# Phosphodiesterase 8B Gene Polymorphism Is Associated with Subclinical Hypothyroidism in Pregnancy

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**Background:** Maternal subclinical hypothyroidism is associated with a number of adverse outcomes in pregnancy. The Endocrine Society's recent consensus guidelines have recommended treatment with $T_4$ for this condition in pregnancy. The single nucleotide polymorphism rs4704397 in the phosphodiesterase 8B (*PDE8B*) gene has been found to be associated with altered serum TSH concentrations in the general population. We aimed to assess whether genetic variation in TSH due to the rs4704397 genotype affects the number of individuals classified as having subclinical hypothyroidism in pregnancy.

**Methods:** Serum TSH, FT4, FT3, and thyroid peroxidase antibodies (TPOAbs) were measured in 970 pregnant women at 28 wk gestation. rs4704397 genotype was available on 877 subjects. Reference range calculations were based on the TPOAb-negative women.

**Results:** TSH, but not FT4, FT3, or TPOAbs, varied with genotype and was highest in those with the AA genotype (median, 2.16, 1.84, and 1.73 mIU/liter for AA, AG, and GG genotypes, respectively; $P = 0.0004$). A greater proportion of women with the AA genotype had TSH concentrations above 4.21 mIU/liter, the upper limit of the reference range, compared with the AG and GG genotypes (9.6 *vs.* 3.5%, respectively; $P = 0.004$). Maternal *PDE8B* genotype was not associated with offspring birthweight or gestational age at delivery.

**Conclusion:** Genetic variation in TSH levels in pregnancy associated with the *PDE8B* rs4704397 genotype has implications for the number of women treated for subclinical hypothyroidism under current guidelines. Consideration should be made to individualization of normal ranges, potential effects on pregnancy outcome, and intention to treat for subclinical hypothyroidism in pregnancy. (***J Clin Endocrinol Metab* 94: 4608–4612, 2009**)

Maternal subclinical hypothyroidism in pregnancy is associated with impaired neuropsychological development of the child (1) and a number of other adverse outcomes for both mother and offspring (2, 3). This has led to a debate regarding the introduction of universal screening for hypothyroidism in pregnancy (4, 5).

The Endocrine Society's recently published Clinical Practice Guidelines (6) recommend treatment with $T_4$ for women found to have subclinical hypothyroidism, defined by a serum TSH concentration above the upper limit of the trimester-specific and assay-specific reference range, with a normal free $T_4$ (FT4). Although this treatment has not been proven

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Abbreviations: FT3, Free $T_3$; FT4, free $T_4$; IQR, interquartile range; PDE8B, phosphodiesterase 8B; SNP, single nucleotide polymorphism; TPOAb, thyroid peroxidase antibody.

to improve neurological development of the child, the panel considers the potential benefits to outweigh the potential risks.

A number of genes have recently been identified that are associated with altered thyroid function in the normal population (7–9). The single nucleotide polymorphism (SNP) rs4704397 in the phosphodiesterase 8B (*PDE8B*) gene shows an association with circulating TSH levels, explaining 2.3% of the variance in TSH in the general population (7). Each copy of the minor A allele was found to be associated with an increase in TSH concentration of 0.13 mIU/liter, an effect size equating to around a 0.42 SD difference between the AA and GG genotypes.

This genetic variation in TSH concentrations, within the normal population, is likely to result in altered "normal ranges" for the three different genotypes. We aimed to assess whether this difference would affect the number of individuals classified as having subclinical hypothyroidism, who would, therefore, potentially be treated with $T_4$ in pregnancy based on the current guidelines.

## Subjects and Methods

### Study subjects

A total of 1014 healthy pregnant women were recruited as part of the Exeter Family Study of Childhood Health (10). Fourteen women on medication for thyroid disorders were excluded from analysis. Detailed anthropometric measurements and serum samples were taken on the mothers at 28 wk gestation, which was determined by the dating scan (±5 d). TSH, FT4, and free $T_3$ (FT3) were measured in the stored serum samples available on 974 women. Results of thyroid peroxidase antibodies (TPOAbs) were available on 970 (99.6%) of the women.

### Analysis of thyroid function and thyroid antibodies

Serum TSH, FT4, and FT3 were analyzed using the electrochemiluminescent immunoassay, run on the Modular E170 Analyzer (Roche, Burgess Hill, UK). Intraassay coefficients of variation were: TSH <5.3%, FT4 <5.3%, and FT3 <5.1%. The manufacturer's population reference ranges were: TSH, 0.35–4.5 mIU/liter; FT4, 11–24 pmol/liter; and FT3, 3.9–6.8 pmol/liter. TPOAbs were analyzed using the competitive immunoassay (Roche), and a titer above 34 IU/ml was considered positive.

### Genotyping

The *PDE8B* rs4704397 genotype was available on 877 subjects. Genotyping of the samples was performed by KBiosciences (Hoddesdon, UK; www.kbioscience.co.uk), using their own system of fluorescence-based competitive allele-specific PCR (KASPar). The genotyping call rate was 96.2%, and the concordance between duplicate samples (13% of total) was 99.6%. There was no evidence of deviation from Hardy-Weinberg equilibrium ($P$ = 0.13).

### Statistics

Maternal TSH results for the whole cohort were not normally distributed, and various transformations were attempted but none led to normal distribution. FT3 and FT4 were approximately normally distributed. Descriptive statistics for the whole group are presented as median and interquartile range (IQR), and nonparametric analysis was carried out. TSH results on TPOAb-negative women only could be normalized using a square root transformation, so these data were used to generate reference ranges that were calculated using a back-transformed mean ± 1.96 SD. The Mann-Whitney $U$ test and Spearman correlation coefficients were used to assess the associations between maternal characteristics and TSH. The Kruskal-Wallis test was used to assess differences between the three genotypes. $\chi^2$ analysis was used to determine differences in frequencies of those outside the reference range between the three genotypes.

The local research ethics committees approved the study, and all participants gave informed written consent.

## Results

### Study population

All women included in the analysis were of white UK Caucasian origin, had a median age of 31 yr (IQR, 27–34), and a median prepregnant body mass index of 23.0 kg/m$^2$ (IQR, 21.1–25.5). A total of 133 (14%) smoked, and 442 (46%) were primiparous. Sixty-nine (7.1%) women were TPOAb-positive. There was no association between TSH concentration and maternal age (r = 0.015; $P$ = 0.65) or body mass index (r = 0.018; $P$ = 0.6). TSH concentrations were similar in primiparous and multiparous pregnancies (median, 1.9 *vs.* 1.8 mIU/liter, respectively; $P$ = 0.1).

### Association of *PDE8B*-SNP with thyroid function in pregnancy

TSH varied with genotype and was highest in those with the AA genotype (Table 1 and Fig. 1A). There was no difference in FT3 and FT4 levels or prevalence of TPOAbs positive between the three genotypes.

Reference ranges specific for the assay and 28th week of gestation were constructed for the TSH, FT4, and FT3 results, based on TPOAb-negative individuals (n = 901). Within this group of normal, healthy, pregnant women, mean ± 1.96 SD for TSH gave a reference range of 0.49–4.21 mIU/liter. FT4 and FT3 reference ranges were 9.13–15.17 and 3.22–5.17 pmol/liter, respectively.

A greater proportion of women with the AA genotype had TSH concentrations above 4.21 mIU/liter, the upper limit of the reference range, compared with the AG and GG genotypes (9.6 *vs.* 2.8 and 4.3%, respectively; $P$ = 0.004; Table 1).

Similar results are obtained if looking at only TPOAb-negative women (7.3% of those with an AA genotype have a TSH above 4.21 mIU/liter compared with 2.2 and 1.3%

**TABLE 1.** TSH level and number and percentage of women with TSH above the upper limit of the reference range (4.21 mIU/liter) by *PDE8B* genotype

| | All women (n = 877) | | TPOAb-negative women only (n = 810) | |
|---|---|---|---|---|
| Genotype | TSH[a] | TSH >4.21 mIU/liter[b] | TSH[a] | TSH >4.21 mIU/liter[b] |
| GG | 1.73 (1.31–2.39) | 15/349 (4.3%) | 1.70 (1.29–2.30) | 4/320 (1.3%) |
| AG | 1.84 (1.39–2.44) | 11/393 (2.8%) | 1.84 (1.38–2.43) | 8/367 (2.2%) |
| AA | 2.16 (1.49–3.04) | 13/135 (9.6%) | 2.06 (1.49–2.84) | 9/123 (7.3%) |
| *P* | 0.0004 | 0.004 | 0.001 | 0.001 |

[a] TSH in mIU/liter expressed as median (IQR).

[b] TSH above upper limit of reference range is expressed as number (percentage).

of those with the AG and GG genotypes, respectively; $P = 0.001$) (Table 1).

Only two women were identified as having overt hypothyroidism (TSH >4.21 mIU/liter and FT4 <9.13 pmol/liter). Both had the AA genotype, and one was TPOAb-positive.

### Association of *PDE8B*-SNP with birthweight and gestational age at birth

There was no difference in offspring birthweight, adjusted for sex and gestational age (median, 3445, 3479, and 3400 g for GG, AG, and AA, respectively; $P = 0.2$) or gestational age (median, 40 wk for all; $P = 0.6$) between the three genotype groups. Similarly, there was no association between genotype and either small for gestational age (defined as birthweight less than 10th centile) (AA 5.3%, AG 7.7%, and GG 9.2% small for gestational age; $P = 0.34$), or prematurity (gestation ≤37 wk) (AA 2.3%, AG 4.3%, GG 5.8%; $P = 0.25$).

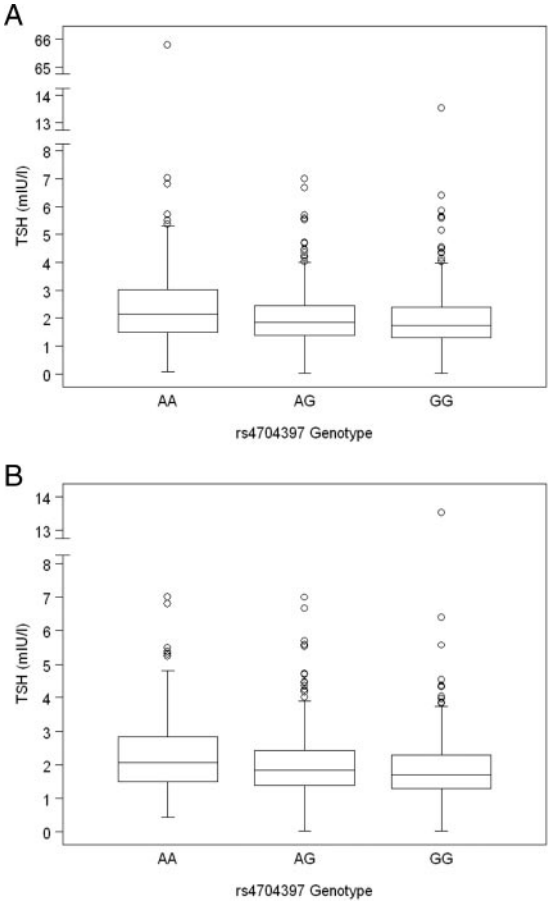


**FIG. 1.** Boxplots to show distribution of TSH results at 28 wk gestation for the three *PDE8B* rs4704397 genotypes in all women (n = 877) (A) and TPOAb-negative women only (n = 810) (B).

## Discussion

Our data suggest that variation in the *PDE8B* gene leads to alteration in serum TSH concentration and different proportions of women picked up with hypothyroidism in pregnancy. Whether this genetic difference conveys increased risk or represents normal variation between individuals is uncertain.

Adequate maternal thyroid function during pregnancy is important for early neurodevelopment of the fetus. Even subclinical hypothyroidism (an elevated TSH level with normal free thyroid hormone levels) has been shown to be associated with poor neuropsychological development of offspring (1). Subclinical hypothyroidism has also been shown to be associated with several obstetric adverse outcomes, including premature birth, placental abruption, impaired fetal growth, and increased fetal mortality (2, 3, 11–13). Although currently there is no clinical trial evidence that $T_4$ replacement in maternal subclinical hypothyroidism improves offspring neuropsychological development, screening in pregnancy is now recommended in high-risk cases, and treatment with $T_4$ has now been advised for any pregnant woman identified with subclinical hypothyroidism (6).

The guidelines recommend measurement of serum TSH for screening subclinical hypothyroidism in pregnancy (6). In recent years, several studies have demonstrated the change in thyroid hormone levels with different stages of pregnancy, and it is now generally accepted that trimester-specific reference ranges for TSH, FT4, and FT3 are necessary for the assessment of thyroid function in pregnancy (14, 15). In this study, we have shown that genetic variation can also alter TSH levels in normal healthy pregnant women, with individuals with the AA genotype of the

*PDE8B* gene being more likely to have elevated TSH concentrations (above the upper limit of the assay-specific reference range) compared with those with the AG or GG genotypes (9.6 *vs.* 3.5%, respectively).

The *PDE8B* gene encodes a high-affinity cAMP phosphodiesterase and has been implicated in thyroid function through its effect on cAMP activity, known to be important in TSH signaling (7). PDE8B catalyzes the hydrolysis and inactivation of cAMP. It is thought that *PDE8B* polymorphisms may reduce cAMP in the thyroid, leading to a decreased thyroid stimulatory response to TSH and hence lower subsequent thyroid hormone production. This is likely to result in a higher TSH being required to maintain the normal range for FT4 and FT3 due to the negative feedback loop, which is consistent with our findings showing association of the *PDE8B*-SNP with TSH but not with free thyroid hormones.

It is uncertain from our data whether the greater proportion classified with TSH above 4.21 mIU/liter are at increased risk of adverse outcomes and under current guidelines would require treatment, or whether this merely reflects normal individual variation. It may be that reference ranges based on genotype would be more appropriate. One limitation of our study is that the results are based on a single thyroid function test, so we do not have follow-up data on these women.

The idea of individualized reference ranges for thyroid function tests has been proposed previously (16–18). It has been shown that individual reference ranges for TSH are narrow, and changes for an individual may not be picked up within normal laboratory population-based reference ranges (16, 18). A recent genome-wide linkage study of dizygotic female twins has suggested that genetic factors may be involved in the regulation of serum TSH, FT4, and FT3 concentrations (8). Our data have provided evidence of a genetic contribution to the individual variation of serum TSH concentrations and how different proportions within each genotype group would be picked up based on conventional definitions for subclinical hypothyroidism. It would be important to establish whether this genetic variation is also associated with increased clinical risks associated with subclinical hypothyroidism. A Mendelian randomization approach examining associations of maternal *PDE8B* genotype with adverse pregnancy outcomes and early neuropsychological development of the child would help tease this out. Our data do not show associations with prematurity or low birth size, and if anything, the trend is in the reverse direction, although we recognize we may be underpowered to see the effect sizes seen in previous studies (3, 19).

In conclusion, we have shown genetic variation in TSH levels in pregnancy that has implications for the number of women treated for subclinical hypothyroidism under current guidelines. Consideration should be given to individualization of normal ranges and potential effects on pregnancy outcome and intention to treat for subclinical hypothyroidism in pregnancy.

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