Inherited defects of thyroid hormone metabolism

Altérations héréditaires du métabolisme des hormones thyroïdiennes

A.M. Dumitrescu a, S. Refetoff a, b, c, *

a Department of medicine, university of Chicago medical center, 5841 S, Maryland avenue, MC3090, room M367, Chicago, IL, 60637, USA
b Department of pediatrics, university of Chicago medical center, 5841 S, Maryland avenue, MC3090, room M367, Chicago, IL 60637, USA
c Committees on molecular medicine and genetics, university of Chicago, 5841 S, Maryland avenue, MC3090, room M367, Chicago, IL 60637, USA

Résumé

Le métabolisme intracellulaire des hormones thyroïdiennes et la disponibilité en l’hormone active, la triiodothyronine, sont régulés par trois sélénoprotéines iodothyronine désiodases (Ds). Alors que les modifications acquises de l’activité des désiodases sont courantes, leurs altérations héréditaires n’ont pas encore été identifiées chez les humains. Le sélénium (Se) est un oligoélément essentiel indispensable pour la biosynthèse des sélénoprotéines et la selenocysteine insertion sequence (SECIS) binding protein 2 (SBP2) représente un facteur clé de transactivation pour l’insertion de la sélénocystéine au sein des sélénoprotéines. En 2005, nous avons rapporté les premières mutations du gène SBP2 dans les familles où les probants présentaient un retard de croissance transitoire, associé à des altérations des tests de la fonction thyroïdienne : baisse de triiodothyronine (T3), augmentation de thyroxine (T4) et de T3 inverse (rT3) et léger accroissement de TSH. Les enfants atteints étaient soit homozygotes, soit hétérozygotes composites pour la mutation du gène SBP2 ; le phénotype relativement discret était lié à un déficit partiel de la protéine SBP2 affectant l’expression d’une sous-population de sélénoprotéines. Les études in vivo de ces sujets ont exploré les effets de la supplémentation en sélénoprotéines et en hormones thyroïdiennes. Les expérimentations in vitro ont apporté des informations nouvelles sur les effets des mutations de SBP2. Un phénotype plus large et plus complexe a été mis en lumière par l’identification ultérieure de trois nouveaux cas issus de différentes familles porteuses de mutations du gène SBP2. Ces mutations sont responsables d’un déficit sévère de SBP2 entraînant une réduction de la synthèse de la plupart des 25 sélénoprotéines humaines identifiées. Dans cette revue, nous synthétisons la présentation clinique des mutations SBP2, leur impact sur la fonction de SBP2 et leurs conséquences délétères pour la synthèse et la fonction de la sélénoprotéine.

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Mots clés : Désiodase ; Sélénoprotéine ; SBP2 ; Hormones thyroïdiennes ; Myopathie ; Retard de croissance ; Infertilité

Abstract

Intracellular metabolism of thyroid hormone and availability of the active hormone, triiodothyronine is regulated by three selenoprotein iodothyronine deiodinases (Ds). While acquired changes in D activities are common, inherited defects in humans have not been identified. Selenium (Se) is an essential trace element required for the biosynthesis of selenoproteins, and selenocysteine insertion sequence (SECIS) binding protein 2 (SBP2) represents a key trans-acting factor for the cotranslational insertion of selenocysteine into selenoproteins. In 2005 we reported the first mutations in the SBP2 gene in two families in which the probands presented with transient growth retardation associated with abnormal thyroid function tests, low triiodothyronine (T3), high thyroxine (T4) and reverse T3, and slightly elevated thyrotropin. Affected children were either homozygous or compound heterozygous for SBP2 gene mutations and the relatively mild phenotype was due to partial SBP2 deficiency, affecting the expression of a subset of selenoproteins. In vivo studies of these subjects have explored the effects of Se and thyroid hormone supplementation. In vitro experiments have provided new insights into the effect of SBP2 mutations. A broader and more complex phenotype was brought to light by the subsequent identification of three new cases from different families with SBP2 gene mutations. These mutations caused a severe SBP2 deficiency resulting in reduced synthesis of most of the 25 known human selenoproteins. Here we summarize the clinical presentation of SBP2 mutations, their effect on SBP2 function and downstream consequences for selenoprotein synthesis and function.

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Keywords: Deiodinase; Selenoprotein; SBP2; Thyroid hormone; Myopathy; Growth delay; Infertility

* Corresponding author.
E-mail address: refetoff@uchicago.edu (S. Refetoff).

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1. Selenoprotein synthesis

Selenoproteins contain the rare aminoacid selenocysteine (Sec) in their active center. Several factors are required for Sec incorporation: cis-acting sequences present in the mRNA of a selenoprotein (UGA codon and Sec insertion sequence, SECIS), and trans-acting factors (Sec-specific elongation factor (eEFSec), Sec-specific tRNA Sec and SECIS-binding protein (SECISBP2 or SBP2)) [1]. However, the list of factors involved in this mechanism is constantly growing, the most recent members being the ribosomal protein L30 [2], the 43 KDa RNA binding protein (Secp43) and the soluble liver antigen protein (SLA) [3–5]. Using the SECIS element as bait, the rat SECIS binding protein, SBP2 was purified and cloned in 2000 [6].

The human selenoproteome comprises at least 25 individual selenoproteins [4,7]. Although the precise function of most selenoproteins is unknown, some characterized mammalian selenoproteins were found to serve as antioxidants or oxido-reductases (glutathione peroxidases [GPx] and thioredoxin reductases), in thyroid hormone metabolism (deiodinases, [Dio or D]), selenium transport and storage (selenoprotein P, SePP) and potential protein folding (Sep15, SelN, SelM, SelS). Some selenoproteins must have a crucial function as supported by the observation that removal of the tRNA Sec gene is lethal to the embryo [8].

A distinct hierarchy exists in the synthesis of selenoproteins as the expression of individual selenoproteins is differentially affected by the cellular content in Se [3]. This may be due to changes in the distribution of the two isoforms of tRNA Sec [3], mRNA degradation by nonsense-mediated decay [9] and preferential SECIS recognition by SBP2 [10,11].

2. Clinical presentation

A total of six families have been identified to harbor recessive SBP2 gene mutations [12–15]. The probands of the initial three families were brought to clinical attention because of growth delay [12,13]. All three were boys ranging in age from six to 14.5 years. The proband of a fourth family was a 12-yr-old girl who presented with delayed bone maturation, congenital myopathy, impaired mental and motor coordination development and bilateral sensorineural loss [14]. In a fifth family, a male child, presented at age two years with progressive failure to thrive in infancy, followed by global developmental delay and short stature that prompted further investigation. Other features in this patient are an early diagnosis of eosinophilic colitis, dermatitis and sun photosensitivity. Pubertal development was normal, but at the age of 15 years, he developed unilateral testicular torsion requiring orchiectomy and fixation of the remaining testis. His final stature (1.67 m), though close to the mean parental height of 1.69 m, was in the ninth centile. The ethnic origins of the first three families are Bedouin from Saudi Arabia, Irish/Kenyan, and African from Ghana [12,13]. The three more recent families are one from Brazil and two from the UK [14,15].

Some of the clinical features, in particular delayed growth and bone age, prompted thyroid function testing. All affected subjects were found to have unusual thyroid function test abnormalities, characterized by high serum thyroxine (T4), low 3,3′, 5-triiodothyronine (T3), high 3,3′, 5′-triiodothyronine (reverse T3 or rT3) and normal or slightly elevated thyrotropin (TSH) concentrations (Fig. 1). None of the subjects had an enlarged thyroid gland confirmed by ultrasound examinations.

3. SBP2 mutations

Extensive in vivo and in vitro studies on the initial family allowed the identification of this new genetic defect [12]. This family of Bedouin origin from Saudi Arabia had seven children three of them being affected. Affected individuals required larger doses of levothyroxine (L-T4) but not liothyronine (L-T3) to suppress their serum TSH concentration, indicating a defect in iodothyronine metabolism. However co-segregation of the phenotype with the three deidinases was excluded.

Skin fibroblasts demonstrated low deiodinase D2 enzymatic activity but normal mRNA content, reflecting a defect in this selenoenzyme synthesis. Affected subjects shared homozygous haplotypes at the SBP2 locus and were found to be homozygous for R540Q mutation. Table 1 summarizes the ten SBP2 gene defects identified so far.

<table>
<thead>
<tr>
<th>Family</th>
<th>SBP2 gene</th>
<th>SBP2 protein</th>
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<tbody>
<tr>
<td>A</td>
<td>R540Q homozygous</td>
<td>R540Q homozygous</td>
</tr>
<tr>
<td>B</td>
<td>K438X</td>
<td>Truncated, missing C-terminus</td>
</tr>
<tr>
<td>C</td>
<td>IVS8+29G→A</td>
<td>Alternative transcripts</td>
</tr>
<tr>
<td>Brazil</td>
<td>120 X</td>
<td>Smaller SBP2 isoforms from downstream ATGs</td>
</tr>
<tr>
<td>UK 1</td>
<td>R770X</td>
<td>Truncated, disrupted C-terminus</td>
</tr>
<tr>
<td></td>
<td>c.668delT fs223 255X</td>
<td>Smaller SBP2 isoforms from downstream ATGs</td>
</tr>
<tr>
<td></td>
<td>Intron 6–155 del C</td>
<td>Abnormal splicing, truncated, missing C-terminus</td>
</tr>
<tr>
<td>UK2</td>
<td>C691R</td>
<td>Increased proteasomal degradation</td>
</tr>
<tr>
<td></td>
<td>(? intronic SNPs)</td>
<td>Transcripts lacking exons 2-4 or 3 and 4, smaller SBP2 isoforms from downstream ATGs</td>
</tr>
</tbody>
</table>

The human *SBP2* gene was cloned in 2002 [16], is located on chromosome 9, contains 17 exons and encodes 854 amino acids. *SBP2* mRNA is expressed at low levels in all tissues tested, with a high expression and an additional smaller transcript in testis [16]. The C-terminal domain of the protein is required for SECIS binding, ribosome binding and Sec incorporation [17] being mandatory for *SBP2* function. The role of the N-terminal region is in part unclear. Recent in vitro studies have characterized a nuclear localization signal (NLS) located in the N-terminal part and nuclear export signal (NES) in the C-terminal part. These domains enable *SBP2* to shuttle between the nucleus and the cytoplasm [18] and play a role in the function of *SBP2* in the nucleus in vivo.

The relative mild phenotype of the affected individuals from the first three families is due to partial *SBP2* deficiency. R540Q mutant *SBP2* behaves as a hypomorphic allele in in vitro studies using the corresponding rat Sbp2 mutation, R531Q, which showed no binding to selective SECIS elements resulting in selective loss of expression of a subset of selenoproteins. The affected child of the second family was compound heterozygous for K438X and IVS8ds+ 29G→A mutations the latter causing alternative splicing, with the amount of normal transcripts in the affected child estimated at 24%.

*SBP2* has several mRNA and protein isoforms as demonstrated with a minigene system. The R128X mutation [13] results in smaller *SBP2* isoforms translated from downstream ATGs, and containing intact C-terminus functional domains including those required for selenocysteine insertion, RNA binding and ribosome interaction (residues present in exons 12 through 16) [19].

The more severe phenotype recently reported in three families is likely due to a more severe impairment in *SBP2* function [20], as it is the case for the Brazilian patient who is compound heterozygous for R120X/R770X [14]. The R770X mutation truncates the C-terminal functional domain in all the isoforms and likely abolishes *SBP2* function. The R120X allele likely generates smaller functionally active *SBP2* isoforms, but the overall amount would be less than that of the homozygous R128X patient [13], thus explaining the more severe phenotype. It is possible that the production of a small amount of active *SBP2* from the R120X allele is the reason for the manifestations not being even more severe. This mechanism was demonstrated also to be true in the case of the two patients from UK. In addition, increased proteasomal degradation was demonstrated for the C691R mutation and Western blotting of skin fibroblasts from both probands showed lack of full length *SBP2* protein expression [15].

4. Consequences for selenoprotein function

*SBP2* is epistatic to selenoprotein synthesis. In the patients with *SBP2* deficiency serum concentrations of selenium, selenoprotein P and other selenoproteins are reduced. Skin fibroblasts have decreased D2 and Gpx activities [12]. Detailed evaluation of three recent cases with severe *SBP2* deficiency [14,15] demonstrated deficiencies in multiple selenoproteins: lack of testis-enriched selenoproteins resulting in failure of the latter stages of spermatogenesis and azoospermia; selenoprotein N (SEPN) like myopathy resulting in axial muscular dystrophy; cutaneous deficiencies of antioxidant selenoenzymes causing increased cellular reactive oxygen species (ROS); and reduced selenoproteins in peripheral blood cells resulting in immune deficits [15]. In addition, deficiencies of other selenoproteins with unknown function SELH, SELT, SELW, SELI were found and their consequences are not yet known [15]. In some of these patients, multiple tissues and organs show damage mediated by ROS, and it is conceivable that other pathologies linked to oxidative damage such as neoplasia, neurodegeneration, premature ageing, may manifest with time.

5. Selenium supplementation and thyroid hormone treatment

Identification of the metabolic pathway responsible for the phenotype of these patients and the demonstration of defects in the *SBP2* gene provided further insight into targeted treatment
zymes deiodinases. Only a few individuals with this defect have affecting selenoprotein synthesis, among which the selenoen-
and thyroid hormone were tested [13,21].

Se bioavailability.

seems to be more efficient as it can be incorporated nonspecifi-
restore normal thyroid hormone metabolism. Se supplemen-
of selenomethionine but not selenite, normalized the serum sele-
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6. Conclusions

The only known inherited defect of intracellular thyroid hor-
mone metabolism is caused by mutations in the SBP2 gene
affecting selenoprotein synthesis, among which the selenoen-
zymes deiodinases. Only a few individuals with this defect have
been described. Typical laboratory findings are high T₄, low
T₃, high rT₃, normal or slightly elevated serum TSH, decreased
serum Se and decreased selenoprotein levels and activity in
serum and tissues. The clinical phenotype is complex; affected
individuals may have delayed growth and puberty, and in severe
cases failure to thrive, mental retardation, infertility, myopa-
thy, hearing impairment, photosensitivity, and immune deficits.
SBP2 defects could have as yet undetermined consequences
and the identification of additional patients, and their long term
follow up, are important in further characterizing this recently
described defect.

Disclosure of interest

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possibilities. Two such options, namely, administration of Se
and thyroid hormone were tested [13,21].

Administration of up to 400 mcg of selenium [21], in the form
of selenomethionine but not selenite, normalized the serum sele-
num concentration but not selenoprotein P levels and did not
restore normal thyroid hormone metabolism. Se supplemen-
tation in form of selenomethionine contained in Se-rich yeast
seems to be more efficient as it can be incorporated nonspecifically
into all circulating serum proteins [22], whereas selenite is
metabolized and inserted as selenocysteine into the growing pep-
tide chain of selenoproteins [23], therefore resulting in different
Se bioavailability.

The effect of L-T₃ administration was tested in three patients
as it was demonstrated to equally suppress serum TSH concen-
tration in affected and unaffected [12]. Delayed linear growth
can be improved with L-T₃ [13] supplementation, but experi-
ence with thyroid hormone administration in these patients is
limited.

Other clinical features of SBP2 defects are treated symp-
tomatically at this point.

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