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Inherited defects of thyroid hormone metabolism

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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 co-translational 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.

Keywords

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

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Conflict of interest statement

1. Selenoprotein synthesis

Selenoproteins contain the rare amino acid 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 transacting factors [Sec-specific elongation factor (eEF^{Sec}), 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 6 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 5th family, a male child, presented at age 2 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, fasting nonketotic hypoglycemia with low insulin levels requiring supplemental enteral nutrition, muscle weakness and mild bilateral high-frequency hearing loss (15).

The only adult with *SBP2* deficiency is the proband of the 6th family, who presented at age 35 years with primary infertility, skin photosensitivity, fatigue, muscle weakness, and severe Raynaud disease (digital vasospasm), impaired hearing, and rotatory vertigo (15). In childhood, both motor and speech developmental milestones were delayed, requiring speech therapy. Hearing problems persisted despite myringotomies for secretory otitis media at 6 years of age. Multiple additional features became obvious as he was advancing in age. He had difficulty walking and running in adolescence, with genu valgus and external rotation of the hip requiring orthotic footwear. At the age of 13 years, marked sun photosensitivity was noted with abnormal UV responses on phototesting. 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 (1.67m), though close to the mean parental height of 1.69 m, was in the ninth centile. The ethnic origins of the first 3 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 (T_4), low 3,3',5-triiodothyronine (T_3), high 3,3',5'-triiodothyronine (reverse T_3 or rT_3) and normal or slightly elevated thyrotropin (TSH) concentrations (Figure 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 ($L-T_4$) but not liothyronine ($L-T_3$) to suppress their serum TSH concentration, indicating a defect in iodothyronine metabolism. However co-segregation of the phenotype with the three deiodinases 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 10 *SBP2* gene defects identified so far.

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 2nd family was compound heterozygous for K438X and IVS8ds+29 G->A mutations the latter causing alternative splicing, with the amount of normal transcripts in the affected child being 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 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 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 selenium concentration but not selenoprotein P levels and did not restore thyroid hormone metabolism dysfunction. Se supplementation 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 peptide 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 concentration in affected and unaffected (12). Delayed linear growth can be improved with L-T₃ (13) supplementation, but experience with thyroid hormone administration in these patients is limited.

Other clinical features of SBP2 defects are treated symptomatically at this point.

6. Conclusions

The only known inherited defect of intracellular thyroid hormone metabolism is caused by mutations in the *SBP2* gene affecting selenoprotein synthesis, among which the selenoenzymes 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, myopathy, 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.

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Figure 1.

Summary of thyroid function tests in families A and B. Affected represented as filled squares, unaffected family members as open circles. Grey boxes indicate the normal range for the respective test.

Table 1Reported *SBP2* mutations.

Family	<i>SBP2</i> gene	<i>SBP2</i> protein
A	R540Q homozygous	R540Q homozygous
B	K438X IVS8ds+29 G->A	truncated, missing C-terminus alternative transcripts
C	R128X homozygous	smaller SBP2 isoforms from downstream ATGs
Brazil	120X R770X	smaller SBP2 isoforms from downstream ATGs truncated, disrupted C-terminus
UK 1	c.668delT fs223 255X Intron 6–155 del C	truncation and smaller SBP2 isoforms from downstream ATGs abnormal splicing, truncated, missing C-terminus
UK2	C691R ? (intronic SNPs)	increased proteasomal degradation transcripts lacking exons 2–4 or 3 and 4, smaller SBP2 isoforms from downstream ATGs