CASE REPORT

Pregnancy in women heterozygous for MCT8 mutations: risk of maternal hypothyroxinemia and fetal care

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Abstract

Context: Monocarboxylate transporter 8 (MCT8 or SLC16A2) mutations cause X-linked Allan–Herndon–Dudley syndrome. Heterozygous females are usually asymptomatic, but pregnancy may modify thyroid function and MCT8 is expressed in the placenta, suggesting that maternal and fetal abnormalities might develop even in the absence of MCT8 fetal mutation. Genetic counseling is so far based on X-linked transmission, and prenatal diagnosis is rarely performed.

Objective: To describe thyroid function and the prenatal diagnosis in pregnant mothers harboring heterozygous MCT8 mutations and management of the persistent maternal hypothyroxinemia.

Patients: Two women heterozygous for MCT8 mutations (c.1690G>A and c.1393-1G>C) were monitored throughout pregnancy.

Methods: Prenatal diagnosis included sex determination, direct MCT8 sequencing, and familial linkage analysis. Ultrasonography and hormonal assays for maternal thyroid function evaluation were performed serially during pregnancy. Neonatal thyroid hormonal status was assessed.

Results: None of the three fetuses (two males and one female) carried MCT8 mutations. One of the two heterozygous mothers revealed gestational hypothyroxinemia, prompting early levothyroxine (L-T4) therapy until delivery. The second heterozygous mother showed normal thyroid function but was preventively treated by L-T4 and all of the three neonates had normal thyroid hormone levels and thyroid gland at birth, suggesting advantages of prenatal care and/or compensatory mechanisms.

Conclusion: Heterozygous MCT8 women should be monitored for requirement of L-T4 therapy to prevent fetal and neonatal hypothyroidism and to avoid risk of potential cognitive delay due to gestational hypothyroxinemia. Moreover, when the disease-causing mutation is known and/or the first child is affected, prenatal diagnosis for male fetuses should be assessed early for MCT8 mutations by direct sequencing.

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Introduction

Monocarboxylate transporter 8 (MCT8 or SLC16A2: solute carrier family 16, member 2) was recently identified as a specific thyroid hormone (TH) transporter expressed in many tissues, including the human brain (1). The pathophysiological importance of the MCT8 transporter has been established by the demonstration of a severe phenotype known as Allan–Herndon–Dudley syndrome (AHDS, OMIM #300523) (2–5) in cases of inactivating MCT8 mutations (6, 7). AHDS is an X-linked disorder characterized by severe psychomotor retardation and abnormal TH levels due to deficient triiodothyronine (T3) entry into neurons (increased free T3 (FT3), low–normal range to low free thyroxine (FT4), and normal to elevated TSH serum levels). In heterozygous females, although mental retardation and/or milder thyroid function impairments were reported (8), usually no thyroid and cognitive dysfunctions were detected (2, 9). Genetic counseling in families having one or more males with AHDS is classically based on X-linked transmission, and, to our knowledge, no specific prenatal diagnosis of MCT8 mutations has been reported yet.

MCT8 is expressed in the placenta starting early in the first trimester, allowing TH transport from mother to fetus (10). This transport is essential for normal fetal brain development, as demonstrated by the
neuropsychological impairments seen in children born to mothers with gestational hypothyroidism (5). Moreover, recent findings, including large population-based studies, have shown harmful effects of maternal hypothyroxinemia on child development (11–16). Mothers with mild MCT8 deficiency related to a heterozygous MCT8 mutation may have alterations in TH transport that thus may affect fetal development, even in the absence of fetal MCT8 mutation (8, 10). In addition, the thyroid gland of heterozygous mothers may be unable to meet the increased TH needs during pregnancy (17), leading to a decrease in THs available to the fetus. However, these fetal and maternal effects of heterozygous MCT8 mutations remain hypothetical as, to our knowledge, no data are available on pregnancies in heterozygous women. The aim of this study is therefore to describe the management of pregnancies in two women heterozygous for MCT8 mutations and to discuss the genetic investigations and thyroid function in the mothers and their neonates.

Patients and methods

Patients

Two pregnant women known to be heterozygous for MCT8 mutations were closely monitored during pregnancy. The genetic studies were performed after the birth of the first boy with AHDS (Fig. 1a and b).

In the first family (A), the mother (M1) was a 31-year-old woman heterozygous for a missense mutation within Exon 6 of the MCT8 gene (c.1690G>A; p.G564R). She had moderate mental retardation, obesity, and a history of transient hypothyroidism during her first pregnancy. TH levels were within the normal range 6 months before the present pregnancy, without replacement therapy. The first prenatal ultrasonogram at 12 weeks of gestation revealed a dichorionic diamniotic pregnancy.

In the second family (D), the mother (M2) was a 30-year-old woman who was heterozygous for a novel intron splice-site MCT8 mutation in Exon 5 (c.1393-1G>C), with no clinical features of MCT8 deficiency.

Sex determination and prenatal genetic diagnosis

In both pregnancies, sex determination was performed early during the first trimester. In male fetuses, prenatal genetic testing included direct sequencing of fetal DNA from chorionic villi using previously described conditions focusing on the known disease-causing mutation (9). To confirm the absence of inherited mutated alleles in the fetuses, linkage analysis was performed using X-linked polymorphic markers close to the MCT8 locus (DXS8101, DXS8066, and DXS8037 in family A; DXS8092 in family B). As shown here, the affected maternal alleles (in red) were not found in any of the three fetuses in the two families studied, strongly suggesting absence of inherited MCT8 mutations.

Figure 1 (a) Pedigrees and linkage analysis using markers covering the X chromosome in the two families studied. Linkage analysis was performed using X-linked polymorphic markers close to the MCT8 locus (DXS8101, DXS8066, and DXS8037 in both families; and ATRX, DXS1225, and DXS8082 in family A or DXS8092 in family D). As shown here, the affected maternal alleles (in red) were not found in any of the three fetuses in the two families studied, strongly suggesting absence of inherited MCT8 mutations.

(b) Chromatograms containing the missense MCT8 mutations. The changed base is indicated by an arrow. In family A, the affected child (P1) has a heterozygous missense c.1690G>A mutation (p.G564R) in Exon 6 of MCT8. In family B, the affected child (P2) has a heterozygous intron splice-site mutation in Exon 5 (c.1393-1G>C MCT8 mutation).
both families; and ATRX, DXS1225, and DXS8082 in family A or DXS8092 in family D (Fig. 1a).

To exclude contamination by maternal DNA, the following polymorphic markers located in autosomal chromosomes were used: D6S1713 in family A and D7S2563 in family D (data not shown).

Pregnancy follow-up
A detailed clinical history with special attention to maternal thyroid function and treatment was obtained retrospectively. Thyroid function was assessed before the pregnancy and at least monthly from the second trimester to delivery in both women. In addition, in the mother M1, thyroid function was assessed every 2 weeks during the first trimester. Serum TSH, FT4, and FT3 levels were measured using an electrochemiluminescence immunoassay (Roche Modular E170 Analyzer) and the results were compared with trimester-specific reference intervals (18).

When hypothyroxinemia was detected, \( \text{L-T}_4 \) replacement therapy was started. Written informed consent was obtained from both women before treatment initiation. Subsequently, the \( \text{L-T}_4 \) dosage was adjusted, usually in \( 25 \mu \text{g} \) increments, to obtain euthyroidism. After delivery, \( \text{L-T}_4 \) therapy was continued and adjusted for maternal thyroid function.

Fetal and neonatal evaluations
Fetal thyroid size was assessed by ultrasonograms obtained during pregnancy and compared with the normative data reported by Ho & Metreweli (19) for volume and Ranzini et al. (20) for diameter. Goiter was defined as a thyroid gland volume equal to or greater than the mean \( +2 \text{ s.d.} \) (19) or as a thyroid gland diameter equal to or greater than the 95th percentile (20).

Conception was estimated to have occurred 2 weeks after the last menstrual period, and gestational age was confirmed by ultrasonography 12 weeks after the last menstrual period.

Fetal skeletal maturation, heart rate, mobility, and growth, as well as the amount of amniotic fluid, were recorded when available as indirect signs of fetal thyroid function (21).

TH levels were assayed from cord blood at birth and in peripheral blood at 4, 7, and 30 days of age.

Results
Genetic analysis
Sex determination revealed two males, one in each family and one twin girl (Fig. 1). Direct sequencing showed that none of the three fetuses had inherited the maternal MCT8 mutation. Linkage analysis confirmed this result (Fig. 1).

Maternal thyroid function and treatment during pregnancy
The M1 mother had low FT4 and mildly elevated FT3 serum levels just before the pregnancy (with normal TSH levels), without \( \text{L-T}_4 \) therapy (Table 1). During the first trimester, TSH levels remained stable, instead of decreasing as usual during pregnancy (22), and FT4 decreased at 12 WG, prompting \( \text{L-T}_4 \) therapy in a dose of 75 \( \mu \text{g/day} \) (0.6 \( \mu \text{g/kg per day} \)). With this dose, FT4 increased to the lower limit of the normal range by 20 WG but decreased again by 24 WG. Thereafter, despite increasing \( \text{L-T}_4 \) dosages up to 150 \( \mu \text{g/day} \) (1.2 \( \mu \text{g/kg per day} \)), FT4 remained below the normal range until the week before delivery (36 WG). FT3 levels remained at the upper limit of the normal range and TSH within the low–normal range throughout the pregnancy. After delivery, \( \text{L-T}_4 \) therapy was continued and 6 months later she was still taking 75 \( \mu \text{g/kg per day} \) (0.7 \( \mu \text{g/kg per day} \)) of \( \text{L-T}_4 \) and had a slightly decreased FT4 level with high FT3 and normal TSH levels (data not shown).

The M2 mother had normal thyroid function before the pregnancy. The first TH assays performed at 24 WG showed that FT4 level was in the lower end of the normal range and based on the history of the first mother M1, \( \text{L-T}_4 \) therapy was started in a dosage of 50 \( \mu \text{g/day} \) (0.75 \( \mu \text{g/kg per day} \)) to prevent maternal hypothyroxinemia. This dosage was maintained throughout the pregnancy, and FT4 remained within the normal range. One week before delivery (40 WG), \( \text{L-T}_4 \) therapy was stopped because of palpitations, despite normal TSH and FT4 levels (Table 1). However, the palpitations persisted despite \( \text{L-T}_4 \) discontinuation.

Fetal and neonatal evaluation
Prenatal ultrasonograms showed normal thyroid fetal measurements. None of the three fetuses had indirect evidence of fetal thyroid dysfunction (normal skeletal maturation, heart rate, and mobility).

Both women delivered at full term and all the three neonates had normal growth measurements. In family A, the mother (M1) delivered at 37 weeks of gestation by Cesarean section. The female and male twin had birth weights of 3060 and 2920 g and lengths of 49 and 48.5 cm respectively. The M2 mother delivered vaginally at 41 weeks, without complications. Birth weight was 3240 g and length 49 cm. No birth defects and/or clinical signs of thyroid dysfunction were detected, and all the three babies had normal Apgar scores. Cord blood TH levels were normal in all the three babies (Table 1), as well as thyroid sonogram measurements (data not shown). No clinical or laboratory evidence of thyroid dysfunction developed in any of the three neonates during the first postnatal month, except a transitory slightly elevated FT4 level in one neonate at 4 days of life (Table 1). Fetal sonograms showed no evidence of goiter in any of the three babies.

Maternal MCT8 mutations during pregnancy
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Serum thyroid hormone levels before and during gestation in women heterozygous for MCT8 gene mutations and their newborns.

**Table 1**

<table>
<thead>
<tr>
<th>MCT8 mutations</th>
<th>Basal</th>
<th>8</th>
<th>12</th>
<th>14</th>
<th>20</th>
<th>24</th>
<th>30</th>
<th>32</th>
<th>36</th>
<th>40</th>
<th>Cord blood</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 30</th>
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<tr>
<td>M1 (c.1690G&gt;A)</td>
<td>0.95</td>
<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
<td>0.9</td>
<td>0.9</td>
<td>5.6/10.6</td>
<td>9.3/9.3</td>
<td>6.7/10.6</td>
<td>8.1/10.6</td>
</tr>
<tr>
<td>TSH (mIU/ml)</td>
<td>6.7/10.6</td>
<td>6.7/10.6</td>
<td>6.7/10.6</td>
<td>16.1/26.8</td>
<td>19.4/33.7</td>
<td>14.9/25.1</td>
<td>19.7/34.5</td>
<td>14.9/25.1</td>
<td>13.7/22.6</td>
<td>11.3/19.3</td>
<td>4.3/10.6</td>
<td>8.9/16.3</td>
<td>11.3/19.3</td>
<td></td>
</tr>
<tr>
<td>FT4 (pmol/l)</td>
<td>5.6/9.3</td>
<td>9.3</td>
<td>7.6</td>
<td>6.9</td>
<td>7.6</td>
<td>7.6</td>
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<td>7.6</td>
<td>7.6</td>
<td>8.1/15.3</td>
<td>14.8/25.1</td>
<td>19.9/34.5</td>
<td>24.5/39.9</td>
<td></td>
</tr>
<tr>
<td>FT3 (pmol/l)</td>
<td>8.1</td>
<td>7.3</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
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<td>10.6/17.6</td>
<td>14.6/21.7</td>
<td>21.7/28.8</td>
<td></td>
</tr>
<tr>
<td>L-T4 dosage</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
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<td>50</td>
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</tr>
</tbody>
</table>

Abnormal values are in bold type. Normal range for FT4 during pregnancy: first trimester (11–19 pmol/l), second trimester (9.7–17.5 pmol/l), and third trimester (8.1–15.3 pmol/l). Trimester-specific distributions of TSH are expressed in values representing the lower and upper quartile (25th–75th percentile): first trimester (1.0–2.2 mIU/ml), second trimester (1.1–2.4 mIU/ml), and third trimester (1.3–2.9 mIU/ml) (18).

**Discussion**

This study provides the first evidence that gestational hypothyroxinemia may develop in women harboring heterozygous for MCT8 mutations. Additionally, we describe the modalities of prenatal testing in male fetuses using linkage analysis and direct sequencing of fetal DNA from chorionic villi to search for the known disease-causing mutation.

To date, genetic counseling of AHDS was based on X-linked condition only. In many X-linked disease, prenatal molecular genetic testing by direct DNA analysis was routinely performed using fetal cells obtained by amniocentesis, usually at ~15–18 WG, or by chorionic villus sampling, at ~10–12 WG. In this study, we showed that AHDS affliction also can be easily and early prenatally determined when MCT8 mutation is identified earlier in the future mother. Additionally, when parental DNA is available, familial linkage analysis could be useful to confirm the absence of affected maternal allele transmission and to exclude contamination by maternal DNA.

Previous studies have shown that thyroid function varies in women heterozygous for MCT8 mutations (3, 23, 24). In this study, we found further evidence of variability, as mild hypothyroxinemia was present before the pregnancy in only one of the two women (M1). This difference may indicate interindividual variability, differences in random X inactivation (8), or differences in the residual activity of the mutant transporter, as the two women had different MCT8 mutations (24). During pregnancy, our experience shows that thyroid function may correlate with the basal condition; indeed, gestational hypothyroxinemia was more severe, requiring higher L-T4 doses, in the M1 mother, who had mild hypothyroxinemia before the pregnancy, than in the M2 mother, whose pre-pregnancy thyroid function was normal. However, this hypothesis based on only two patients needs to be evaluated in larger numbers of heterozygous woman.

Note that neither woman displayed any symptoms of hypothyroidism, probably due to the hypermetabolic state that characterizes pregnancy (25). Nevertheless, our findings of potential maternal hypothyroxinemia emphasize the need for close TH monitoring before and during pregnancy in women heterozygous for MCT8 mutations. In our study, TSH level remain within the low–normal range and we did not observe real gestational hypothyroidism, but recent large population-based studies have debated that maternal hypothyroxinemia during pregnancy, and not TSH levels across the entire range, appears as the main factor leading to adverse cognitive outcomes (15). Indeed, a certain threshold in pregnant women with normal TSH levels should be reached before low concentrations of FT4 affect children’s neurodevelopmental outcomes (15). In the M1 mother, the gestational hypothyroxinemia was persistent despite
increasing L-T4 dosages, with no detected fetal or neonatal abnormalities in the twin’s thyroid function and morphology by ultrasound. However, maternal L-T4 therapy might prevent fetal growth restriction, neonatal morbidity, and risk of verbal or nonverbal cognitive delay (13, 15, 16, 26). The normal neonatal growth measurements and neurological findings indicate that active compensatory mechanisms in the fetoplacental unit might increase the transplacental transfer of TH. These mechanisms may include increased expression of MCT8 or other TH transporters such as MCT10 (6) and/or increased expression of TH receptors. Another possible mechanism is an increase in the enzyme selenocysteine monodeiodinase, as the fetal brain depends on T4 as a substrate for local T3 generation by local T4 deiodination, rather than on circulating T3, whose levels are normal or slightly elevated in pregnant women heterozygous for MCT8 mutations (27–29). To confirm this hypothesis, evaluations of fetal thyroid function during pregnancy would be of interest. Two large population-based Dutch studies are divergent in establishing a threshold for FT4 in pregnant women (11.7–11.8 vs 10.4 pmol/l) and this might indicate differences in FT4 measurement methods (13, 15). As FT4 threshold is still not clearly precised and based on our first observation, L-T4 was introduced in the M2 mother, even in the absence of abnormal thyroid function to prevent potential maternal hypothyroxinaemia (Table 1) and FT4 measurement remained normal for the method used.

Altogether, based on our case reports, we suggest some recommendations for managing pregnancy in women heterozygous for MCT8 mutations. Prior to conception, genetic counseling for X-linked disease should be offered and maternal thyroid function should be assessed. Then, fetal sex should be determined early during the first trimester and prenatal molecular testing of male fetuses should be performed promptly. Whatever the result, maternal thyroid function should be monitored closely from the 1st month of pregnancy to delivery, and serial sonograms should be performed to detect any indirect signs of fetal hypothyroidism. Furthermore, L-T4 replacement therapy should be considered, even in the absence of MCT8 mutation in the fetus, to prevent neonatal hypothyroidism and potential neurological damage.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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