nervous system (CNS)-mediated regulation of global energy balance.

Methods: In vivo studies in the murine model.

Results: The authors showed that either whole-body hyperthyroidism or central administration of triiodothyronine (T_3) decreased the activity of hypothalamic AMP-activated protein kinase (AMPK), increased the sympathetic nervous system (SNS) activity and upregulated thermogenic markers in brown adipose tissue (BAT). Inhibition of the lipogenic pathway in the ventromedial nucleus of the hypothalamus (VMH) prevented CNS-mediated activation of BAT by thyroid hormone and reversed the weight loss associated with hyperthyroidism. Similarly, inhibition of thyroid hormone receptors in the VMH reversed the weight loss associated with hyperthyroidism. This regulatory mechanism depended on AMPK inactivation, as genetic inhibition of this enzyme in the VMH of euthyroid rats induced feeding-independent weight loss and increased expression of thermogenic markers in BAT. These effects were reversed by pharmacological blockade of the SNS.

Conclusion: Thyroid hormone-induced modulation of AMP-activated protein kinase activity and lipid metabolism in the hypothalamus is a major regulator of whole-body energy homeostasis.

Traditionally, it was assumed that the effect of thyroid hormones on energy homeostasis was exerted peripherally. The presented study shows in an elegant set of experiments that a central regulation of energy balance by thyroid hormones exists and that this central regulation is mediated by AMP-activated protein kinase in the ventromedial nucleus of the hypothalamus. Specifically, central T_3 or hyperthyroidism inhibits AMPK activity, thereby increasing de novo hypothalamic lipogenesis. Enhanced fatty acid metabolism acts via the sympathetic nervous system and results in upregulated thermogenesis in brown adipose tissue and finally in weight loss. However, whether modulation of AMPK in the ventromedial nucleus is pharmacologically possible in a selective manner to treat disorders of compromised energy balance remains to be shown in the future.

New mechanism

Mice deficient in MCT8 reveal a mechanism regulating thyroid hormone secretion

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Background: While the consecutive steps of thyroid hormone synthesis and the proteins involved are well characterized, the mechanism of thyroid hormone secretion from the thyrocyte into the bloodstream has long been assumed to be a passive process. However, the precise mechanism has not been investigated. MCT8 has been identified as a key transmembrane transporter of T_3 and T_4 in the peripheral

tissues and in the brain. Based on preliminary observations in the Mct8 knockout mice, the authors hypothesized that Mct8 could also be expressed in thyrocytes and be involved in regulation of thyroid hormone secretion.

Methods: The authors compared thyroid hormone synthesis, thyroid hormone content of the thyroid gland, and kinetics of thyroid hormone secretion in WT versus Mct8 knockout mice.

Results: The authors showed first expression of Mct8 in the basolateral membrane of thyrocytes in the WT mice. In the Mct8 knockout mice, the authors described reduced serum T₃ and T₄ shortly after pharmacological depletion of the thyroid gland from thyroid hormones. They then showed that thyroid glands of Mct8 knockout mice contained more non-thyroglobulin-associated T₄ and triiodothyronine than thyroid glands from WT mice. Further, after administration the secretion rate and appearance of ¹²⁵I in the serum was greatly reduced. Finally, depletion of thyrocytes thyroid hormone content was slower during iodine deficiency and after injection of TSH compared to WT mice.

Conclusions: This is the first study to demonstrate that Mct8 is involved in the secretion of TH from thyrocytes and contributes in part to the low level of T₄ observed in MCT-8 deficient patients.

So far, it was assumed that Mct8 was regulating thyroid hormone uptake through the cell membrane of peripheral target cells. By an extensive in vivo study in Wt and Mct8 knockout mice, the authors are able to propose a new function for Mct8. The results clearly show that Mct8 is (1) expressed at the basolateral membrane of thyrocytes and (2) involved in thyroid hormone secretion from thyrocytes. Thus, this is the first study so far to provide a molecular mechanism for thyroid hormone release from the thyrocyte and revises the concept that thyroid hormone secretion is a passive diffusion process through the lipid bilayer of the basolateral membrane. Further studies are needed to determine involvement of other putative thyroid hormone transporters in this process.

Concept revised

Lack of action of exogenously administered T₃ on the fetal rat brain despite expression of the monocarboxylate transporter 8

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Endocrinology 2011;152:1713–21.

Background: Mct8 is a thyroid hormone (T₄ and T₃) transmembrane transporter in target tissues. Mutations of the monocarboxylate transporter 8 gene (MCT8, SLC16A2) cause the Allan-Herndon-Dudley syndrome, an X-linked syndrome of severe intellectual deficit and neurological impairment. It is generally assumed that this syndrome is caused by lack of T₃ transport to neurons during critical periods of fetal brain development. The authors questioned this concept.

Methods: To evaluate the role of Mct8 in the fetal brain, the effect of T₄ vs. T₃ rescue was investigated on fetal cerebral cortex in a murine model of fetal hypothyroidism by gene expression and immunohistochemical studies.

Results: T₄ increased the expression of thyroid hormone target genes in the fetal cerebral cortex, but surprisingly T₃ was without effect. They then investigated the cause for the lack of T₃ action by analyzing, with confocal microscopy, the expression of organic anion transport polypeptide (Oatp14, Slco1c1), a T₄ transporter, and Mct8 (Slc16a2), a T₄ and T₃ transporter. Both proteins were present in the brain capillaries forming the blood-brain barrier and in the epithelial cells of the choroid plexus forming the blood-cerebrospinal fluid barrier.

Conclusion: T₄ from the maternal compartment influences gene expression in the fetal cerebral cortex, possibly after transport via organic anion transporter polypeptide and/or Mct8, and conversion to T₃ in the astrocytes. On the other hand, T₃ does not reach the target neurons directly despite the presence of Mct8. The data indicate that T₄, through local deiodination in astrocytes, provides most T₃ in the fetal rat brain. The role of Mct8 as a T₃ transporter in the fetal rat brain is therefore uncertain.

This provocative study emphasized the role of systemic T_4 and not that of systemic T_3 as major regulator of thyroid hormone target genes expression in the developing cerebral cortex, as already shown by a previous publication by the same authors. Further, based on normal Mct8 protein expression in the endothelial cells of the brain capillaries, the authors questioned the role of Mct8 as T_3 transporter in the fetal cerebral cortex and propose an indirect T_3 delivery of neurons by initial T_4 uptake and deiodination to T_3 in astrocytes and secondary release from astrocytes to neurons. The role of different known and putative thyroid hormone transporters in the cerebral cortex remains a fascinating and important field of research in thyroidology. Every new piece of information on physiological mechanisms will help in defining adequate measures for preventing developmental delay in infants of pregnant mothers with thyroid disorders.

Hes1 is required for appropriate morphogenesis and differentiation during mouse thyroid gland development

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Background: The thyroid follicular cells or thyrocytes, which produce thyroxine (T_4), originate mainly in the median anlage while C-cells (parafollicular cells), which produce calcitonin (CT), originate only in the ultimobranchial bodies. Hes1 is an important target gene in the Notch signaling pathway. This pathway through Hes1, a bHLH transcriptional repressor, influences progenitor cell proliferation and differentiation and is involved in other endocrine gland development.

Methods: To investigate the role for Hes1 in thyroid development, the authors analyzed Hes1 expression during thyroid differentiation and studied thyroid development in mice lacking Hes1.

Results: During normal mouse thyroid development, Hes1 was detected from E9.5 onwards in the median anlage, and at E11.5 in the ultimobranchial bodies. Hes1^{-/-} mouse embryos had a significantly lower number of Nkx2-1-positive progenitor cells ($p < 0.05$) at E9.5 and at E11.5. Moreover, Hes1^{-/-} mouse embryos showed a significantly smaller total thyroid surface area (-40 to -60%) compared to wild-type mice at all study time points (E9.5–E16.5). In Hes1^{-/-} mouse embryos, fusion of the median anlage with the ultimobranchial bodies was delayed by 3 days (E16.5 vs. E13.5 in wild-type mice). After fusion of thyroid anlagen, hypoplastic Hes1^{-/-} thyroids revealed a significantly decreased labeling area for T_4 (-78%) and calcitonin (-65%) normalized to Nkx2-1-positive cells. Decreased T_4 synthesis might be due to reduced Nis labeling area (-69%).

Conclusion: These data provided for the first time evidence that Hes1 is an additional regulator of murine thyroid development and differentiation, beside the known thyroid enriched transcription factors. Hes1 maintained an adequate pool of thyroid progenitor cells via a p57-independent mechanism, determining normal thyroid size. Moreover, Hes1 was necessary for normal endocrine function of both thyrocytes and C cells in the differentiated thyroid gland.

This study identified a new player in the thyroid developmental network in the murine model. Remarkably, Hes1 is important for development and differentiation of two cell types in the thyroid, namely thyrocyte as well as C cells. Hes1 is involved in controlling the number of thyrocyte and C-cell progenitors, and at a later embryonic stage, the adequate differentiation of both cell types.

In vivo role of different domains and of phosphorylation in the transcription factor Nkx2-1

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Background: The transcription factor Nkx2-1 (also known as TTF-1, Titf1 or T/EBP), a marker of precursor and mature thyroid follicular cells, contains two apparently redundant activation domains and is posttranslationally modified by phosphorylation.

Methods: The authors have generated mutant mouse strains to assess the respective roles of the two activation domains and of phosphorylation in mouse development and differentiation *in vivo*.

Results: Phenotypic analysis showed for each mutant a distinct set of defects demonstrating that distinct portions of the protein endow diverse developmental functions of Nkx2-1. The mouse strain with mutated phosphorylation site showed a thyroid gland with deranged follicular organization and gene expression profile demonstrating the important functional role of phosphorylation of Nkx2-1. Further data demonstrated that for correct pituitary development only one of the functional domains of Nkx2-1, indicated as activation domain 1, was indispensable while both activation domains were required for thyroid development.

Conclusions: The different functions of Nkx2-1 are not all due to the protein as a whole. Some of them can be assigned to separate domains of the protein or to specific posttranslational modifications, such as phosphorylation.

These results show elegantly how different functions of a transcription factor can be mapped to distinct portions of the protein. Further, the overlaid posttranslational modification of the protein by phosphorylation adds another level of regulatory potential. These data are important in the understanding of regulation of organogenesis in NKX2-1-expressing organs during embryogenesis, such as hypothalamus, lung, and thyroid, and the wide phenotypic spectrum of mutations in the NKX2-1 gene in humans with brain-lung-thyroid syndrome [10].

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