Hypothyroidism and Pregnancy

MICHAELA GRANFORS
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Abstract

Hypothyroidism is a common endocrine disorder affecting women of reproductive age. On a global level, iodine deficiency is still the most common cause of hypothyroidism. Also genetic variations, in particular SNP rs4704397 in the PDE8B gene, are responsible for a significant proportion of TSH variations. Untreated hypothyroidism has significant adverse effects on pregnancy and fetal outcome. Most international guidelines suggest targeted thyroid testing in pregnant women with risk factors for thyroid disturbances.

In a case-control study, an association between homozygous A/A as well as homozygous G/G carriers of SNP rs 4704397 in PDE8B and recurrent miscarriage was found. The explanation for this association is unknown.

In a nationwide survey, all guidelines for thyroid testing and management of hypothyroidism during pregnancy in Sweden were collected and compared with international guidelines. The local guidelines were variable and poorly compliant with the international guidelines.

In a follow-up in one district, 5,254 pregnant women were included for subsequent review of their medical reports. We found a targeted thyroid testing rate of 20.1% in clinical practice, with an overall frequency of women with trimester-specific elevated TSH of 18.5%. More disturbingly, half of the women who were on levothyroxine treatment at the time of conception had an elevated TSH level at thyroid testing.

In a subsequent cohort study of the 5,254 women, we found the prevalence of trimester-specific elevated TSH and overt hypothyroidism to be equal in targeted thyroid tested and untested women.

In a cross-sectional study, a median urinary iodine concentration (UIC) of 98 μg/l was found in the study population. According to WHO/UNICEF/IGN criteria, the population-based median UIC during pregnancy should be 150-249 μg/l.

In conclusion, genetic variations may contribute to adverse pregnancy outcomes. In clinical practice, thyroid testing and the management of hypothyroidism during pregnancy is unsatisfactory, regarding the whole chain from development of local guidelines to their implementation and to targeted thyroid testing. Moreover, our results indicate insufficient iodine status in the pregnant population of Sweden.

Keywords: phosphodiesterase 8B, recurrent miscarriage, single nucleotide polymorphism, thyroid, guidelines, hypothyroidism, pregnancy, survey, thyroid testing, screening, iodine, iodine deficiency, median urinary iodine concentration

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The more you know, the better you realize how little you actually know.

After Aristotle, 384-322 BC

To all women who suffer from hypothyroidism during pregnancy
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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### Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>AOR</td>
<td>Adjusted Odds Ratio</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>G</td>
<td>Guanine</td>
</tr>
<tr>
<td>hCG</td>
<td>Human Chorionic Gonadotropin</td>
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<tr>
<td>IGN</td>
<td>The Iodine Global Network</td>
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<tr>
<td>IQ</td>
<td>Intelligence Quotient</td>
</tr>
<tr>
<td>LT4</td>
<td>Levothyroxine</td>
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<tr>
<td>OR</td>
<td>Odds Ratio</td>
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<tr>
<td>PDE8B</td>
<td>Phosphodiesterase Type 8B</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>T3</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>Thyroxine</td>
</tr>
<tr>
<td>TBG</td>
<td>Thyroxine-Binding Globulin</td>
</tr>
<tr>
<td>Tg</td>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>TPO</td>
<td>Thyroid Peroxidase</td>
</tr>
<tr>
<td>TPOAb</td>
<td>Thyroid Peroxidase Antibody</td>
</tr>
<tr>
<td>TRH</td>
<td>Thyrotropin Releasing Hormone</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid Stimulating Hormone</td>
</tr>
<tr>
<td>UIC</td>
<td>Urinary Iodine Concentration</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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Preface

After having worked with obstetrics for several years, I was astonished by the various attitudes different doctors had to hypothyroidism during pregnancy. While some advocated testing and treating a large number of women, others seemed to feel that the issue was not important. Most colleagues, however, seemed, as I was, to be confused. Even more confusing, iodine status was never mentioned or discussed. The idea of hopefully being able to contribute knowledge to the subject was an inspiration for me.
Introduction

Historical perspective

In a historical perspective, iodine deficiency has been both an extensive and the most important reason for hypothyroidism worldwide, often visible as a goiter. Until barely a hundred years ago, hypothyroidism and goiter due to iodine deficiency was common in, for instance, parts of central Europe around the Alps and also in parts of Scandinavia. The primary scenario and consequences of severe hypothyroidism was often described as cretinism, which includes impairment of mental and physical growth, development, and has a negative impact on most organ systems. Infertility was common and, in case of pregnancy, the child was often similarly affected. As endemic cretinism was widespread in certain areas, it was often attributed to stagnant air in the valleys or bad water.

Marine and Kimball showed in 1917 that, in most cases, goiter was caused by iodine deficiency and could be prevented by iodine supplementation. In 1928-9 medical doctor and scientist Axel Höjer conducted an extensive nationwide survey to map the goiter rate in Sweden. His group systematically examined 29,000 men and women from all over Sweden, and reported several cases of cretinism and a goiter prevalence of more than 15% in men and more than 33% in women in several parts of the country (Figure 1).

Figure 1. Goiter prevalence in Sweden 1929
Switzerland and the United States were the first countries to introduce goiter prophylaxis through salt iodization. In Sweden, iodine prophylaxis with iodized salt was established in 1936.  

### Definitions and causes of hypothyroidism

Definitions used in this thesis:
- **Overt hypothyroidism**: a TSH level above the defined upper limit of the reference range in combination with a serum free T4 below the lower limit of the reference range
- **Subclinical hypothyroidism**: elevated TSH levels, combined with normal free T4 levels
- **Hypothyroxinemia**: normal TSH levels in conjunction with free T4 levels below the lower limit of the reference range
- **Thyroid autoimmunity**: presence of thyroid autoantibodies

Iodine deficiency is still the most common cause of hypothyroidism worldwide, but in areas of iodine sufficiency, chronic autoimmune thyroiditis (Hashimoto’s thyroiditis) is the main cause of hypothyroidism. Other causes of hypothyroidism include prior thyroidectomy or ablative radioiodine therapy, whereas secondary (pituitary) and tertiary (hypothalamic) causes of hypothyroidism are rare.

### The thyroid gland during fetal life and pregnancy

An overview of thyroid homeostasis is shown in Figure 2.

![Figure 2](image.png)

**Figure 2.** The hypothalamic–pituitary–thyroid axis with positive (+) and negative (−) feedback control. During pregnancy, also hCG stimulates the thyroid gland due to similarities in TSH and hCG glycoprotein.
The thyroid gland during fetal life

During fetal life, the thyroid gland completes its formation at the end of the first trimester of pregnancy. Before that, maternal free T4 and perhaps T3 in very low concentrations are the only sources of thyroid hormones in the developing fetus. From 16-20 weeks of gestation, secretion of thyroid hormones in the fetus reaches significant levels, but the regulatory feedback system including the pituitary and hypothalamus is not fully developed until birth. Besides the thyroid hormones, iodine passes the placenta and accumulates in the fetal thyroid from 12 weeks of gestation, where it is used for thyroid hormone production. Thyroid hormones are essential for neural development and myelination of nerve cells in all the stages of fetal life.

The thyroid gland during pregnancy

Pregnancy is a kind of “stress test” for the thyroid gland. If the thyroid gland is unable to adapt to these physiological changes during pregnancy, hypothyroidism may occur. Production of T4 and T3 increases by 50%, along with a 50% increase in the daily iodine requirement from early pregnancy. During normal pregnancy, there are several maternal physiological changes to adapt to the demands of the mother and the fetus. TBG levels increase, which leads to lower levels of free T4, resulting in a feedback stimulation of the thyroid gland. Moreover, fast increasing hCG-levels in maternal plasma in early pregnancy transiently stimulate the thyroid gland due to similarities in TSH and hCG glycoprotein.

Ten to fifteen percent of pregnant women are estimated to be positive for thyroid autoantibodies, which are indicators of underlying chronic autoimmune thyroiditis such as Hashimotos thyroiditis. TPOAb are the most sensitive and specific autoantibodies for thyroid autoimmunity. Even in euthyroid women, thyroid autoimmunity is a risk factor for incurring hypothyroidism later in pregnancy or postpartum thyroiditis.

Prevalence of hypothyroidism during pregnancy

Hypothyroidism is one of the most common endocrine disorders in women of reproductive age. The prevalence of overt hypothyroidism during pregnancy has commonly been estimated to be 0.3-0.5% and, in addition, 2-3% of pregnant women may suffer from subclinical hypothyroidism. Of course, the prevalence of overt or subclinical hypothyroidism depends on the upper TSH cut-off levels used. There is strong evidence that the reference range for TSH is lower throughout pregnancy compared with the non-pregnant state. The lowest TSH levels are observed during the first tri-
mester of pregnancy, and are apparently related to hCG stimulation of the thyroid gland as hCG levels are highest early in gestation\textsuperscript{15-17}.

According to several guidelines, TSH reference intervals should be established from the 95\% confidence limits of the log-transformed values of at least 120 apparently healthy individuals without any personal or family history of thyroid disease, goiter, thyroid autoantibodies or medications\textsuperscript{18 19}. Due to thyroid hormone changes during pregnancy, separate, trimester-specific TSH reference ranges, as defined in populations with optimal iodine intake, should be established. However, in the absence of local normative data, the following upper cut-off levels for TSH are commonly recommended: 2.5 mIU/L in the first and 3.0 mIU/L in the second and third trimester of pregnancy\textsuperscript{10 20 21}. As few laboratories have established trimester-specific reference ranges, the use of these latter TSH reference ranges is widespread. When applying these reference ranges, several studies report a prevalence of hypothyroidism of 12-15\% during pregnancy\textsuperscript{22-24}.

Reference intervals for free T4 are important to be able to differentiate overt from subclinical hypothyroidism, and universally accepted free T4 reference ranges would be desirable. In the non-pregnant state, 0.03\% of total T4 is unbound to serum proteins and is available for tissue uptake. The remaining 99.97\% of total T4 is bound to transport proteins, mainly to TBG (70\%) and to a lesser extent to albumin and transthyretin (around 15\% each). Pregnancy implies an abnormal binding-protein state with higher levels of TBG and lower levels of albumin.

Free T4 levels are usually measured by immunoassays that are known to be sensitive to alterations in binding proteins\textsuperscript{25}. Due to the abnormal binding-protein state during pregnancy, these commonly used immunoassays do not accurately reflect free T4 levels during pregnancy\textsuperscript{26}. Methods of equilibrium dialysis and tandem mass spectrometry have been considered to be the gold standard for the measurement of T4 during pregnancy\textsuperscript{27}. However, the method is expensive and demanding, and its use is not widespread\textsuperscript{10 28}. Alternative strategies, for example, the use of free T4 indexes have been suggested\textsuperscript{26} and criticized by others\textsuperscript{28}. In case of using immunoassays, the establishment of method- and trimester-specific reference ranges for free T4 are usually recommended\textsuperscript{10}. Moreover, it is pointed out that serum TSH is a more accurate indicator of thyroid function during pregnancy than any free T4 measurement apart from equilibrium dialysis and tandem mass spectrometry\textsuperscript{10}.

The Committee for Standardization of Thyroid Function Tests (C-STFT) keeps on working on the development of a reference measurements system for standardization, investigating the status of between-assay comparability, and assessing the key performance attributes of the individual assays for thyroid hormones\textsuperscript{27}. 
Genetic influences on thyroid hormones

More than 99.9% of human DNA sequences are the same, even in unrelated individuals. Single nucleotide polymorphisms, SNPs, make up about 90% of all human genetic variation. SNPs are DNA sequence variations that occur when a single nucleotide in the genome sequence is altered. Many SNPs have no known effect on cell function, while others are believed to predispose people to disease or influence their response to a drug.

Heritability studies have suggested that up to two thirds of circulating thyroid hormone and TSH levels are genetically determined\textsuperscript{29, 30}. However, until now, polymorphisms in only a few genetic loci have been identified to be associated with circulating TSH levels\textsuperscript{30, 31}. One of those genetic loci is the human phosphodiesterase type 8B (PDE8B), which is predominantly expressed in the thyroid gland. PDE8B is located on chromosome 5 and encodes a high affinity cAMP specific nucleotide phosphodiesterase\textsuperscript{32-34}. The SNP that to date has been shown to have the strongest association to TSH levels is SNP rs 4704397 in PDE8B, being responsible for approximately 2.3% of TSH variation\textsuperscript{30, 35}. While this association was initially detected in a genome-wide association study\textsuperscript{35}, it has been confirmed in several subsequent studies\textsuperscript{36, 37}. In addition, the association between SNP rs 4704397 in PDE8B and levels of TSH has been confirmed in pregnant women\textsuperscript{38}.

In SNP rs 4704397 of PDE8B an adenine (A) nucleotide is replaced by a guanine (G). The association between the polymorphism and higher levels of TSH and lower free T4 levels, indicating relative hypothyroidism, is found in homozygous carriers of A/A\textsuperscript{37}. The SNP rs 4704397 is present in intron 1 of the PDE8B gene, and is thus not directly involved in a coding region\textsuperscript{37}. Based on previous results it has been proposed that the SNP rs 4704397 in PDE8B and in particular the presence of A alleles might induce increased phosphodiesterase activity in PDE8B, thereby reducing the ability of the thyroid gland to generate free T4 when stimulated by TSH\textsuperscript{35}.

The possible impact of SNP rs4704397 in PDE8B on clinical outcomes has barely been studied. As A/A has been associated with higher TSH levels, we hypothesized that homozygous carriers of A/A might be at increased risk of recurrent miscarriage.

Consequences of hypothyroidism during pregnancy

Recurrent miscarriage

Recurrent miscarriage is commonly defined as three or more consecutive pregnancy losses before 20 weeks of amenorrhoea and affects approximately 1% of all couples trying to conceive\textsuperscript{39, 40}. Predisposing factors include maternal age, parental chromosomal aberrations, uterine abnormalities, antiphos-
pholipid syndrome, immunological and thrombophilic disorders, and endocrine diseases such as hypothyroidism and diabetes mellitus. Unlike sporadic spontaneous miscarriage, recurrent miscarriage more often occurs despite normal fetal cytogenetic findings, and in 50% of cases the underlying cause remains unexplained. There is a known relation between hypothyroidism and recurrent miscarriage.

Adverse pregnancy outcomes

In relation to overt hypothyroidism

Overt hypothyroidism during pregnancy has been clearly associated with adverse pregnancy outcomes such as preeclampsia, gestational hypertension, fetal death, premature delivery, spontaneous abortions, recurrent abortions and cretinism. Numerous observational studies confirm the benefit of treating overt hypothyroidism during pregnancy. A recent review summarized that levothyroxine is effective in reducing the risk of miscarriage and preterm delivery in women with overt hypothyroidism during pregnancy. Treatment is generally recommended.

In relation to subclinical hypothyroidism

Numerous studies have investigated possible associations between subclinical hypothyroidism and adverse pregnancy outcomes such as preeclampsia, gestational hypertension, fetal death, premature delivery, spontaneous abortions or recurrent abortions. In comparison to overt hypothyroidism, the data regarding subclinical hypothyroidism are not as consistent. A recent review summarized that subclinical hypothyroidism in early pregnancy, compared with normal thyroid function, was associated with the occurrence of preeclampsia and an increased risk of perinatal mortality.

Concerning the treatment of subclinical hypothyroidism during pregnancy, current evidence is insufficient, and controlled, randomized trials are lacking. Pregnancy outcomes related to subclinical hypothyroidism are currently being investigated as secondary outcomes in an ongoing, prospective randomized controlled trial by the National Institute of Child Health and Human Development, USA.

In relation to hypothyroxinemia

Some studies have investigated possible associations between hypothyroxinemia and pregnancy outcomes. Findings are divergent. Korevaar et al found an association between hypothyroxinemia and premature delivery, while several other studies did not find hypothyroxinemia to be associated with different pregnancy outcomes.
Studies concerning the treatment of hypothyroxinemia and the effect on pregnancy outcome are lacking. Pregnancy outcomes related to hypothyroxinemia are also being investigated as secondary outcomes in the ongoing, prospective randomized controlled trial by the National Institute of Child Health and Human Development, USA.48

In relation to thyroid autoimmunity

In observational studies, the presence of thyroid autoantibodies (mainly TPOAb) in euthyroid pregnant women or women of childbearing age has been associated with an increased risk of unexplained subfertility, miscarriage, recurrent miscarriage, preterm birth and maternal post-partum thyroiditis when compared with the absence of thyroid antibodies.47 One prospective, randomized trial including 984 euthyroid pregnant women showed higher rates of miscarriage and premature deliveries (<37 weeks) in TPOAb-positive women not treated with levothyroxine compared with treated TPOAb-positive or TPOAb-negative women53. However, it is worth noticing that most cases of miscarriage occurred prior to the initiation of treatment/no treatment.

To date, there is insufficient evidence to recommend for or against levothyroxine treatment in euthyroid TPOAb-positive women 1) undergoing assisted reproduction technologies, 2) with sporadic or recurrent abortion and 3) during pregnancy (to prevent miscarriage or preterm delivery)10 20 46.

Two ongoing RCTs assess the effect of levothyroxine treatment on pregnancy outcome in euthyroid TPOAb-positive women with a history of (recurrent) miscarriage or with an ongoing treatment for infertility: The TABLET (Thyroid Antibodies and Levothyroxine) trial in the UK, and the T4 Life trial in the Netherlands. Planned closing dates for these trials are in 2015 and 2016, respectively.54 55. Hopefully, these studies will help to clarify whether to treat euthyroid, TPOAb-positive women in the situations described.

Neurocognitive development of the child

In relation to overt hypothyroidism

Overt, untreated hypothyroidism during pregnancy has consistently been shown to be associated with impaired neurodevelopment of the child56 57. In a sub-analysis within an observational study by Haddow et al, children to women with untreated overt hypothyroidism during pregnancy had lower IQ-scores at the age of seven compared with children of women with levothyroxine treated hypothyroidism or euthyroidism during pregnancy.56 In line with this, several observational studies indicate that child neurocognitive development is normal when overt hypothyroidism is detected and subsequently treated during pregnancy58-60.
There are no randomized controlled trials concerning levothyroxine treatment in overt hypothyroidism during pregnancy. This seems unethical, however, and most likely such a study will never be conducted. It is generally considered that the available data confirm the benefits of treating overt hypothyroidism during pregnancy.

In relation to subclinical hypothyroidism or hypothyroxinemia

The effect of subclinical hypothyroidism or hypothyroxinemia on neurodevelopment of the child is less clear. Countless observational studies have investigated the association between these mild forms of thyroid dysfunction and outcomes in offspring as, for example, (non)-verbal cognitive function, behavioral problems, motor or IQ scores or vision or hearing development. However, study designs were variable, and the findings in the studies are not consistent.

Some studies found an association between subclinical hypothyroidism and neurocognitive delay in offspring, while others did not. Others found subclinical hypothyroidism to predict externalizing problems in specific ages or ADHD symptoms in girls.

Consistently, also studies concerning isolated hypothyroxinemia were inconsistent. While several studies suggested an association between, for example, isolated hypothyroxinemia during pregnancy and neurocognitive delay in offspring, others could not confirm these findings.

To date, only one placebo-controlled, randomized trial has assessed the effect of levothyroxine therapy in mild maternal thyroid failure on offspring IQ. The primary outcome was the children’s IQ at the age of three years. There were no differences between the groups in mean IQ or the proportion of children with an IQ less than 85. As a secondary outcome, there were no differences in IQ scores between the treatment and control groups when divided into subgroups (overt or subclinical hypothyroidism or hypothyroxinemia). Thus, the study does not support screening for, and treatment of, mild thyroid failure during pregnancy to prevent impaired childhood cognitive function.

The children from this study are now aged between 7 and 10 years, and a follow-up study is ongoing (CATS II), assessing aspects of the children’s cognitive functioning including their IQ.

Yet another study is also ongoing: a large-scale, prospective randomized controlled trial sponsored by the National Institute of Child Health and Human Development (USA) is screening pregnant women with less than 20 weeks gestation for subclinical hypothyroidism or hypothyroxinemia, and randomizing to treatment with levothyroxine or placebo until delivery. The primary outcome is IQ at five years of age in offspring. The results are expected in 2015. Hopefully, these studies will contribute to clarity.
In relation to thyroid autoimmunity
Studies on the association between thyroid autoimmunity and neurocognitive development of the child are rare. One observational study reported that TPOAb positivity in euthyroid women at 16-20 weeks of gestation was associated with lower scores on intellectual and motor development in their children at 25 to 30 months of age.

Management of hypothyroidism during pregnancy
Guidelines
In recent years, three evidence-based international and European guidelines have been published: 1) The Endocrine Society Guidelines from 2007 with a recent update in August 2012, 2) the guidelines from the American Thyroid Association from 2011, and 3) the European Thyroid Association Guidelines from 2014. The implementation of guidelines into clinical practice is of course essential. Despite existing guidelines, a European survey demonstrated a lack of consensus in thyroid testing and the treatment of hypothyroidism during pregnancy. Moreover, only 11.5% of providers in an American survey had read the Endocrine Society Guidelines.

According to these data, proper implementation of international guidelines into clinical practice is not obvious. Beyond these surveys, few studies have evaluated how international guidelines are implemented into clinical practice.

Treatment of hypothyroidism during pregnancy
Hypothyroidism during pregnancy is recommended to be treated with levothyroxine. In Figure 3, the recommendations of different international guidelines are illustrated. Isolated hypothyroxinemia or isolated thyroid autoimmunity during pregnancy should not be treated. According to the European Thyroid Association, however, levothyroxine therapy may be considered when isolated hypothyroxinemia is detected in the first trimester of pregnancy, but not when detected in the second or third trimester. In hypothyroid women already treated with levothyroxine before conception, the demand of increase in levothyroxine may vary from 25 to 50% from very early in pregnancy. It has been proposed and widely accepted to increase the dosage of levothyroxine by two additional tablets weekly (nine tablets per week instead of seven tablets per week; 29% increase) at confirmation of pregnancy, with subsequent TSH monitoring every four weeks through midgestation. The goal of treatment is to maintain TSH levels within trimester-specific reference ranges.
Universal screening or targeted thyroid testing?

To screen or not to screen – that is the question. Whether all pregnant women should be screened in order to identify and treat thyroid dysfunction has been, and still is, extremely controversial. The Endocrine Society Guidelines 2007 recommend targeted case finding in high-risk women for thyroid disease at the first prenatal visit or at diagnosis of pregnancy, suggesting 10 indications for targeted case finding. However, according to their updated guidelines from 2012, there is insufficient evidence to recommend universal TSH screening at the first trimester visit, leaving options open for either universal screening or targeted thyroid testing. This is in agreement with the guidelines from the American Thyroid Association. If applying targeted thyroid testing, verbal screening of all pregnant women at the initial prenatal visit is recommended, and subsequent TSH testing in case of any present risk factors for thyroid dysfunction (Table 1). Finally, the ambivalence on this topic is nicely illustrated by the guidelines of the European Thyroid Association: while concluding that universal screening for subclinical hypothyroidism in early pregnancy is not recommended because of the lack of grade 1 evidence, it is noted that the majority of the authors anyway recommend universal screening.
It has been shown that targeted case finding in research settings miss about one to two thirds of pregnant women with overt or subclinical hypothyroidism\textsuperscript{79, 80}. The impact of screening method has been ambiguous in the only prospective study concerning this topic: as the main outcome, universal screening compared with case finding did not result in an overall decrease in adverse obstetrical outcomes in the whole study population. As a secondary outcome, when only considering low-risk women, treatment of hypothyroidism or hyperthyroidism identified by screening a low-risk group was associated with a lower rate of adverse outcomes\textsuperscript{80}.

However, these data apply to the research setting, when detailed study protocols are used to identify pregnant women with risk factors for thyroid disease. The efficacy of targeted thyroid testing when implemented in clinical practice is currently unknown. Older data suggest that identifying high risk women for targeted thyroid testing appears to not be very successful outside the research setting. In an audit from 2002, for example, less than 20\% of high-risk women for thyroid disease in a district were screened, despite existing local guidelines\textsuperscript{81}.

Table 1. Indications for targeted thyroid testing during pregnancy according to the American Thyroid Association and 2007 and 2012 Endocrine Society Guidelines

| History of thyroid dysfunction or prior thyroid surgery |
| Family history of thyroid dysfunction |
| Presence of goiter |
| TPOAb positivity (when known) |
| Symptoms or clinical signs of thyroid dysfunction |
| Type 1 diabetes |
| Other autoimmune disorders |
| Infertility (as part of the infertility work-up) |
| History of head or neck radiation |
| History of preterm delivery |
| Age >30 years* |
| History of miscarriage* |
| Residing in an area of known moderate to severe iodine insufficiency* |
| Morbid obesity (BMI > 40 kg/m\textsuperscript{2})† |
| Use of amiodarone or lithium, or recent administration of iodinated radiologic contrast† |

* In American Thyroid Association and 2012 Endocrine Society Guidelines
† Only in American Thyroid Association Guidelines

Iodine

Although required only in very small amounts, iodine is an essential nutrient as part of thyroid hormones. This is illustrated in Figure 4.
Dietary sources and requirements

Iodine is unevenly distributed in the earth’s environment. While iodine-deficient soils are common in, for example, mountain areas far from the sea, most iodine is found in the oceans. The iodine content of crops and plants is reflective of the iodine content of the soil or water they are growing in. Consequently, the iodine levels of, for example, meat, chicken, eggs, dairy products and fish or seafood are reflective of the iodine content of the animal feed used. The native content of most foods and beverages is low, with the exception of fish, seafood, and especially certain seaweeds.

The composition of iodine from dietary sources in a typical food basket can be completely different in different countries, regions, or even within a region. This depends on, for example, the country’s iodine prophylaxis program, and the content of iodine in food or personal food habits. In Sweden, iodized salt is the main source of iodine in the diet. Iodine fortification of edible table salt is, however, not mandatory in Sweden. Dairy products and fish are the second and third contributors to iodine intake in Sweden.

According to WHO/UNICEF/IGN, the recommended daily nutrient intake for iodine is:

- 90 μg for pre-school children (0-5 years);
- 120 μg for schoolchildren (6-12 years);
- 150 μg for adolescents (above 12 years) and adults;
- 250 μg for pregnant and lactating women.
Assessment of iodine status

Currently, there are four different methods generally recommended for assessing iodine nutrition in populations: 1) assessment of thyroid size and goiter rate, 2) urinary iodine concentration (UIC), 3) serum TSH and 4) serum Tg.[85]

Assessment of thyroid size by inspection and palpation is the historical method used for evaluation of goiter prevalence and iodine nutrition. Today, ultrasonography provides a more precise and objective method. Goiter rates in a population reflect long-term iodine nutrition (months to years). Although the assessment of thyroid size is not as widely used as previously, it remains important in assessing goiter rates previous to the introduction of any intervention to control iodine deficiency. School-aged children are the preferred group for this kind of assessment.

Urinary iodine is a good marker of recent iodine intake, as more than 90% of dietary iodine is excreted in the urine within 24-48 hours.[86] Epidemiological criteria for the median UIC for different populations are given in Table 2.

<table>
<thead>
<tr>
<th>Median UIC (μg/L)</th>
<th>Iodine intake</th>
<th>Iodine nutrition status</th>
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<tbody>
<tr>
<td>Adults and school-aged children*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>Insufficient</td>
<td>Severe iodine deficiency</td>
</tr>
<tr>
<td>20-49</td>
<td>Insufficient</td>
<td>Moderate iodine deficiency</td>
</tr>
<tr>
<td>50-99</td>
<td>Insufficient</td>
<td>Mild iodine deficiency</td>
</tr>
<tr>
<td>100-199</td>
<td>Adequate</td>
<td>Optimal</td>
</tr>
<tr>
<td>200-299</td>
<td>Above requirements</td>
<td>May pose a slight risk of more than adequate intake in the overall population</td>
</tr>
<tr>
<td>≥ 300</td>
<td>Excessive</td>
<td>Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)</td>
</tr>
<tr>
<td>Pregnant women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 150</td>
<td>Insufficient</td>
<td>Iodine deficiency</td>
</tr>
<tr>
<td>150-249</td>
<td>Adequate</td>
<td>Optimal</td>
</tr>
<tr>
<td>250-499</td>
<td>Above requirements</td>
<td>-</td>
</tr>
<tr>
<td>≥ 500</td>
<td>Excessive</td>
<td>-</td>
</tr>
<tr>
<td>Lactating women and children &lt; 2 years of age†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 100</td>
<td>Adequate</td>
<td>Optimal</td>
</tr>
</tbody>
</table>

* Applies to adults, but not to pregnant and lactating women
† For lactating women and children <2 years of age, a median urinary iodine concentration of 100 μg/l can be used to define adequate iodine intake, but no other categories of iodine intake are defined. Although lactating women have the same requirement as pregnant women, the median urinary iodine is lower because iodine is excreted in breast milk.
According to WHO/UNICEF/IGN, population-based prevalence surveys of iodine status should be done by measurement of urinary iodine levels from spot urine samples. Surveys should be done separately in school-age children and women of childbearing age/pregnant women. Due to large intra-individual variations of spot UIC, single UIC measurements cannot be used as an individual marker of iodine deficiency. Ideally, an assessment of iodine status should include a concurrent assessment of household use of iodized salt.

TSH levels are poor indicators of iodine status. The exceptions are neonates, where TSH is a valuable indicator for iodine deficiency.

Thyroglobulin (Tg) is a thyroid protein that is a precursor in the synthesis of thyroid hormones. Over a range of iodine intake from severely deficient to excessive, Tg levels show a clear U-shaped curve with the lowest levels in iodine sufficient populations. According to WHO, dried whole blood spots Tg can be used for monitoring of iodine status in school-age children after iodine repletion.

The WHO advises each country to assess the iodine nutrition situation in the population every five years. In case of suboptimal iodine status, supplementation with iodine for vulnerable groups, such as pregnant women, is recommended. Recent European and international guidelines have implemented this and recommend supplementation to pregnancy-planning, pregnant and breastfeeding women with formulas containing around 150 μg iodine daily.

Consequences of iodine deficiency during pregnancy
Iodine deficiency can have multiple adverse effects on fetal and infant growth and neurodevelopment, resulting from inadequate thyroid hormone production.

Effects on neurodevelopment of the child
Exposure to severe iodine deficiency during fetal life and infancy has a detrimental effect on neurocognitive development and may result in cretinism. Several randomized studies have proven that endemic cretinism can be reduced or eliminated with iodine prophylaxis.

Evidence concerning the effect of mild to moderate iodine deficiency during pregnancy on neurocognitive development of the child is not equally clear. Observational studies have, with few exceptions, reported impaired intellectual function and motor skills in children born to mothers with mild to moderate iodine deficiency during pregnancy compared with those born to iodine sufficient mothers.

Results from large-scale controlled trials are lacking, but needed, to clarify whether iodine supplementation will benefit infant and childhood neurodevelopment in countries with mild to moderate iodine deficiency. Results of
randomized controlled trials from Thailand, India, Australia and New Zealand on this topic are expected in the near future.\textsuperscript{96,97}

**Effects on thyroid function**

Severe iodine deficiency results in severe maternal hypothyroidism, but the impact of mild to moderate iodine deficiency on maternal thyroid function during pregnancy is not as consistent. In mild to moderate iodine deficient areas, studies among pregnant women have shown that: 1) thyroid volume increased up to 31\%, 2) TSH levels either increased slightly or remained stable, 3) free T4 levels either decreased or remained stable and 4) Tg levels increased up to 50\% throughout pregnancy.\textsuperscript{98}

Randomized controlled trials have shown that iodine supplementation during pregnancy in mild to moderate iodine deficient areas results in: 1) thyroid volume increasing to a lesser content than in untreated controls, 2) stable TSH levels, 3) either decreasing or stable free T4 levels and 4) in most studies decreasing Tg levels throughout pregnancy.\textsuperscript{98}

Concerning neonatal thyroid function, iodine supplementation during pregnancy resulted in lower Tg levels or smaller thyroid volumes in neonates compared with neonates born after non-supplemented pregnancies. No differences in TSH or free T4 levels were seen between the groups.\textsuperscript{98}

**Global and national iodine status**

On a global level, only a few countries were completely iodine sufficient before 1990. Substantial progress has been made since then, mainly due to effective salt iodization programs.\textsuperscript{99} However, Europe is lagging behind. Populations in several countries remain mildly to moderately iodine deficient, and some countries have also described re-emerging iodine deficiency after decades of iodine sufficiency.\textsuperscript{100} The few countries that have performed iodine nutrition surveys in pregnant women suggest iodine deficiency in as many as 2/3 of the examined pregnant populations in Europe.\textsuperscript{21}

Nationwide surveys indicate adequate iodine nutritional status in the general population in Sweden.\textsuperscript{82,101} Information on iodine nutrition during pregnancy in Sweden is, however, scarce. Two small studies from Uppsala examined the effect of pregnancy on thyroid hormone homeostasis and tested the usefulness of thyroglobulin as an indicator of iodine status during pregnancy.\textsuperscript{102,103} However, the iodine data were not presented in currently used units, and no conclusions were drawn about the iodine status in Swedish pregnant women. Hence, there are no recent data on iodine status of pregnant women in Sweden and no national recommendations concerning iodine supplementation during pregnancy.
Aims

The general aim of this thesis was to examine the implementation of international guidelines and a targeted thyroid testing approach for clinical practice, and to map iodine nutritional status in pregnant women.

The specific aims of the studies were

- To investigate whether there is an association between SNP rs 4704397 in the PDE8B gene and recurrent miscarriage. (Study I)

- To determine the adherence of nationwide local guidelines concerning testing for, and treatment of, hypothyroidism in pregnancy to corresponding international guidelines. (Study II)

- To analyze the degree of implementation of guidelines into everyday clinical practice. (Study II)

- To examine the real-life efficacy of a targeted thyroid testing approach in identifying women with elevated TSH and overt hypothyroidism during pregnancy. (Study III)

- To evaluate iodine nutrition during pregnancy in two regions in Sweden. (Study IV)
Materials and Methods

All the studies included in this thesis were conducted in Sweden. Antenatal care in Sweden is standardized, decentralized to maternity care centers, and free of charge. In Sweden, all pregnant women are invited to an ultrasound examination at 17-19 weeks of gestation, and approximately 97% of the Swedish pregnant population participates\textsuperscript{104}.

Within each maternity care district, a consultant in obstetrics is responsible for the development and implementation of guidelines. In the guidelines of Uppsala County, a targeted thyroid testing approach was implemented in 2004.

Overview of the studies

Table 3. Overview of the studies

<table>
<thead>
<tr>
<th>Study and topic</th>
<th>Design</th>
<th>Inclusion date</th>
<th>Population / materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE8B polymorphism and recurrent miscarriage (Study I)</td>
<td>Case-control</td>
<td>April 2009-June 2010 (cases); May 2007-June 2010 (controls)</td>
<td>n=188 (cases); n=392 (controls)</td>
</tr>
<tr>
<td>Thyroid testing (a) (Study II)</td>
<td>Cross-sectional</td>
<td>2011-2012</td>
<td>Endocrine Society Guidelines 2007; 29 local guidelines n=5254</td>
</tr>
<tr>
<td>Thyroid testing (b) (Study II)</td>
<td>Descriptive</td>
<td>Jan 2009-Dec 2011</td>
<td>n=5254</td>
</tr>
<tr>
<td>Targeted thyroid testing (Study III)</td>
<td>Cohort</td>
<td>Jan 2009-Dec 2011</td>
<td>n=5254</td>
</tr>
<tr>
<td>Iodine status (Study IV)</td>
<td>Cross-sectional</td>
<td>Jan 2006-July 2007, Jan 2010-Sept 2012</td>
<td>n=459</td>
</tr>
</tbody>
</table>

27
Study populations
Uppsala Biobank of Pregnant Women

Data from this biobank have been used for three studies (Studies I – III).

Starting from May 31 2007, Swedish-speaking women aged 18 years or older from the whole of Uppsala County, without blood-borne disease, have been approached to participate in the Uppsala Biobank of Pregnant Women when attending the second-trimester routine ultrasound screening at 17 to 18 weeks of gestation. In Uppsala County, all routine ultrasound examinations are performed at Uppsala University Hospital, which is also the only available delivery ward within the county. Hence, the biobank participants represent a population-based sample. The biobank is a convenience sample based on the availability of a research nurse. Approximately 30% of the respondents decline participation, mainly due to lack of time or fear of blood or needles. Participation in this biobank includes both clinical approaches, such as, for example, medical record reviews and biobank research. A blood sample is collected before the routine ultrasound examination from all women and is stored at -70°C. Moreover, brief maternal demographic data are collected, including ongoing chronic disorders, medication, smoking, and height and weight. It is estimated that the Biobank covers approximately half of the pregnant population of Uppsala County.

Study I

Cases

Cases (n=188) were recruited from the Department of Obstetrics and Gynecology at Uppsala University Hospital, Karolinska University Hospital, Huddinge University Hospital and Danderyd University Hospital, Sweden. Women with a diagnosis of recurrent miscarriage during 1989-2009, defined as three or more verified consecutive miscarriages in the first or second trimester of pregnancy (5-21 completed weeks of gestation), were identified in the out-patient registers of the participating clinics and invited to participate in the study. Inclusion occurred between April 29, 2009 and June 30, 2010. Women with known risk factors for recurrent miscarriage, such as systemic lupus erythematosus, diabetes mellitus type 1, severe thrombophilia and major chromosomal aberrations were not included. Furthermore, two women with hyperthyroidism were excluded.

Controls

The control subjects (n=391) were matched for age at first planned pregnancy and were randomly chosen from the Uppsala Biobank of Pregnant Women (May 31, 2007 until June 30, 2010). In the control group, none had a history of miscarriage and 74.9% had at least two spontaneous pregnancies,
including the ongoing pregnancy, resulting in a term (≥37 weeks) birth of a live infant. Beyond that, the same inclusion and exclusion criteria were applied for the control group as for the cases.

Studies II-III
All women from the Uppsala Biobank of Pregnant Women with an ultrasound-estimated date of delivery between January 1, 2009, and December 31, 2011, were included in the second part of Study II and in Study III (n=5,254).

Study IV
The study population consisted of two cohorts of pregnant women (total n=459). The cohorts were from the counties of Värmland (n=273) and Uppsala (n=186), situated in the western and eastern part of Sweden, respectively. Only Swedish-speaking women older than 18 years of age and with singleton pregnancies were included in the cohorts. For the purpose of the study, women with known thyroid disease before pregnancy, thyroid disease detected during pregnancy, preexisting diabetes mellitus or women who continued smoking during pregnancy had not been included in the cohorts. Spot urine samples were collected during the third trimester of pregnancy (from 28 weeks of gestation or later).

The Värmland cohort
A cohort of pregnant women without chronic hypertension was enrolled in gestational weeks 8–12 at five participating antenatal maternity care centers in Värmland, Sweden. The cohort was initially gathered for the study of preeclampsia risk factors. According to the study protocol, spot urine samples were collected around gestational week 33. The urine samples analyzed in this study were collected between January 2006 and July 2007. We reviewed the medical records of the women in 2014.

The Uppsala cohort
Participants were recruited within the pregnancy cohort ‘Biology, Affect, Stress, Imaging, and Cognition in Pregnancy and the Puerperium’ (BASIC), which is a longitudinal study investigating biological correlates of mood and anxiety disorders during pregnancy and in the postpartum period. All pregnant women in Uppsala County were invited to participate in BASIC at the time of their routine ultrasound around gestational week 18. According to the study protocol, spot urine samples were collected in a subgroup of 200 randomly invited participants around gestational week 38. The urine samples analyzed in this study were collected between January 2010 and September
2012. The demographical data was collected at inclusion, and the medical records were reviewed after childbirth.

Study procedures
Study I
At inclusion, both cases and controls attended a brief health examination and answered standardized questions on reproductive history. Further, we reviewed all medical records. According to routine clinical procedures, TSH-levels were analyzed in all women in the case-group when diagnosed with recurrent miscarriage. In the control-group, only women at risk of thyroid disease were subjected to targeted thyroid testing via TSH according to respective local guidelines. In both groups, hypothyroidism was defined as a TSH above the current defined upper limit of the reference range at the different hospitals.

Study II
Part one: adherence of local guidelines to international guidelines
All existing local guidelines from Sweden concerning thyroid testing or treatment of maternal hypothyroidism during pregnancy were compared with The Endocrine Society Guidelines 2007\textsuperscript{12}. To identify local guidelines concerning thyroid testing or treatment of maternal hypothyroidism during pregnancy, a nationwide survey by e-mail was conducted in 2011-12. All maternity care consultants (n=41) replied to this inquiry. The guidelines were analyzed with respect to four different aspects: 1) the degree of adherence to the Endocrine Society Guidelines, where 10 high-risk groups as reasons for thyroid testing are described, and the proportion of these reasons that were found in the local guidelines was calculated; 2) recommended thyroid function tests; 3) the trimester-specific TSH upper reference limit for intervention with levothyroxine; and 4) the trimester-specific TSH upper reference limit for monitoring women on treatment with levothyroxine.

Part two: degree of implementation of guidelines into everyday clinical practice
We reviewed all the women’s medical records. During the time-frames for this study, there were about 12,000 deliveries in Uppsala County, and 5,254 pregnant women were included in the biobank. Thus, nearly half of the women from the whole district, pregnant in the second trimester, were included in our study.

The included women’s medical reports were reviewed between February and May 2012 concerning:
1. If and when thyroid testing had been performed. In case of repeated testing, only the first test during the pregnancy (from gestational week 4+0 until delivery) was considered and defined as thyroid testing.

2. The specified reasons for thyroid testing.

3. The frequency of women with trimester-specific elevated TSH, overall, and within the groups with specified reasons for thyroid testing.

   According to international guidelines, we used trimester-specific TSH upper reference limits of 2.5 mIU/L in the first trimester and 3.0 mIU/L in the second and third trimester.12 20

   Pregnancy trimesters were defined as follows: first trimester until 13 completed weeks, second trimester week 14+0 to 27+6, and third trimester 28 completed weeks or later.

   In the district guidelines, valid during the entire study period, five reasons for thyroid testing were found: personal history of thyroid dysfunction with or without ongoing levothyroxine treatment, family history of thyroid dysfunction, goiter, type 1 diabetes, and other autoimmune diseases (Mb Addison, celiac disease, and atrophic gastritis with vitamin B12 deficiency were specified).

Study III

This study was a continuation of Study II. Targeted thyroid testing was performed on 1,054 women and, consequently, 4,200 women were not tested for thyroid dysfunction during pregnancy. All women who were on levothyroxine treatment at conception (n=163) were excluded from the study. Thus, the targeted thyroid testing group consisted of 891 women. From the 4,200 untested women, 1,006 were randomly selected in three blocks (representing equal samples of women from the years 2009, 2010, and 2011, respectively). The women’s stored Biobank samples were assayed for levels of TSH, free T4, and TPOab in December 2012. The flow diagram of the study protocol is shown in Figure 5.
Figure 5. Flow diagram of the study protocol

Overt hypothyroidism was defined as trimester-specific elevated TSH in conjunction with free T4 less than 9.7 pmol/L (according to local reference range; see further below) or TSH greater than 10.0 mIU/L irrespective of free T4. The remaining women with trimester-specific elevated TSH levels were considered to have subclinical hypothyroidism. According to local reference ranges, women with TPOab levels 34 kIE/L or greater were considered to be TPOab positive. The second trimester-specific free T4 reference range was calculated in the untested group among those who had no detectable TPOab. Hence, samples from 949 untested women were available for determination of the free T4 reference range. The second trimester 2.5-97.5th percentile for free T4 was 9.7-15.7 pmol/L. TSH, free T4, and TPOab analyses were performed using of the same laboratory methods in the targeted thyroid testing group and the untested group. TSH, free T4 and TPOab analyses were run on an automatic immune analyzer. The total assay variation in the individual assays was less than 5%. All analyses were performed at the routine laboratory of the Department of Clinical Chemistry at the University Hospital in Uppsala.

Study IV

Spot urine samples from both cohorts were collected between 0800 and 1600 hours and were frozen immediately. The samples collected in Värmland County were transported on ice to Uppsala. All samples were stored at -70°C in the laboratory at the Department of Women's and Children's Health, Uppsala University until analysis. For analysis, the samples were sent to the Swiss Federal Institute of Technology in Zürich (ETH). There, UIC was
measured in duplicate using a modified Sandell-Kolthoff reaction with spectrophotometric detection\textsuperscript{107,108}.

Statistics

The statistical software package SPSS was used for all data analyses. All significance tests were two-tailed. P-values $< 0.05$ were considered as statistically significant. One sample t-tests (Study II), paired t-tests (Study I), unpaired t-tests (Studies I, III and IV) and ANOVA (Study I) were used for normally distributed data. For non-normal distributions, the Mann-Whitney U test was used (Study IV). For categorical data, comparisons were performed using Fisher’s exact test (Study III) and the chi-squared test (Study I and III).

Adjusted odds ratios (AOR) for recurrent miscarriage were calculated in multivariate regression analyses (Study I). Only variables with a possible association with each outcome (p<0.25) in the bivariate analyses were entered into the final model.

Ethics

Informed consent was obtained from all women included in the studies. The studies were approved by the Ethical Review Board at Uppsala University (Studies I-IV) and the Ethical Review Board at Karolinska Institutet, Stockholm (Study I).
Results

Study I

Homozygous A/A and G/G carriers, compared with heterozygous carriers, were more common among women with recurrent miscarriage (Table 4).

**Table 4. Distribution of SNP rs 4704397 genotype among controls and cases**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=391)</th>
<th>Cases (n=188)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>69 (17.6%)</td>
<td>41 (21.8%)</td>
</tr>
<tr>
<td>A/G</td>
<td>193 (49.4%)</td>
<td>73 (38.8%)</td>
</tr>
<tr>
<td>G/G</td>
<td>129 (33.0%)</td>
<td>74 (39.4%)</td>
</tr>
</tbody>
</table>

The distribution of women with hypothyroidism among controls and cases related to SNP rs 4704397 is shown in Table 5.

**Table 5. Distribution of women with hypothyroidism among controls and cases related to SNP rs 4704397 genotype**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=391)</th>
<th>Cases (n=188)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>4/69 (5.8%)</td>
<td>3/41 (7.3%)</td>
</tr>
<tr>
<td>A/G</td>
<td>7/193 (3.6%)</td>
<td>4/73 (5.5%)</td>
</tr>
<tr>
<td>G/G</td>
<td>1/129 (0.8%)</td>
<td>9/74 (12.2%)</td>
</tr>
</tbody>
</table>

Based on the finding that homozygous A/A and G/G carriers were more common among women with recurrent miscarriage (Table 4), heterozygous A/G carriers were chosen as the reference group in the subsequently carried out logistic regression analyses. Bivariate associations between homozygous A/A as well as G/G carriers of SNP rs 4704397 in PDE8B and recurrent miscarriage were verified and a test for trend across all three genotypes was performed (Table 6). After adjustment for known confounders such as age, BMI and smoking, the association between homozygous A/A (AOR 1.63, 95% CI 1.01-2.64, p=0.045) and G/G (AOR 1.52, 95% CI 1.02-2.27, p=0.039) carriers of SNP rs 4704397 in PDE8B and recurrent miscarriage remained.
Table 6. Factors associated with recurrent miscarriage

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted OR (95%CI)</th>
<th>Adjusted OR* (95%CI)</th>
<th>P-value (Adjusted OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Years</td>
<td>1.00 (0.97-1.03)</td>
<td>0.99 (0.96-1.02)</td>
<td>0.500</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>0.96 (0.92-1.00)</td>
<td>0.96 (0.92-0.96)</td>
<td>0.026</td>
</tr>
<tr>
<td>Smoker No/Yes</td>
<td>1.84 (1.12 - 3.00)</td>
<td>1.86 (1.12-3.09)</td>
<td>0.017</td>
</tr>
<tr>
<td>SNP rs 4704397</td>
<td>A/A</td>
<td>1.57 (0.98-2.52)</td>
<td>1.52 (1.02-2.25)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>1.63 (1.01-2.64)</td>
<td>1.52 (1.02-2.27)</td>
</tr>
</tbody>
</table>

* adjusted for all other variables in the Table

Study II

Part one: adherence of local guidelines to international guidelines

Throughout Sweden, 29 local guidelines on thyroid testing and management of hypothyroidism during pregnancy were available. One maternity care area had no written guideline.

The 10 reasons for thyroid testing identified in the Endocrine Society Guidelines 2007 and the implementation of these thyroid testing reasons in the Swedish local guidelines are displayed in Table 7. The most commonly stated reasons for thyroid testing were personal and/or family history of thyroid disease (both 86.2%).

Table 7. Adherence of local guidelines (n=29) to the Endocrine Society Guidelines 2007 concerning reasons for thyroid testing

<table>
<thead>
<tr>
<th>Reason for thyroid testing</th>
<th>Numbers (n) and proportion (%) of Swedish guidelines listing the reason</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>Personal history of thyroid disease</td>
<td>25</td>
</tr>
<tr>
<td>Family history of thyroid disease</td>
<td>25</td>
</tr>
<tr>
<td>Goiter</td>
<td>20</td>
</tr>
<tr>
<td>Type I diabetes</td>
<td>17</td>
</tr>
<tr>
<td>Other autoimmune disorders</td>
<td>15</td>
</tr>
<tr>
<td>Symptoms or clinical signs</td>
<td>10</td>
</tr>
<tr>
<td>History of miscarriage or preterm delivery</td>
<td>4</td>
</tr>
<tr>
<td>Infertility</td>
<td>1</td>
</tr>
<tr>
<td>Prior head or neck irradiation</td>
<td>1</td>
</tr>
<tr>
<td>Thyroid antibodies (when known)</td>
<td>0</td>
</tr>
</tbody>
</table>

Of the local guidelines, only 17.2% recommended thyroid testing solely with TSH. All other local guidelines recommended additional analyses.

The first trimester TSH cut-off levels in the local guidelines for intervention with levothyroxine (95% CI 2.37–2.81) did not differ significantly from
the cut-off 2.5 mIU/L recommended by the Endocrine Society. However, the TSH upper cut-off levels for women on levothyroxine treatment were significantly lower in the local guidelines, both in the first trimester (95% CI 2.08–2.36) and in the second and third trimesters (95% CI 2.14–2.49). Here, the Endocrine Society Guidelines recommended 2.5 mIU/L and 3.0 mIU/L, respectively.

**Part two: degree of implementation of guidelines into everyday clinical practice**

In the follow-up population, the thyroid testing rate was 20.1%. Most women had their tests taken in the first trimester (65.7%), whereas 23.9% and 10.4% were tested in the second or third trimester, respectively. Approximately 91% of the 163 women who were on levothyroxine treatment at the time of conception were tested in the first trimester of pregnancy.

The overall frequency of women with trimester-specific elevated TSH was 18.5% in the follow-up population.

In Table 8, the proportion of women with trimester-specific elevated TSH in relation to the respective reason for thyroid testing is shown. More than half of the women who were already on levothyroxine treatment at conception had trimester-specific elevated TSH when first tested.

**Table 8. Women with trimester-specific elevated TSH in relation to reason for thyroid testing in the follow-up population**

<table>
<thead>
<tr>
<th>Reason for thyroid testing</th>
<th>Number (total)</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal history of thyroid disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ongoing levothyroxine treatment</td>
<td>83 (163)</td>
<td>50.9%</td>
</tr>
<tr>
<td>No ongoing levothyroxine treatment</td>
<td>15 (60)</td>
<td>25.0%</td>
</tr>
<tr>
<td>Family history of thyroid disease</td>
<td>51 (353)</td>
<td>14.4%</td>
</tr>
<tr>
<td>Goiter</td>
<td>2 (14)</td>
<td>14.3%</td>
</tr>
<tr>
<td>Type I diabetes</td>
<td>2 (8)</td>
<td>25.0%</td>
</tr>
<tr>
<td>Other autoimmune disorders</td>
<td>1 (12)</td>
<td>8.3%</td>
</tr>
<tr>
<td>Symptoms or clinical signs</td>
<td>23 (226)</td>
<td>10.2%</td>
</tr>
<tr>
<td>History of miscarriage or preterm delivery</td>
<td>2 (16)</td>
<td>12.5%</td>
</tr>
<tr>
<td>Infertility</td>
<td>2 (5)</td>
<td>40.0%</td>
</tr>
<tr>
<td>Prior head and neck irradiation</td>
<td>0 (2)</td>
<td>0%</td>
</tr>
<tr>
<td>Thyroid antibodies (when known)</td>
<td>1 (3)</td>
<td>33.3%</td>
</tr>
<tr>
<td>Blood sampling as part of a medical investigation</td>
<td>6 (15)</td>
<td>40.0%</td>
</tr>
<tr>
<td>Unclear reason</td>
<td>7 (177)</td>
<td>4.0%</td>
</tr>
</tbody>
</table>

**Study III**

Mean gestational age at targeted thyroid testing was 15.6±8.1 weeks (median 11.9 weeks, range 4.4–40.3 weeks), whereas untested women donated their blood sample at a mean gestational age of 17.9±1.2 weeks (median 18 weeks, range 10.3–24.6 weeks).
The proportion of trimester-specific TSH elevation was 12.6% in the targeted thyroid testing group and 12.1% in the untested group (p=0.8; OR 1.04, 95% CI 0.79–1.37). Rates of overt and subclinical hypothyroidism were similar in the targeted thyroid testing and untested groups. The proportion of women with thyroid disturbances is shown in Table 9.

Table 9. Thyroid dysfunction in the targeted thyroid testing and untested groups, respectively

<table>
<thead>
<tr>
<th>Thyroid dysfunction</th>
<th>Targeted thyroid testing group (n = 891)</th>
<th>Untested group (n = 1006)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimester-specific elevated TSH, n (%)</td>
<td>112 (12.6)</td>
<td>122 (12.1)</td>
<td>0.8</td>
</tr>
<tr>
<td>TSH &gt; 10 mU/L, n (%)</td>
<td>2 (0.2)</td>
<td>2 (0.2)</td>
<td>1.0</td>
</tr>
<tr>
<td>Overt hypothyroidism, n (%)</td>
<td>7 (1.1)a</td>
<td>7 (0.7)</td>
<td>0.5</td>
</tr>
<tr>
<td>Subclinical hypothyroidismb, n (%)</td>
<td>87 (13.3)a</td>
<td>115 (11.4)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

† Fisher’s exact test was used for TSH > 10 mU/L, otherwise chi-square tests were used.
a Analyzes were made in 656 women who were tested after April 23 2009, i.e. with the same free T4 assay as later used for the untested women.
b Free T4 was not available in 10 women in the targeted thyroid testing group and in one untested woman with trimester-specific elevated TSH. Those 11 women were defined to have subclinical hypothyroidism.

In Table 10, rates of thyroid dysfunction in untested women with or without additional risk factors for thyroid disease as suggested by later international guidelines are shown.

Table 10. Thyroid dysfunction in untested women with or without additional risk factors (age ≥ 30 years, BMI > 40 kg/m², and autoimmune disorders)

<table>
<thead>
<tr>
<th>Thyroid dysfunction</th>
<th>Untested women with additional risk factors (n = 558)</th>
<th>Untested women without additional risk factors (n = 448)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overt hypothyroidism, n (%)</td>
<td>3 (0.5)</td>
<td>4 (0.9)</td>
<td>0.7</td>
</tr>
<tr>
<td>Subclinical hypothyroidism, n (%)</td>
<td>68 (12.2)</td>
<td>47 (10.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>TPOAb positive, n (%)</td>
<td>30 (5.4)</td>
<td>26 (5.8)</td>
<td>0.8</td>
</tr>
<tr>
<td>Subclinical hypothyroidism and TPOAb positive, n (%)</td>
<td>11 (2.0)</td>
<td>9 (2.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Isolated hypothyroxinemia, n (%)</td>
<td>16 (2.9)</td>
<td>12 (2.7)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

† Fisher’s exact test was used for overt hypothyroidism, otherwise chi-square tests were used.

The majority of women with additional risk factors were aged 30 years or older (n=548). Only a few women younger than 30 years of age had exclusively BMIs of 40 or greater (n=3) or autoimmune disorders (n=7). The addition of these risk factors did not result in improved efficacy because no difference in rates of thyroid dysfunction between untested women with or without additional risk factors for thyroid disease was found.
Pregnancy outcomes in untested women who had elevated or normal TSH levels are shown in Table 11. Bearing in mind that the study was unpowered for this analysis, no increased prevalence of adverse pregnancy outcomes was noted in untested women who had elevated TSH levels.

Table 11. Obstetric and neonatal outcome in the low-risk group in relation to TSH levels

<table>
<thead>
<tr>
<th></th>
<th>Untested women with normal TSH (n=854)</th>
<th>Untested women with elevated TSH (n=119)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrauterine demise, n (%)</td>
<td>2 (0.2)</td>
<td>1 (0.8)</td>
<td>0.3</td>
</tr>
<tr>
<td>Premature birth, n (%)</td>
<td>50 (5.9)</td>
<td>4 (3.4)</td>
<td>0.4</td>
</tr>
<tr>
<td>Post-term delivery, n (%)</td>
<td>48 (5.6)</td>
<td>9 (7.6)</td>
<td>0.4</td>
</tr>
<tr>
<td>Gestational hypertension or preeclampsia, n (%)</td>
<td>42 (4.9)</td>
<td>5 (4.32)</td>
<td>0.8</td>
</tr>
<tr>
<td>Birth weight, g ± SD</td>
<td>3589 ± 581</td>
<td>3689 ± 640</td>
<td>0.1</td>
</tr>
<tr>
<td>Gestational length, days ± SD</td>
<td>278 ± 12</td>
<td>279 ± 12</td>
<td>0.6</td>
</tr>
<tr>
<td>pH &lt; 7.05 at delivery*, n (%)</td>
<td>16 (2.1)</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Apgar &lt; 7 at 5 minutes, n (%)</td>
<td>14 (1.7)</td>
<td>2 (1.7)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data available in 973 women who delivered at Uppsala University hospital. Cases with intrauterine demise were excluded from remaining analyses.

† Fisher’s exact test was used for all variables with n < 5, otherwise chi-square tests were used.

* umbilical artery

Study IV

The median UIC concentration in the total study population of pregnant women was 98 μg/L (interquartile range 57-148 μg/L). The median UIC was higher in the Värmland than in the Uppsala cohort (105 versus 85 μg/L; p<0.001). Figure 6 shows the frequency distribution of UIC in the total study population. The distribution was slightly skewed towards elevated values.
**Figure 6.** Distribution of Spot Urinary Iodine Concentration in the Total Study Population of 459 Pregnant Women in Sweden.
Discussion

Briefly, our principal findings were:

- There was an association between homozygous A/A as well as homozygous G/G carriers of SNP rs 4704397 in PDE8B and recurrent miscarriage (Study I).
- Local guidelines for thyroid testing and the management of hypothyroidism during pregnancy differed in several aspects from international guidelines (Study II).
- We found a thyroid testing rate of 20.1% among pregnant women, with an overall frequency of women with trimester-specific elevated TSH of 18.5%. Half of the women who were on levothyroxine treatment at the time of conception had an elevated TSH level at thyroid testing (Study II).
- In clinical practice, the prevalence of trimester-specific elevated TSH and overt hypothyroidism was equal in targeted thyroid tested and untested pregnant women (Study III).
- The median UIC of 98 μg/l obtained in the study population was far below the recommended range of median urinary iodine of 150-249 μg/l during pregnancy and indicates insufficient iodine intake in the pregnant population (Study IV).

Methodological considerations

Study designs and representativeness

Study I

This study was designed as a case-control study. A case-control study is in general a design useful to initially establish an association between a risk factor (different alleles of SNP rs 4704397 in PED8B) and a rare disease or outcome (recurrent miscarriage). To our knowledge, this was the first study to examine this possible association and the study design can be considered as appropriate. However, case-control studies can only test a hypothesis and do not prove an association or causation.

The major weakness of case-control studies is risk of bias, mainly recall bias. However, the different alleles of SNP rs 4704397 in PED8B are definite in the DNA, and do not underlie any recall bias. Also, the outcome, re-
current miscarriage in the cases and the combination of no history of miscarriage and at least one pregnancy resulting in a term (≥37 weeks) birth of a live infant in the controls, is unlikely to be influenced by recall bias, which otherwise may lead to incorrect classifications of outcome. In contrast, smoking, which was investigated at inclusion in the case-group, is typically at risk of underlying recall bias.

In the bivariate analysis in this study, we adjusted for all known confounders such as age, BMI and smoking. However, it can be debated if these factors are confounders in a more modern way of epidemiological thinking\textsuperscript{109}. Age, BMI and smoking are known risk factors for recurrent miscarriage but, reasonably, they cannot affect the set of alleles of SNP rs 4704397 in PED8B in an individual. According to this way of thinking, no adjustments should have been made for the estimation of the total effect of PED8B genotype on the outcome recurrent miscarriage. In the unadjusted bivariate analysis, only G/G SNP rs 4704397 in PED8B was associated with recurrent miscarriage.

In any case, both unadjusted and adjusted associations of A/A and G/G of SNP rs 4704397 in PED8B and recurrent miscarriage are just around the limits of significance, and to confirm our results, further larger studies are needed.

**Study II**

**Part one: adherence of local guidelines to international guidelines**

This part of Study II was designed as a cross-sectional study, comparing local guidelines concerning several aspects with, at that time available, international guidelines of the Endocrine Society\textsuperscript{12}. The study design was simple, but nevertheless illustrative of the first step of implementation of international guidelines into clinical practice. The response frequency was 100%, and bias, e.g. recall bias, was avoided as we only asked for the written local guidelines and did not send out any questionnaires. Consequently, to map the adherence of local guidelines to international guidelines, the study design was appropriate.

However, there are some limitations regarding the comparisons. We did not know the proportion of pregnant women covered by respective local guidelines, but counted the local guidelines as equal parts when comparing them with the international guidelines.

**Part two: degree of implementation of guidelines into everyday clinical practice**

This part of Study II was descriptive, conducted at a given time.

Due to the retrospective character of this study, the caregivers were not aware of the study, thus avoiding, for example, recall bias among caregivers. This study provides a good illustration of the reasons for thyroid testing in
clinical practice and the proportion of women with trimester specific elevated TSH in relation to reason for thyroid testing. This can hardly be done with another study design.

**Study III**

This study was designed as a cohort study.

Randomized controlled trials are considered to be the gold standard of study design. The only randomized controlled trial in this field randomized women in the first trimester of pregnancy to either targeted thyroid testing or screening and investigated obstetrical and neonatal outcomes. According to the study protocol, women were stratified as either high or low risk based on risk factors for thyroid disease. All in all, 4.2% of the high risk women were hypothyroid, while only 2.2% of the low risk women were, indicating a twice as high detection rate in high risk women. In contrast, we did not find any difference in prevalence of hypothyroidism between high and low risk pregnant women.

Obviously, a lack of external validity is a problem in RCT’s in this field. To group pregnant women into high and low risk women and, thus, to targeted thyroid testing or non-testing in clinical practice, is completely different from a stratification process in a RCT. At the first antenatal visit during pregnancy many different health aspects have to be considered. Thyroid testing or non-testing, based on a long list of risk factors, is probably not a high priority for many caregivers. At the end, the efficacy of targeted thyroid testing in clinical practice is reasonably more interesting than the efficacy under perfect study conditions. Thus, our study design may be appropriate with regard to this specific question.

**Study IV**

This study was designed as a cross-sectional study conducted at two different time points: between January 2006 and July 2007 in the Värmland cohort and between January 2010 and September 2012 in the Uppsala cohort. Both cohorts involved secondary analyses of data primarily collected for other purposes. Thus, for example, no information about household use of iodized salt was available.

To assess iodine status in a population, WHO suggests a multi-stage cluster sampling, including concurrent assessment of household use of iodized salt. Consequently, the study design was not optimal.

Moreover, some concerns have to be raised about inclusion criteria. As Sweden has been considered to be an iodine sufficient country, we hypothesized that even the pregnant population would be iodine sufficient. With the initial intention to be able to subsequently develop reference ranges for TSH and free T4 from the cohorts, we did not include women with factors known to affect thyroid function, such as known thyroid disease before pregnancy, thyroid disease detected during pregnancy, preexisting diabetes mellitus, or...
smoking during pregnancy. According to recommendations from the WHO, no exclusion criteria should be applied for obtaining the median UIC in a population. As thyroid disturbances are known to be more common in iodine insufficient populations, the “true” median UIC in the cohorts is probably even lower than the median UIC obtained in our study.

However, this study is the first survey on iodine nutrition in pregnant women in Sweden and the size of the study population was adequate. The number of spot urine samples needed to estimate the iodine level in a population with 95% confidence within a precision range of ±10% has been described to be about 125, and within a precision range of ±5% to be about 500. Even though the two cohorts are slightly different with regards to the gestational age at assessment and the time-period in which the cohorts were gathered, they are both population-based and still likely to give a good indication of the overall national status in Sweden.

Significance of the results in a general context

Study I

PDE8B is known to be abundantly expressed in the thyroid and is important for thyroid function. Increased levels of TSH have previously been shown in homozygous A/A carriers of SNP rs 4704397 in PDE8B and we hypothesized that there might be an association between A allele carriers and recurrent miscarriage. In our study, associations between homozygous A/A as well as homozygous G/G carriers of SNP rs 4704397 in PDE8B and recurrent miscarriage were found.

According to a genome-wide association scan in a Sardinian population, the specific SNP rs 4704397 in PDE8B has a strong association with increased levels of TSH and explains 2.3% of the variance in TSH. Each copy of the A allele was associated with an average increase of 0.13 μIU/ml in TSH serum concentrations. This trend could be found also in our material, as there was a slightly lower prevalence of hypothyroidism in A/G than in A/A allele carriers, both in controls and cases. Further, there was an even lower prevalence of hypothyroidism among G/G-carriers in the control group.

Limitingly, as the study population was evaluated at clinics with different reference ranges for TSH analyses, and serum was not stored at the time of diagnosis, TSH levels were unavailable for further analysis. Thus, we were not able to draw conclusions about whether the increased risk of recurrent miscarriage in homozygous A/A carriers in our material was mediated through relatively increased TSH levels. A further limitation was that cases and controls were tested for thyroid disease in different ways. According to routine clinical procedures, TSH levels in all women with recurrent miscar-
riage were analyzed, while controls were subjected to targeted thyroid testing when considered to be at a high risk of thyroid disease. It is highly probable, that the frequency of hypothyroidism has been underestimated in the control group, which is of importance when the results are evaluated.

Based on the fact that homozygous A/A and G/G carriers, compared with heterozygous, were more common among women with recurrent miscarriage, we decided to use heterozygous carriers as references. This concept has been used before in other studies. This finding that carriage of the G/G allele of SNP rs 4704397 in PDE8B was also associated with recurrent miscarriage was unexpected, and the mediator of the association is unknown. The association is presumably not mediated by hypothyroidism, since no association between homozygous G/G carriers and elevated TSH levels has been identified in previous studies. Probably there are other, still unknown, underlying factors which associate homozygous G/G carriers with recurrent miscarriage.

It is noteworthy, however, that G/G carriers were more common among hypothyroid women with recurrent miscarriage, compared with both hypothyroid controls and all cases (Table 5). Thus, concerning recurrent miscarriage, it seems to be a disadvantageous combination to be a G/G carrier and to suffer from hypothyroidism. However, it is important to remember the limitations concerning the diagnosis of hypothyroidism in this material and that the number of women with hypothyroidism was quite small.

There might be other reasons for the detected associations between SNP rs 4704397 and recurrent miscarriage. The importance of PDE8B in the placenta and human ovaries is unknown, but the relevance of phoshodiesterases (PDE) in human reproduction has been discussed. The mechanisms that regulate oocyte maturation in vivo and in vitro are still not well understood but the second messenger, cAMP, plays a critical role in maintaining the oocytes at meiotic arrest in the diplotene stage of the first meiotic prophase. The PDEs inactivate and degrade cAMP in oocytes as a response to the ovulatory luteinizing hormone pulse and have thus been proposed as important factors for regulating oocyte maturation. Furthermore, it has been shown that levels of cAMP in the oocyte at meioic re-sumption correlate with oocyte competence and embryonic development. Based on the knowledge that PDE activity is relevant for levels of cAMP in oocytes, the importance of SNP rs 4704397 in PDE8B for regulation of oocyte maturation would be one pathway of interest for further study.

Of course, we cannot exclude our results concerning SNP associations to be false positive. Generally, there is a high false positive rate in small genetic association studies due to low prior probability. However, SNP rs 4704397 in PDE8B has been shown to be associated with variations in serum TSH levels in several studies, and there is a well-known association between hypothyroidism and recurrent miscarriage. To our knowledge, this is the first study examining a possible association between SNP rs 4704397.
in PDE8B and recurrent miscarriage. To confirm our findings, it would be important to increase the sample size and replicate them in an independent cohort.

Genetic variability plays a role in many different diseases and has been proposed to be of relevance in screening for distinct syndromes with known genetic defects, and it might also be used for the prediction of success related to disease treatment\textsuperscript{122} \textsuperscript{123}. Hypothetically, this specific SNP may be used for individual counselling and for optimising treatment to prevent recurrent miscarriage, based on which allele the woman is a carrier of. But this is currently still in the future.

Studies II and III

In summary, our studies show that thyroid testing is unsatisfactory in clinical practice. This applies to the entire chain; from development of local guidelines to targeted thyroid testing in clinical practice. As a final result of the implementation of guidelines into clinical practice, the prevalence of trimester-specific elevated TSH and overt hypothyroidism was equal in targeted thyroid tested and untested women in our study population. Thus, to identify pregnant women with thyroid disturbances, targeted thyroid testing in clinical practice was no better than simply flipping a coin.

As a first step for implementing targeted thyroid testing during pregnancy, evidence-based guidelines that are usable in everyday clinical practice should be available for all caregivers. Though international evidence-based guidelines had been available for some time, the local guidelines in our study were variable and poorly compliant with the international ones\textsuperscript{12} \textsuperscript{124}. Probably, the consultants in obstetrics in our setting, who were responsible for the development and implementation of the local guidelines, were not completely aware of the international ones. This would be in line with a survey from Wisconsin, where only 11.5\% of providers had read the Endocrine Society’s guidelines\textsuperscript{75}.

As a second step for implementing targeted thyroid testing during pregnancy, the guidelines should be followed at all points. Therein, the research setting, when detailed study protocols are used to identify pregnant women with risk factors for thyroid disease, illustrates the “perfect world”. However, even under these perfect conditions, targeted thyroid testing has been shown to miss between 33\% and 67\% of pregnant women with overt or subclinical hypothyroidism\textsuperscript{79} \textsuperscript{80}. In our study population, the proportion was significantly higher, and, when extrapolating to all 5,254 included in the study, approximately 82\% of women with elevated TSH were missed by targeted thyroid testing in clinical practice\textsuperscript{125}. Also other studies have illustrated the difficulty in implementing targeted thyroid testing. Despite the
availability of local guidelines in a district, less than 20% of high-risk preg-
nant women were tested for thyroid dysfunction81.

Probably, there is no specific failure in the implementation process of tar-
geted thyroid testing; rather it is a combination of several failures. Moreover,
similarities in study results suggest that the results from our study might also
be generalized to other settings. For example, similar to our results, in a
large national sample of 500,000 pregnant women in the United States, 23% 
underwent targeted thyroid testing during pregnancy. Of those, 15.5% had 
elevated trimester-specific TSH-levels126. However, the proportion of gesta-
tional hypothyroidism in the untested group is unknown.

As a next step, when adding extra indications for targeted case finding, as
exemplified in Table 10, targeted thyroid testing should have had to be per-
formed in approximately 63% of women in our study population. In line
with earlier studies, this would improve the targeted thyroid testing strategy
efficiency substantially, but only as a result of the increased number of 
women being tested127. It is questionable whether it is worthwhile to apply
targeted thyroid testing when the goal is to test almost two thirds of the 
pregnant population, while still missing a significant number of women with 
hypothyroidism.

Thus, concerning thyroid testing, improvements seem to be necessary and
urgent. Two options for improvement seem to be possible. The first possibil-
ity, based on ongoing targeted thyroid testing, might be a two-step proce-
dure: establishment of short but concise evidence-based guidelines with local
or national modifications for differences in maternal health care and subse-
quent efforts to improve the implementation of these guidelines. The other
possibility might be to apply universal thyroid testing to all pregnant women.

Dosiou et al investigated the cost-effectiveness of targeted or universal
thyroid testing in the scenario of untreated maternal hypothyroidism result-
ing in decreased child intelligence, with levothyroxine therapy being preven-
tive. Universal thyroid testing remained cost-effective in various clinical
scenarios, including when only overt hypothyroidism (estimated prevalence
0.43%) was assumed to have adverse obstetrical outcomes128.

This concerns the heart of the matter: the main problem is that, to date, it
is unknown at which degree of hypothyroidism levothyroxine treatment
might be advantageous. In the only RCT in this field, levothyroxine treat-
ment for mild maternal thyroid failure did not result in improved cognitive
function in children at three years of age72. The combined prevalence of 
over and subclinical hypothyroidism and isolated hypothyroxinemia was
4.8% in the total study population of 21,848 women72. Thus, levothyroxine

treatment in a study population with a comparatively low prevalence of mild 
maternal thyroid failure did not demonstrate any benefit of treatment con-
cerning childhood cognitive function72. Consequently, it seems unreasonable
to expect any benefits from treatment of about 12-15 % of a pregnant popu-
lation, a prevalence of hypothyroidism found in our and other studies when
applying upper cut-off levels for TSH of 2.5 mIU/L in the first and 3.0 mIU/L in the second and third trimester of pregnancy. The recommended TSH upper cut-off level of 2.0 mIU/L throughout pregnancy in some of the local guidelines in our study setting just seems unreasonably low in this context.

However, to date, most national and international guidelines recommend applying upper cut-off levels for TSH of 2.5 mIU/L in the first and 3.0 mIU/L in the second and third trimester of pregnancy if no laboratory dependent cut-off levels are available. Further, they recommend treatment of even subclinical hypothyroidism during pregnancy, though evidence for the treatment of subclinical hypothyroidism during pregnancy is lacking (see Introduction). That means suggesting treatment to a large number of pregnant women for thyroid disturbances. Obstetric and neonatal outcomes in populations with such a high prevalence of elevated TSH have to our knowledge not been previously investigated. Possibly, concerns about legal consequences may be a contributing reason for these numerous recommendations?

It is obvious that few concerns have been raised about the possible adverse effects of levothyroxine treatment during pregnancy, even when the recommendations will lead to treatment of a high percentage of pregnant women. Adverse effects of levothyroxine treatment are usually considered to be improbable, but can in fact not be excluded to exist. Data about a possible association of levothyroxine use and congenital malformations in offspring are scarce. In a recent epidemiological study from Sweden on 1,567,736 children born during 1996-2011, there was a slight but not significant increased risk of “relatively severe malformations” in children born to women who reported levothyroxine use in early pregnancy (adjusted OR 1.06; 95% CI 0.98-1.14). Risk ratios were significantly increased in levothyroxine users for anal and choanal atresia. However, two main points have to be considered: firstly, no information on malformed fetuses aborted after prenatal diagnosis was available in the registers, which may clearly lead to an underestimation of a possible association between levothyroxine treatment and malformations. Secondly, malformations may be associated to the underlying disease, hypothyroidism itself, instead of the treatment.

Moreover, levothyroxine treatment may have other adverse health effects. For example, in adults 70 years or older, current levothyroxine treatment was associated with a significantly increased risk of fractures, with a strong dose-response relation. There are no data on possible adverse outcomes on maternal health other than pregnancy-related outcomes.

According to several guidelines, TSH reference intervals should be established in iodine sufficient populations from the 95% confidence limits of the log-transformed values of at least 120 apparently healthy volunteers without
any personal or family history of thyroid disease, goiter, thyroid autoantibodies or medications. As a consequence of this definition, reasonably not more than about 3% of an iodine-sufficient, pregnant population should have TSH levels exceeding the upper TSH cut-off level. Mild to moderate iodine deficiency might influence TSH levels to a mild degree, but very probably not to an extent of 12% of the pregnant population having elevated TSH levels. Thus, the establishment and use of population-based reference ranges for TSH and thyroid hormones should be clearly preferable to the use of fixed TSH cut-off levels, and this should also be pointed out clearly in all guidelines.

Two additional aspects should be pointed out. First, only 17.2% of the local guidelines in our study recommended thyroid testing solely with TSH. There is no reason to perform additional analyses to TSH at primary testing, as there is no evidence for the treatment of thyroid autoimmunity or isolated hypothyroxinemia in pregnancy (see Introduction). Second, we found that more than half of the women who were already on levothyroxine treatment at conception had trimester-specific elevated TSH when first tested (50.9% of cases). This prevalence is in line with earlier studies. Fetal loss has been shown to be significantly greater in levothyroxine-treated women with abnormal first trimester TSH values compared with those with normal TSH values, and also other adverse pregnancy outcomes might be more common. Yassa et al suggest an intake of two additional levothyroxine doses per week, initiated immediately at confirmation of pregnancy. In contrast to the international guidelines, none of the local guidelines contained this recommendation.

Study IV

The median UIC of 98 μg/l obtained in a study population of pregnant women indicates insufficient iodine intake in the pregnant population (Study IV). These results are in contrast to earlier studies of non-pregnant populations in Sweden, which report adequate iodine nutrition. However, in line with our results, earlier studies have shown that the median UIC of school-aged children should not be used as a surrogate for monitoring the iodine status of pregnant women.

No UIC cut-off for distinguishing severe and moderate/mild iodine deficiency during pregnancy has been proposed. According to existing literature, the degree of iodine deficiency in our study population would probably be defined as mild to moderate. Several observational studies have described the negative effects of mild to moderate iodine deficiency during pregnancy on the motor and cognitive functions of the progeny. Furthermore, iodine deficiency during pregnancy has been shown to have a negative impact on both infant and maternal thyroid function. We found a very high prevalence
of thyroid disturbances during pregnancy, especially regarding subclinical hypothyroidism (Study III)\(^\text{125}\). Whether the mild to moderate iodine deficiency contributes to this high prevalence is only a matter of speculation.

When feasible, the most effective way to eliminate and control iodine deficiency is through salt iodization\(^\text{85}\). Salt intake is quite consistent throughout the year and is one of the few nutrients consumed by almost everyone. Iodization technology is simple and inexpensive, and in many countries, salt production or importation is limited to a few sources. Moreover, the quantity of iodine in salt can be easily monitored at the production, retail, and household levels\(^\text{90}\). However, it is important not to achieve excessive iodine intake in the population. Overconsumption of iodine has been shown to have a negative health impact, especially in populations where chronic iodine deficiency has been known to exist\(^\text{138}\). Zimmermann et al suggest a new method for the calculation of iodine deficiency prevalence and for estimations on how much iodine intake should be increased in the population\(^\text{139}\).

According to sales figures from 2012, 35% of all salt sold in Sweden was iodized (data from Salinity Group). The main proportion of all salt in 2012 was sold to the food industry (56.5%), with a remarkably low proportion being iodized (7%). Thirty-six percent of all salt sold was table salt, whereof only 77% iodized salt. The remaining 7.5% of all salt was sold to foodservices, with a proportion of 45% being iodized.

The level of iodine fortification in Swedish salt (50 mg/kg) exceeds the recommended optimal level of 20-40 mg/kg and is likely adequate\(^\text{85}\). Countries with fortification levels within this range and high iodized salt coverage report adequate iodine nutrition in pregnant women\(^\text{108}\). We have no data on the use of iodized salt in our two study cohorts, but the observed low median UIC indicates poor coverage of iodized discretionary salt and/or low use of iodized salt in the food production.

At present, the reason for the modest but observed difference in median UIC between the Värmland and Uppsala cohorts is unclear. Geographical differences in iodine intake is an unlikely reason, as the 2006-07 national iodine nutrition survey in school children did not find any regional differences in iodine status and thyroid size in girls\(^\text{82,101}\). The Värmland cohort was collected five years earlier than the Uppsala cohort. The difference in median UIC between the cohorts might reflect decreasing iodine intake in the Swedish population in this 5-year interval, which is supported by market basket studies carried out by the national food agency in 1999 and 2010\(^\text{84}\). These studies found a decreasing average per capita exposure to iodine from 200 μg/day in 1999 to 126 μg/day in 2010. Decreased iodine concentrations in milk as mentioned in the market basket study 2010 or changes in the use of iodized salt in the food industry during this period may be explanations. To note, since the use of table salt was not included in either basket, the total exposure to iodine was probably underestimated\(^\text{84}\).
During a transitional period, while efforts to improve the salt iodization program continue, but when iodization efforts are still unable to meet the requirement of vulnerable groups such as pregnant women, WHO suggests that the iodine supplementation of pregnant women and children less than two years of age should be considered. International guidelines recommend, more or less in general, to all pregnant or pregnant-planning women the use of supplements containing iodine. However, in mild to moderate iodine deficient pregnant women, there is a lack of evidence concerning this recommendation. To assess efficacy and safety, data from two ongoing randomized trials could be awaited before recommending any supplements in our or – with respect to the iodine situation – similar populations.

How to deal with the current situation

In summary, how should we deal with the current situation while waiting for the results of ongoing studies? To date, there is no definite answer, only suggestions that can be discussed.

First, it seems important to recommend the exclusive use of iodized table salt to women of childbearing age and to pregnant women. Especially in case of low or non-existent dairy, fish and seafood consumption, as in vegans, iodine supplements might be considered.

Second, adjustments to local guidelines or the development of national guidelines seems to be important. Very recently, national guidelines have been developed and published in Sweden.

Third, ongoing levothyroxine treatment should be adjusted as soon as pregnancy has been confirmed.

Fourth, to apply targeted thyroid testing in clinical practice seems unreasonable, as well as treating a large proportion of pregnant women for small aberrations in thyroid function tests in the absence of evidence. As even suggested by Laurberg et al, screening and treatment for overt thyroid dysfunction in early pregnancy may be indicated, rather than focusing on identifying and treating small aberrations in thyroid function tests.

Fifth, purely pragmatic and practically, in case of focusing on overt hypothyroidism, TSH upper cut-off-levels of 2.5 mIU/L in the first and 3.0 mIU/L in the second and third trimester might be used transitionally. What to do until method-specific and trimester-specific reference ranges of free T4 are determined however still remains unanswered.
Conclusions

- Our findings suggest that there is an association between homozygous A/A as well as homozygous G/G carriers of SNP rs 4704397 in PDE8B and recurrent miscarriage. The explanation for this association is unclear. (Study I)

- On a nationwide basis, local guidelines on thyroid testing and the treatment of hypothyroidism during pregnancy were variable and poorly compliant with international guidelines. (Study II)

- Performance of thyroid testing in clinical practice was not optimal, and rates of elevated TSH at thyroid testing were extremely high in women already on levothyroxine treatment at the time of conception. (Study II)

- A targeted thyroid testing approach in clinical practice was unsatisfactory, as the prevalence of trimester-specific elevated TSH and overt hypothyroidism was equal in targeted thyroid tested and untested women. Once, and if, evidence to support treatment of pregnant women with elevated TSH becomes available, universal thyroid testing appears the most reasonable approach. (Study III)

- The median UIC of 98 μg/l obtained in this study population of pregnant women indicates an insufficient iodine intake in the pregnant population in Sweden. This might have a negative impact on child motor and cognitive functions. There is an urgent need for further assessments in order to optimize iodine nutrition during pregnancy. (Study IV)
Future perspectives

- Our results emphasize the importance of: 1) establishment of short but concise evidence-based guidelines with local or national modifications for differences in maternal health care and 2) subsequent efforts to improve the implementation of these guidelines.

- To date, targeted thyroid testing in clinical practice seems to be an unreasonable approach. Efforts as mentioned above might improve the efficacy of targeted thyroid testing, but should be proven in studies.

- Which mother and fetus benefit from treatment for thyroid disturbances during pregnancy? This core issue is still unresolved. Hopefully, ongoing studies are able to contribute to clarity on this subject.

- Closely associated with the point above, our results highlight the use of appropriate trimester-specific reference ranges for TSH. Treating 12-15% of a pregnant population for hypothyroidism seems to date unreasonable, and the possible adverse effects of such a broad treatment approach have not been investigated. Thus, only laboratory- and trimester-specific reference ranges for TSH, as defined in populations with optimal iodine intake, should be applied.

- Our results illustrate the importance of conducting surveys on iodine status in pregnant women, even though iodine sufficiency might have been determined in the general population. The results from our study should be confirmed in a national survey, conducted as a multi-stage cluster sampling for the measurement of UIC. This should also include repeat urine samples from a subset of the study population, which could be used for estimations on how much the iodine intake should be increased in the population.
Till för cirka 100 år sedan var hypotyreos till följd av svår jodbrist en folk-sjukdom som drabbade människor över hela världen. Vid svåra former av hypotyreos var fertiliteten mycket nedsatt, och vid uppkomen graviditet var risken för missfall och andra graviditetskomplikationer stor. Om ett barn föddes, kunde exponeringen för svår hypotyreos under foster- och späd-barnslivet få allvarliga konsekvenser för den mentala och fysiska utvecklingen. I de flesta länder har olika jodprogram, ofta genom jodering av bordssalt, lett till att svår jodbrist så småningom blivit alltmer sällsynt. Under graviditeten föreligger ett ökat jodbehov, men i Sverige liksom i de flesta andra länder saknas studier, som har utvärderat jodintaget under graviditeten.

I länder med tillräckligt jodintag är kronisk autoimmun tyreoidit den vanligaste orsaken till hypotyreos, följd av hypotyreos som har uppstått som konsekvens av behandling för en tidigare överfunktion av sköldkörteln. Obehandlad klinisk hypotyreos har förknippats med en ökad risk för graviditetskomplikationer, medan konsekvenserna av obehandlad subklinisk hypotyreos inte är lika entydiga.

De flesta internationella riktlinjer förespråkar en riktad provtagning med TSH i tidig graviditet hos kvinnor med riskfaktorer för tyreoideasjukdom. Hur väl dessa internationella riktlinjer tillämpas och hur väl denna riktade provtagning fungerar i klinisk praxis är föga undersökta.

Upprepade missfall drabbar 1 – 2 % av alla par. Hypotyreos är en av flera kända orsaker till upprepade missfall. Phosphodiesteras 8B (PDE8B) har stor betydelse för tyreoideametabolismen hos människan. En SNP i PDE8B-genen (rs 4704397) har visats vara associerad med variationer i tyreoideahormonnivåer, hos både gravida och icke-gravida.

**Delarbete I**

Syftet med studien var att undersöka om det finns ett samband mellan SNP rs 4704397 och upprepade missfall, vilket tidigare inte har studerats. Studien är en fall-kontroll studie, där 188 fall med upprepade missfall jämfördes med 391 kontroller som hade åtminstone ett barn och ingen anamnes på missfall eller assisterad befruktning. Vi fann ett signifikant samband mellan homozygota A/A och G/G bärare av SNP rs 4704397 i PDE8B-genen och upprepade missfall.
Delarbete II och III

Syftet med dessa delarbeten var att utvärdera hur internationella riktlinjer gällande provtagning för och behandling av hypotyreos under graviditet följs i klinisk praxis (II), samt att kartlägga precisionen av riktad provtagning för hypotyreos i klinisk praxis (III).

Alla under 2011 och 2012 i Sverige gällande lokala riktlinjer om tyreoidesjukdom och graviditet jämfördes med de då aktuella internationella riktlinjerna. Samtliga lokala riktlinjer rekommenderade en riktad provtagning av kvinnor med riskfaktorer för tyreoidesjukdom. Dock fann vi att de lokala riktlinjerna var sinsemellan olika och avvek i flera punkter från de internationella riktlinjerna: 1) det angavs genomgående färre än de tio i de internationella riktlinjerna angivna indikationerna för provtagning, 2) riktad provtagning med fler prover än enbart TSH rekommenderades i 83 % av de lokala riktlinjerna samt 3) det angavs signifikant lägre övre referensgränser för TSH för start/dosökning av behandling.

Vidare studerade vi en kohort på 5254 gravida kvinnor från Uppsala län. 1054 (20,1%) av dessa kvinnor hade genomgått en riktad provtagning under sin graviditet, dvs. man hade kontrollerat ett TSH på mödravårdscentralen, sjukhuset eller annan sjukvårdsinrättning. Av dessa 1054 kvinnor hade 18.5 % ett förhöjt TSH-värde vid provtagningen. Av de 163 gravida som hade en pågående behandling mot hypotyreos redan innan graviditeten hade mer än hälften (50,9%) ett förhöjt TSH-värde vid provtagningen.

I nästa steg undersökte vi bara de 891 riktat provtagna kvinnor, som inte hade stått på behandling mot hypotyreos när de blev gravida. Dessa 891 kvinnor definierade vi som ”högriskgrupp” för att utveckla hypotyreos under graviditeten. Vi jämförde nu dessa 891 kvinnor mot 1006 ”lägrisk”-kvinnor ur kohorten, dvs. gravida där man inte hade kontrollerat något TSH under graviditeten. Alla kvinnor ur kohorten hade i början av sin graviditet lämnat ett blodprov för forskningssyfte, vilket vi nu använde för att kontrollera TSH-värden i lägriskgruppen. I högriskgruppen hade 12,6 % ett förhöjt trimesterspecifikt TSH-värde, medan motsvarande andel i lägriskgruppen var 12,1% (p=0,8).

Delarbete IV

Syftet med det sista delarbetet var att undersöka jodstatus under graviditeten i två svenska regioner. Den totala studiepopulationen på 459 gravida bestod av regionala kohorter från Uppsala län (n=186) och Värmlands län (n=273). Alla kvinnor hade lämnat ett slumpmässigt urinprov under graviditetens tredje trimester som sedan analyserades gällande jodkoncentrationen. Medianvärdet för jodkoncentration i urinen i den totala studiepopulationen var 98 μg/L, klart under det från WHO angivna referensintervall på 150-249 μg/L.
Konklusion
Vi fann ett samband mellan homozygota A/A och G/G bärare av SNP rs 4704397 i PDE8B-genen och återkommande missfall. Orsaken till det funna sambandet är oklar.

Lokala riktlinjer gällande upptäckt och behandling av hypotyreos under graviditet var olika utformade och dåligt kompatibla med internationella riktlinjer. Genomförandet av den riktade provtagningen var inte optimal, och andelen av gravida med förhöjda TSH-värden vid provtagning var hög, speciellt bland kvinnor med en redan pågående behandling av hypotyreos.

Riktad provtagning i klinisk praxis var otillfredsställande och inte bättre än slumpen på att identifiera kvinnor med förhöjda TSH-värden. Om pågående studier visar att behandling av gravida med förhöjda TSH-värden förbättrar graviditetsutfallet vore det rimligt att genomföra en allmän TSH-provtagning tidigt under graviditeten.

Våra resultat indikerar att det förekommer jodbrist bland gravida i Sverige, vilket kan ha en negativ inverkan på ofödda barns framtida hälsa. Studien pekar på ett akut behov av en mera omfattande kartläggning och optimering av jodsituationen för gravida i Sverige.
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References


48. Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). Thyroid Therapy for Mild Thyroid Deficiency in Pregnancy (TSH) ([clinicaltrials.gov/ct2/show/NCT00388297](https://clinicaltrials.gov/ct2/show/NCT00388297)).
54. Thyroid AntiBodies and LEvoThyroxine study (TABLET). ([isrctn.com/ISRCTN15948785](http://www.isrctn.com/ISRCTN15948785)).
55. T4Lifetrial ([trialregister.nl/trialreg/admin/rctview.asp?TC=3364](http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=3364)).


97. ANZCTR. A Randomised controlled trial of iodine supplementation in pregnancy to enhance neurodevelopment in children - (PINK). *Trial ID ACTRN12610000411044.*


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