

The Syndrome of Inherited Partial SBP2 Deficiency in Humans

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Abstract

Selenium (Se) is an essential trace element required for the biosynthesis of selenoproteins. 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. Intracellular metabolism of thyroid hormone (TH) 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 were not known. Affected children were either homozygous or compound heterozygous for *SBP2* mutations. Other selenoproteins, glutathione peroxidase, and selenoprotein P were also reduced in affected subjects. Since our initial report, another family manifesting a similar phenotype was found to harbor a novel *SBP2* mutation. *In vivo* studies of these subjects have explored the effects of Se and TH supplementation. *In vitro* experiments have provided new insights into the effect of *SBP2* mutations. In this review we discuss the clinical presentation of *SBP2* mutations, their effect on protein function, consequence for selenoproteins, and the clinical course of subjects with *SBP2* defects. *Antioxid. Redox Signal.* 12, 905–920.

Introduction

THYROID HORMONES (TH) are iodinated compounds which, by controlling the expression of specific genes, affect the differentiation, growth, and metabolism of all vertebrates. TH homeostasis is maintained by a feedback system involving the hypothalamus, pituitary, and thyroid glands (Fig. 1A). Thyrotropin releasing hormone (TRH), a tripeptide secreted by the hypothalamus, stimulates the synthesis and secretion of thyroid stimulating hormone (TSH or thyrotropin) by the thyrotrophs, located in the anterior pituitary gland. TSH is a circulating glycoprotein made of two subunits that binds a G-protein coupled receptor expressed in thyrocytes to stimulate TH synthesis and secretion. TSH is also regulated by TH through a negative feedback system. Thus, a high serum TSH concentration is indicative of TH deficiency, while TH excess suppresses TSH. The effects of TH are dependent on the quantity of the active hormone that reaches peripheral tissues, their intracellular availability, and the presence of unaltered TH receptors and cofactors.

Entry of TH into cells is an active process that involves several classes of TH membrane transporters with different kinetics and substrate preferences (33). After entering a cell, the hormone precursor 3,3',5,5'-tetraiodothyronine (thyroxine, T₄) is metabolized by removal of the outer ring iodine (5'-

deiodination) to form the active hormone, 3,3',5-triiodothyronine (liothyronine, T₃). Alternatively, T₄ and T₃ are inactivated by inner ring (5-deiodination) to form 3,3',5'-triiodothyronine (reverse T₃, rT₃) and 3,3'-diiodothyronine (T₂), respectively (6). The deiodinases that activate TH are D1 and D2, while the enzyme that inactivates TH is principally D3 (Fig. 1B). The presence of these enzymes in changing concentrations in various cell types contributes an additional mechanism in regulating the amount of active hormone supplied to the cell (6).

While genetic defects of thyroid gland development, TH synthesis, secretion and action have been identified, until few years ago, inherited defects in TH metabolism were not known (71). Deiodinases are selenoenzymes containing 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)] (26) (Fig. 2). However, the list of factors involved in this mechanism is constantly growing, the most recent members being the ribosomal protein L30 (15), the 43 kDa RNA binding protein (Secp43), and the soluble liver antigen protein (SLA) (3, 66, 97). Sec, the 21st amino acid, is structurally identical to

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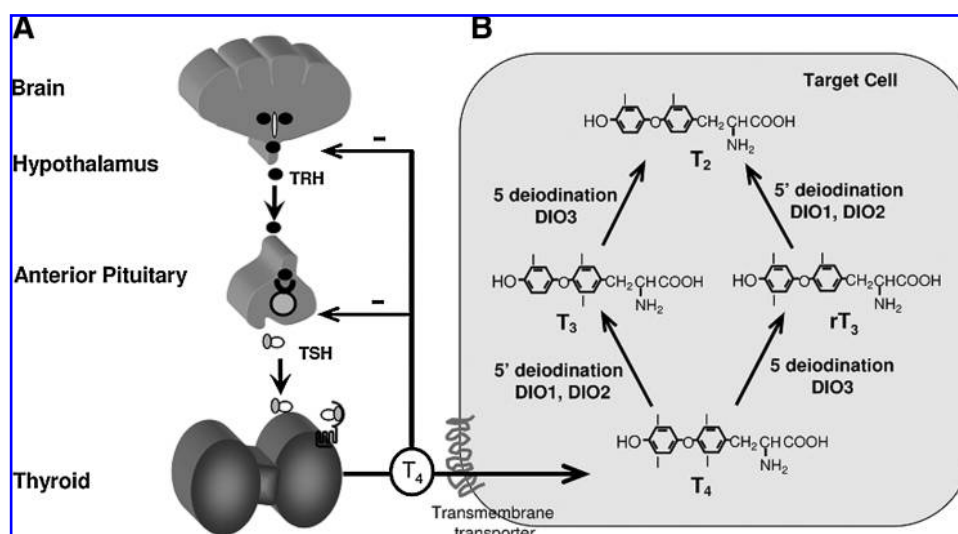


FIG. 1. Central regulation of TH synthesis and TH metabolism. (A) Feedback system maintaining TH homeostasis. TRH secreted by the hypothalamus, stimulates the synthesis and secretion of TSH by the thyrotrophs, located in the anterior pituitary gland. TSH stimulates TH synthesis and secretion by the thyroid gland. TSH is also regulated by TH through a negative feedback system. (B) Activation and metabolism of TH. After active cellular uptake of TH through transmembrane transporters, the precursor 3,3',5,5'-tetraiodothyronine (thyroxine, T₄) is converted into the active 3,3',5-triiodothyronine (T₃) hormone or inactive 3,3',5'-triiodothyronine metabolite (reverse T₃, rT₃). D1 and D2 are the principal enzymes that catalyze 5'-deiodination, converting T₄ to T₃ and rT₃ to 3,3'-diiodothyronine (T₂), while D3 catalyzes 5-deiodination, converting T₄ to rT₃ and T₃ to T₂.

cysteine (Cys), except for the selenium replacing sulfur. Sec has a distinct functional advantage at physiological pH. When Sec is replaced with Cys, the catalytic activity of a selenoenzyme is drastically reduced (38).

Sec is incorporated through recoding of a UGA codon present in the mRNAs of selenoproteins. This is achieved by

the presence of SECIS, a characteristic stem-loop RNA structure in the 3' untranslated region. Using the SECIS element as bait, the rat SECIS binding protein, SBP2, was purified and cloned in 2000 (21).

The human selenoproteome comprises at least 25 individual selenoproteins (52, 66). 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), selenium transport and storage (SePP), and potential protein folding (Sep15, SelN, SelM, SelS). Several approaches have been used to study the role of selenoproteins in rodents (61, 80). These include dietary restriction in Se, the knockout of individual selenoproteins (82), or targeting tRNA^{Sec} (7, 11, 12, 53). That some selenoproteins must have a crucial function is supported by the observation that removal of the tRNA^{Sec} gene is lethal to the embryo (7). The efficiency of Se retention during exposure to Se-depleted diets is different among tissues (82). A distinct hierarchy exists in the synthesis of selenoproteins as the expression of individual selenoproteins is differentially affected by the cellular content in Se (12). This may be due to changes in the distribution of the two isoforms of tRNA^{Sec} (12), mRNA degradation by nonsense-mediated decay (94) and preferential SECIS recognition by SBP2 (9, 85). Components of the Sec incorporation machinery other than tRNA^{Sec} have not been targeted in animal models and no Sec insertion defects have been described in humans.

In 2005, we described two families with mutations in the *SECISBP2*, (MIM 607693; also called *SBP2*) gene. The probands presented with growth retardation associated with abnormal thyroid function tests (28), consisting of high serum total and free T₄ concentrations, a modest reduction in serum T₃, high rT₃ and normal or slightly elevated TSH. Because

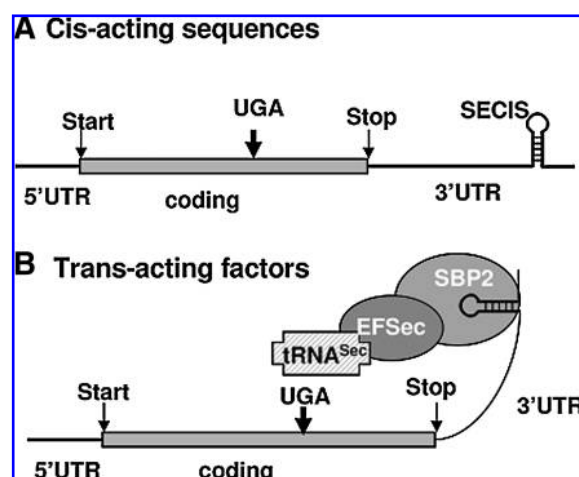


FIG. 2. Some of the important components involved in Sec incorporation. (A) Cis-acting sequences present in the mRNA of selenoproteins: an in frame UGA codon, and Sec incorporation sequence (SECIS) element, a stem loop structure located in the 3'UTR (untranslated region). (B) Binding of the trans-acting factor SECIS-binding protein (SBP2) recruits the Sec-specific elongation factor (EFSec) and Sec-specific tRNA (tRNA^{Sec}), resulting in the recoding of the UGA codon and Sec incorporation.

SBP2 is a major determinant in the incorporation of selenocysteine during selenoprotein synthesis (20, 67), the absence of lethality and mild phenotype were attributed, respectively, to the preservation of partial SBP2 activity and the hierarchy in the synthesis of selenoproteins (12). The latter allows the differential preservation of some selenoproteins in the presence of unfavorable conditions for their synthesis.

Since the initial description of the two families with SBP2 mutations (28), another family with a similar phenotype was reported by our laboratory (25). This family had a new SBP2 gene mutation producing an early stop codon. Another family was identified in England (59) and its full report is in progress. In addition, *in vitro* studies of a rat Sbp2 harboring a mutation corresponding to that identified in family A, and immunoprecipitation studies using the mutant SBP2 R540Q have been performed by Bubenik *et al.* (9) and Squires *et al.* (85), respectively. The effectiveness of Se supplementation was tested in several affected individuals (25, 79) and TH supplementation was tried in two affected children (25, 28). All these studies and observations are the subject of this review.

Clinical Presentation

The probands of three families were brought to clinical attention because of short stature. All three were boys ranging in age from 6 to 14.5 years (25, 28). The proband of a fourth family was a 32-year-old man, and infertility led to thyroid investigation (59). All affected subjects had thyroid function test abnormalities, characterized by high serum T4, low T3, high rT3, and normal or slightly elevated TSH concentrations. This unusual combination of thyroid test results, in otherwise healthy appearing individuals, were the reason for referral for further investigation. None of the subjects had an enlarged thyroid gland confirmed by ultrasound examinations.

Family A is Bedouin from Saudi Arabia. Though the parents were not consanguineous, they belong to the same tribe. Three of the seven siblings of the proband had the characteristic thyroid function test abnormalities (Fig. 3A). The proband, the oldest of the three affected, was the second born. He was 14.5 years old when brought to medical attention because of short stature. Since age 11 he has been growing below the 3rd percentile and bone ages were 7.5 and 9 at

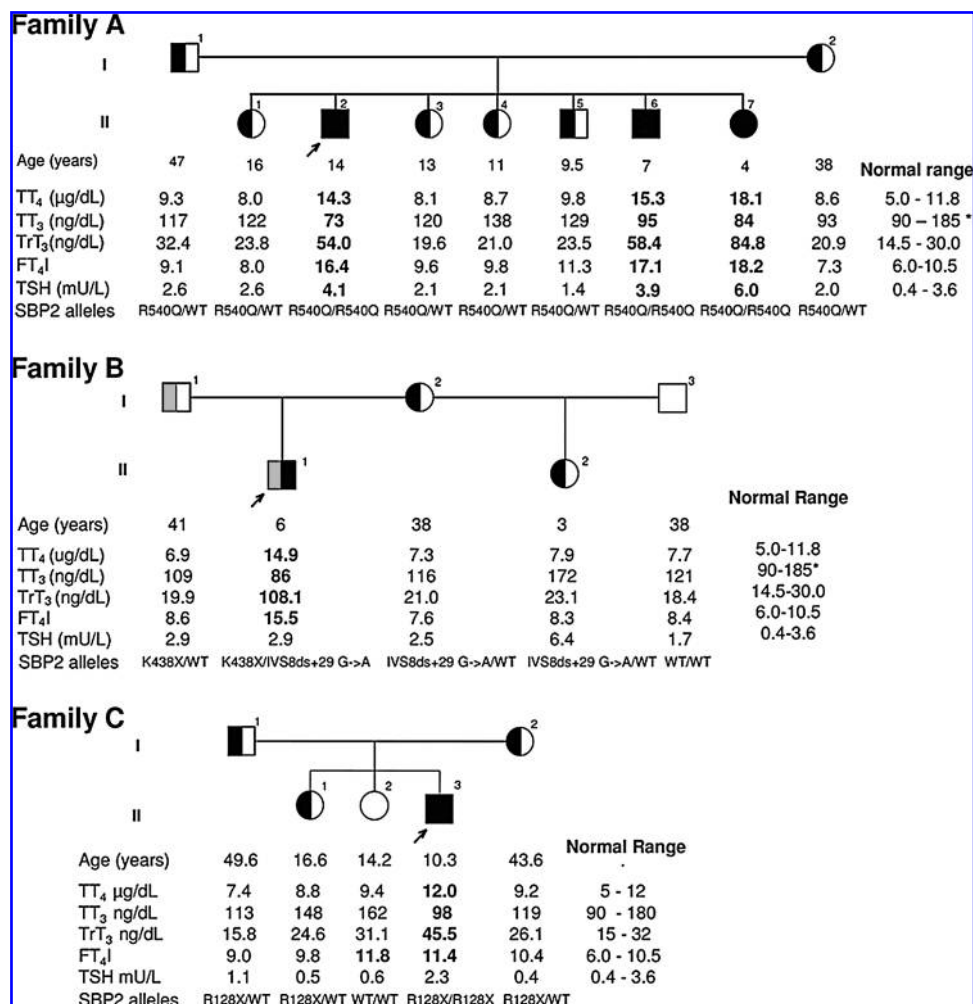


FIG. 3. Pedigrees and thyroid function tests of the three families. (A, B, and C) Values are aligned under each subject symbol. TT₄, total T₄; TT₃, total T₃; TrT₃, total reverse T₃; FT₄I, free T₄ index. Abnormal values are in **bold characters**. Note that the blood sample from the proband of family C was obtained 5 days after interruption of L-T₃ treatment. *The normal range for children younger than 10 years is 100–205 μg/dL. SBP2 alleles present in each individual are indicated.

chronological ages of 11.5 and 14.5, respectively, while still prepubertal (28). Insulin-like growth factor (IGF) 1 and stimulated growth hormone values were within the normal range. He was the product of full-term pregnancy and spontaneous vaginal delivery. Birth weight was 3.4 kg, length 54 cm, and head circumference 25.5 cm (all within the normal range). It is of note that, on the second day of life, because of protruding tongue suggestive of possible hypothyroidism, thyroid tests were obtained. TSH was >60 mU/L (normal <20) but total T4 was high at $21.6 \mu\text{g/dl}$ (normal range 6–14). Treatment with levothyroxine (L-T4) was not given because serum T4 concentration was not low. Growth and development appeared to be normal but at age 2 his height was 75 cm [standard deviation score (SDS) -3.0 ; <1 centile]. Thyroid function tests obtained at ages 2, 11.5, and 14.5 years showed TSH values that ranged from 1.8 to 4.1 mU/L, thus straddling the upper limit of normal of 3.8 mU/L; total T4 ranged from 214 to 245 nmol/L and free T4 29.2 pmol/L, both above the respective upper limits of normal of 141 nmol/L and 19 pmol/L. In contrast, a free T3 determination gave a low result of 0.65 pmol/L (normal range 1.3–2.5). The TSH response to TRH was normal, rising from a baseline level of 3.1 to a peak value of 8.9 mU/L at 30 min following the intravenous administration of TRH. Ultrasound showed that the thyroid gland was of normal size and a pertechnetate scan revealed a homogeneous uptake of 3% (normal range 0.5–4). Audiogram showed no hearing impairment.

At age 13.5 years, the proband was treated for 8 months with $20 \mu\text{g}$ L-T3 daily. During this period of time his height increased from 133 to 133.8 cm and weight from 24.2 to 24.8 kg. Although T3 had no obvious effect on growth, TSH was suppressed to 0.2 mU/L. Six months later, off L-T3, TSH returned to 2.0 mU/L and his height and weight were 136 cm and 26.1 kg, respectively. The concentrations of total T4, free T4, and total rT3 were high, while that of total and free T3 were low. The same pattern of thyroid function tests was found in a 7-year-old brother and 4-year-old sister (Fig. 3A), both clinically euthyroid. However, the height of the affected brother was in the 3rd percentile. Thyroid function tests of the parents and four other siblings were normal and their stature ranged from 10 to 50 centile.

Family B is of mixed ethnic origin, the mother being Irish of European descent and the father Kenyan of African origin. Their single affected child was 6 years old when brought to medical attention by the mother concerned about his growth, because she was buying clothes for a child younger than his age. However, growth retardation was excluded by measurements. The mother reported syncopal episodes of the child after exertion. One episode witnessed in the physician's office, consisted of unresponsiveness, jerky movements of the arms and legs, followed by drowsiness for 5 min. Clinical, biochemical, and electroencephalographic investigations did not detect abnormalities. Thyroid function tests revealed a TSH at 8.1 mU/L (normal range 0.4–4) with a normal alpha subunit and a free T4 by equilibrium dialysis of 45.0 pmol/L (normal range 10–25). The possibility of resistance to thyroid hormone (RTH) was considered due to the combination of high free T4 with nonsuppressed TSH. However, RTH was excluded by sequencing the *thyroid hormone receptor beta* (*TR β*) gene (72). Results of more detailed studies of thyroid function were similar to those of affected members of family A. The child had a maternal half sister. Her thyroid function tests

results and those of the parents were within the range of normal (Fig. 3B).

Family C came to our attention after the report of families A and B (28). The proband, of African origin, was born to non-consanguineous parents from the same town in the eastern region of Ghana. Pregnancy was normal and delivery at term. Birth weight was 3.1 kg and early development was thought to have been normal. It is not clear when his growth slowed down, as there were no records of height measurements, but short stature was noted at age 8 years, 2 months after his family moved to the United Kingdom. He was 8.9 years old when the family sought medical attention regarding his stature. He was proportionately short for age (height 113.5 cm, SDS -3.36 ; centile <1) and for the height of his parents (target height 170.9 cm, SDS -1.06 , target centile 14). His bone age was delayed at 3.1 years (height for bone age SDS $+4.7$). He was slim, weighing 18.5 kg (BMI 14.4 kg.m^2 , weight for height 93%) whilst his head circumference was 51.0 cm (centile 2.4). No goiter or other somatic abnormalities were found and he was clinically euthyroid (25). Thyroid function tests showed high total T4, free T4 index (FT4I), and rT3 with low total T3 and normal TSH (Fig. 3C). All members of the immediate family were tested. They were of normal stature (10 to 80 centile) and tests of thyroid function were within normal range, except for the slight elevation of FT4I in one of the sisters (Subject II-2, Fig. 3C). Additional studies in the proband showed normal plasma IGF1 and IGF binding protein 3 at 11.1 nmol/L and 2.2 mg/L, respectively. Peak growth hormone response to insulin induced hypoglycemia was 11.2 mU/L and cortisol 668 nmol/L (25). Blood selenium was low but assessment for malabsorption, including upper and lower gastrointestinal endoscopy and biopsy, were negative. Immunoglobulins, CD 3:4:8, and inflammatory markers were negative. Karyotype was 46XY and normal. Electrocardiography and echocardiography showed no evidence of cardiomyopathy (25).

Initial *In Vivo* and *In Vitro* Studies

Although the clinical presentation and abnormalities of thyroid function were not typical of resistance to TH (72) or defects in serum (71) or cell membrane TH transport defects (29, 32), these were excluded by further testing (isoelectric focusing, measurement of thyroxine binding globulin and transthyretin) and sequencing of the corresponding genes. The relative ability of L-T4, compared to L-T3, to suppress TSH was used to test the hypothesis of a defect in iodothyronine metabolism. The *in vivo* and *in vitro* studies were done in members of family A, as it was the largest family available, with three affected and four unaffected siblings. When incremental doses of L-T4 were given, higher doses [up to $4.4 \mu\text{g/kg}$ body weight (BW)] were required to reduce the TSH level in the two affected children as compared to $2.2 \mu\text{g/kg}$ BW to have the same effect on the two normal siblings. These doses achieved on average 1.8-fold higher T4 levels in the affected as compared to the unaffected siblings (Fig. 4A). In contrast, similar doses of L-T3, on average $2.48 \mu\text{g/kg}$ BW compared to $2.46 \mu\text{g/kg}$ BW, used in the affected and unaffected siblings, respectively, had equal suppressive effects on TSH (Fig. 4B). These results of apparent resistance to L-T4 but not L-T3, suggest a defect in T4 to T3 conversion. However, linkage of the phenotype to loci of the

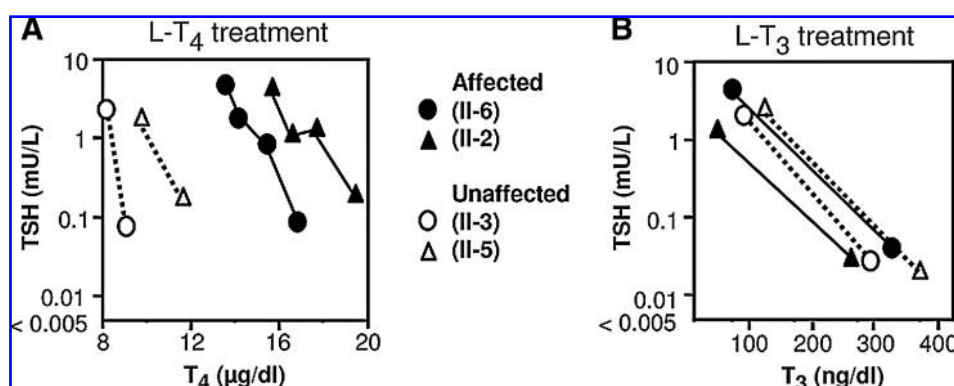


FIG. 4. Evidence for abnormal TH metabolism. *In vivo* studies in members of family A. Serum TSH and corresponding serum T4 and T3 levels, before and during the oral administration of incremental doses of L-T4 (A) and L-T3 (B). Note that higher concentrations of T4, but not T3, are required to reduce serum TSH in the affected subjects. Affected and normal individuals are identified in the legend and described in detail in Fig. 3A.

three deiodinases was excluded and their coding sequences were normal (28).

We used cultured skin fibroblasts from members of family A to test *in vitro* the hypothesis of abnormal T4 to T3 conversion. As D1 and D3 are not expressed in these cells, the expression and function of D2, an integral membrane ER-

resident selenoenzyme (2) was measured. The synthetic analogue dibutyrylcAMP (db-cAMP) was used to stimulate D2 expression and activity, as the *DIO2* gene contains a cAMP response element. Baseline D2 activity in fibroblasts from the affected subjects was reduced to the limit of detection (Fig. 5A). In fibroblasts from normal individuals, D2 mRNA and

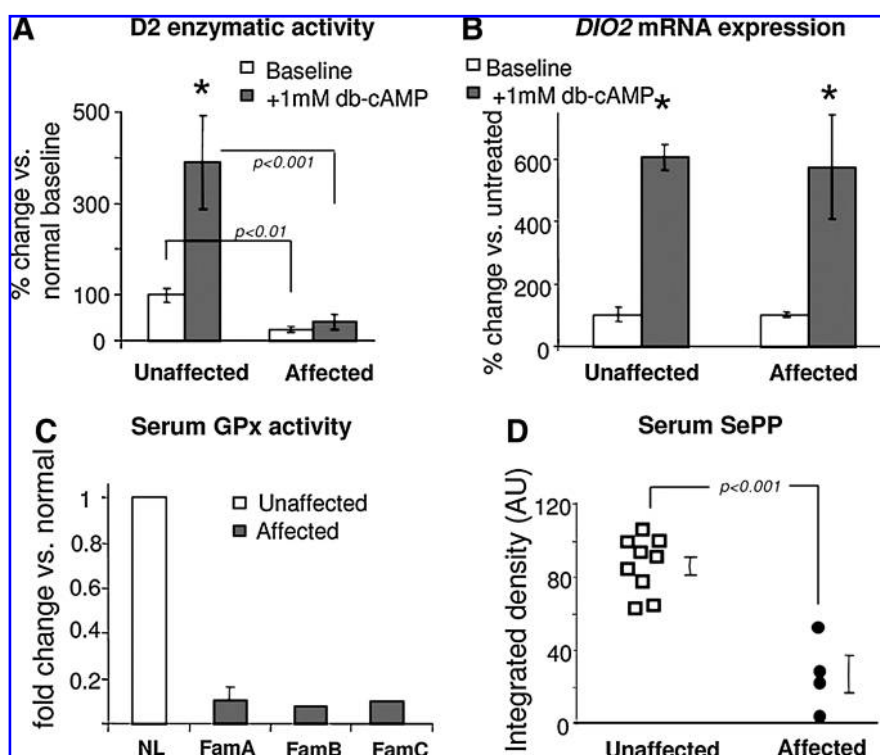


FIG. 5. *In vitro* studies. (A) Deiodinase 2 enzymatic activity and (B) *DIO2* mRNA expression in cultured fibroblasts from two affected children and four unaffected individuals from family A. Bars indicate \pm SEM. Baseline and stimulated D2 activity is significantly lower in affected. There is significant increase of *DIO2* mRNA with db-cAMP, in both normals and affected ($*p < 0.001$). There are no significant differences in baseline and db-cAMP-stimulated *DIO2* mRNA in affected versus the unaffected. (C and D) Effect of SBP2 deficiency on selenoproteins other than deiodinases. (C) Glutathione peroxidase (GPx) enzymatic activity in serum from all five affected children and nine unaffected individuals (NL) from families A, B, and C. Results are expressed as fold change compared to unaffected subjects. Bars indicate mean \pm SD. (D) SePP levels in serum from four affected and available unaffected members of families A and B were quantified by scanning the Western blot (AU, arbitrary units).

enzymatic activity increased with dbcAMP stimulation, whereas in the affected subjects, the increase in D2 mRNA was not accompanied by a corresponding increase in enzymatic activity (Fig. 5A and B). These results support the clinical findings of defective TH metabolism and provided direct evidence for abnormal D2 function. Furthermore, the reduction in D2 enzymatic activity without corresponding change in mRNA levels suggested that the defect may be post-transcriptional.

Identification of *SBP2* Gene Mutations

Based on the results of *in vitro* studies in fibroblasts and absence of co-segregation between phenotype and haplotypes of all three iodothyronine deiodinases, a post-transcriptional defect involving D2 metabolism or synthesis was considered as possibly responsible for the reduced enzymatic activity (28). Supply of active D2 enzyme is regulated by degradation in proteosomes through ubiquitination (8) and by de-ubiquitination (23). Linkage studies were carried out in family A having three affected and four unaffected siblings. There was absence of co-segregation for genes encoding proteins involved in D2 ubiquitination (*UbE2G1*, *UbE2G2*, *UbE2L3*) (8) and in D2 de-ubiquitination (*VDU1*, *VDU2*) (23). Defects in some components of the Sec incorporation machinery (*cis* sequences, tRNA^{Sec} and EFSec) (26) were also excluded by co-segregation analysis or by sequencing. However, affected subjects shared homozygous haplotypes at the *SBP2* locus (Fig. 6). Sequencing revealed a missense mutation in exon 12, a G to A transition in a CpG dinucleotide that changes the wild-type (WT) codon CGG to CAG. This results in the replacement of the normal arginine-540 with a glycine (R540Q) (28). Affected children were homozygous and the parents and the four unaffected siblings were heterozygous carriers (Fig. 3A). The haplotype harboring the mutation is most likely inherited identical by descent in this Bedouin family. Extensive genotyping with markers flanking the locus showed a 16–18cM segment shared by the parents, suggestive of a recent common ancestor.

Two different *SBP2* gene mutations were found in family B. The affected child was compound heterozygous. He inherited from his father an A to T transversion that changes the WT codon AAA with TAA, a nonsense mutation replacing the normal lysine-438 with a stop (K438X). From the mother he

inherited an intronic mutation (IVS8ds+29 G->A) which, by altering the donor splice site, produces an alternative transcript that incorporates 26bp into exon 8 (Fig. 3B) (28). The abnormally spliced transcripts represented 52% of the transcripts generated from the mutant maternal allele in lymphocytes and change the reading frame to produce a putative truncated protein (28). The total amount of normal transcripts in the affected child was estimated to be 24%.

For family C, an *SBP2* defect was suspected based on the thyroid function tests abnormalities, growth delay, and the detection of a low serum Se concentration. Sequencing of the *SBP2* gene revealed a novel mutation, a C to T transition in codon 128, located in exon 3, that results in the replacement of the normal arginine (CGA) with a stop (TGA), R128X (25). This early stop predicts an *SBP2* defect in the proband and is in agreement with the observed thyroid phenotype. Both parents were heterozygous for the same mutation as was one of his sisters (subject II-1) but not the other (Subject II-2, Fig. 3C) (25).

Population screens showed that these *SBP2* gene sequence differences were not polymorphic in the respective populations.

Consequences for Other Selenoproteins

As *SBP2* is epistatic to selenoprotein synthesis, identification of decreased D2 activity due to recessive *SBP2* defect prompted us to investigate whether other selenoproteins were also affected. As D1 and D3 are too low to measure or absent in skin fibroblasts and lymphocytes, other selenoproteins were assessed (25, 28). Compared to that of normal individuals, serum GPx activity (GPx3) was 9.9-fold lower in the three (mean value) affected subjects of family A, 7.7-fold lower in the affected of family B (28), and 9.2-fold in that of family C (25) (Fig. 5C). GPx1 mRNA expression and activity in fibroblasts of the affected subjects from family A was 7.2-fold and 3.3-fold lower, respectively, compared to normal subjects. No fibroblasts from individuals of families B and C were available. Furthermore, selenoprotein P (SePP) serum levels measured in members of families A and B were significantly lower in the affected compared with unaffected individuals (Fig. 5D).

SBP2 is expressed at low levels in all tissues tested, with a high expression and an additional smaller transcript in testis (55). The human *SBP2* gene, cloned in 2002 (55), has 854 amino acids. The C-terminal domain of the protein is required for SEC IS binding, ribosome binding, and Sec incorporation (20). 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 (67) and these motifs play a role in the function of *SBP2* in the nucleus, *in vivo*. Oxidative stress induces nuclear accumulation of *SBP2* via oxidation of cysteine residues within a redox-sensitive cysteine-rich domain. Sec incorporation is reduced substantially after treatment of cells with agents that cause oxidative stress, suggesting that nuclear sequestration of *SBP2* under such conditions may represent a mechanism to regulate the expression of selenoproteins (67).

There appears to be a hierarchical preservation of selenoproteins during Se deprivation, conserving the enzymatic

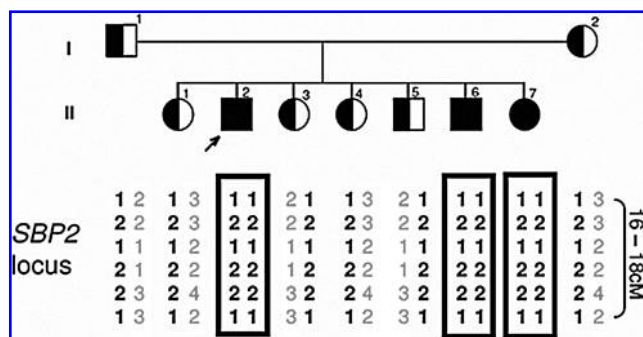


FIG. 6. Identification of a *SBP2* gene mutation; Haplotyping of family A with genetic markers overlapping the *SBP2* locus. Affected share homozygous haplotypes while the unaffected parents and siblings are heterozygous.

activity of selected selenoproteins (20, 50). This hierarchy is supposedly produced by the rates of selenoprotein degradation and by the functional demands of particular selenoproteins. More recently, selective binding of SBP2 to different SECIS elements was demonstrated *in vitro*, thus further contributing to selenoprotein hierarchy (9,85). Therefore, SBP2 defects manifest as deficiencies in the synthesis of selective selenoproteins.

Effect of the SBP2 Gene Mutations on Protein Function

Given the expected devastating effect from complete impairment of selenoprotein synthesis (7), we hypothesized that the mild phenotype of the affected individuals of these families, limited to growth retardation, possible infertility, and partial defect in thyroid hormone metabolism, must be due to preservation of sufficient SBP2 function in the affected subjects. Indeed, the mutation IVS8ds+29 G->A of family B results in partial alternative splicing and abnormal transcripts. The total amount of normal transcripts in the affected child was estimated to be 24%, resulting in a partial, rather than a complete SBP2 defect, thus predicting a partial deficiency of selenoprotein synthesis and a mild phenotype (28). An experiment that mimics this situation was performed by Squires *et al.* (85) using knockdowns of SBP2 expression in cell lines to 25%–30% of normal. These studies assessed the effects on selenoprotein mRNA levels and quantified the *in vivo* binding of selenoprotein mRNAs by SBP2 via immunoprecipitation. SBP2 exhibited strong preferential binding to some selenoprotein mRNAs over others, and SBP2 was a major determinant in dictating the hierarchy of selenoprotein synthesis via differential selenoprotein mRNA translation and sensitivity to nonsense-mediated decay. A more recent study also demonstrates loss of telomeric reserve when SBP2 expression is attenuated (84).

The homozygous mutation R540Q in family A is a non-synonymous change located in a conserved amino acid across species (9) and likely creates a hypomorphic rather than a null allele. This mutation is located in the RNA-binding domain of SBP2 and suggests that the mutant protein could be defective in SECIS binding. Bubenik *et al.* (9) examined its ability to bind to various SECIS elements using the rat Sbp2 R531Q protein. This amino acid substitution is equivalent to the same change in humans located at position 540. In agreement with our initial findings of defects in GPx1 and D2 activity in subjects homozygous for SBP2 R540Q, RNA electrophoretic mobility shift assays (REMSA), using the rat mutant Sbp2 R531Q, showed no binding to the SECIS elements of GPx1 and Dio2 (9) (Fig. 7A), while the WT protein had normal binding. In addition, the R531Q mutant did not bind the SECIS element of Dio1 (9), which also catalyzes 5' deiodination as D2 does (49) and this likely contributes to the overall thyroid phenotype seen in SBP2 defects.

Because SBP2 is required for selenoprotein synthesis, the expectation would be that a SBP2 defect would have a generalized effect on all selenoproteins. However, given the mild phenotype of the affected individuals, the hypothesis was that the expression of other selenoproteins might be in part preserved in these individuals. In particular, phospholipid hydroperoxide glutathione peroxidase (PhGPx) and thioredoxin reductase (TR1) are considered essential selenoproteins, be-

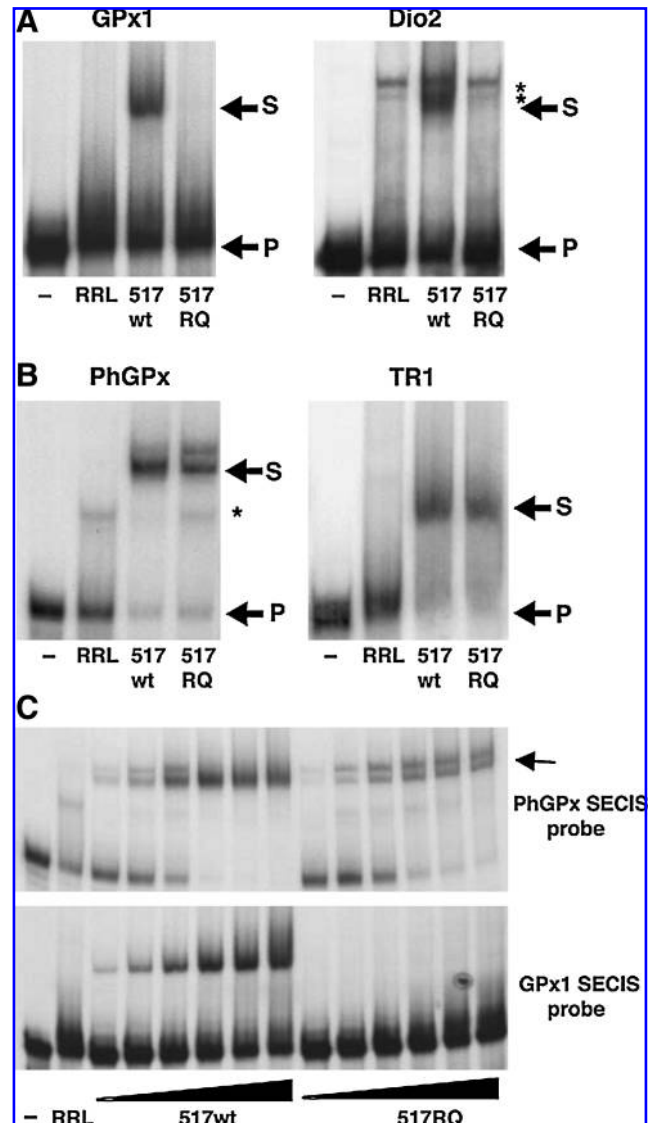


FIG. 7. *In vitro* studies using the rat Sbp2 R531Q, the equivalent mutation to the human SBP2 R540Q mutation identified in family A. Rat Sbp2 R531Q results in selective perturbations in SECIS interactions. (A) REMSA assay with *in vitro* translated rat Sbp2[517–777] or rat Sbp2[517–777,R531Q] using rat GPx1 and human Dio2 SECIS elements as probes. P, unbound probe; S, shift caused by the functional SBP2/SECIS interaction; *shift caused by interaction with endogenous protein in the lysate. (B) REMSA assay as above, using rat PhGPx and TR1 SECIS as probe. (C) REMSA with increasing concentrations of rat Sbp2[517–777] or Sbp2[517–777,R531Q], corresponding to a 24-fold range, with probes as indicated; wt, wild type. Arrow indicates the functional band, the lower band of the two. [Figure reproduced from Bubenik *et al.* (9) JBC 282: 34658, 2007, with permission from the authors and publisher].

cause knock-out of these proteins are lethal to mice in the embryonic stage (45, 98). Thus, the loss of expression of these proteins in humans is expected to have more severe consequences. When the SECIS elements from these two selenoprotein mRNAs were used as probes in REMSA assays (9), the R531 Q Sbp2 mutant was able to bind both the PhGPx SECIS

and TR1 SECIS. However, the R531Q Sbp2 mutant was not capable of binding the PhGPx SECIS as well as the WT Sbp2 (Fig. 7B), suggesting that the R531Q mutant has a weaker RNA binding affinity than the WT. This is further demonstrated using increasing amounts of protein in REMSA assay. When the SECIS element of PhGPx was used as probe (Fig. 7C, upper gel), increasing the concentrations of WT protein resulted in an increase of the intensity of the lower functional band and the loss of the upper band. Although the R531Q protein is able to interact with the PhGPx SECIS, the binding does not exactly recapitulate that of the WT protein because the doublet remains, even at higher concentrations. If GPx1 SECIS is used as probe (Fig. 7C, lower gel), only the binding of the WT protein is detected. Increasing the amount of R531Q protein does not result in complex formation. Similarly, using selenocysteine insertion assays, Bubenick *et al.* (9) demonstrated that the R531Q mutation further decreases the affinity of SBP2 for a subset of SECIS elements, implying that in the affected children of family A harboring the equivalent mutation, this additional decrease in affinity results in the loss of a subset of selenoproteins (9).

Independently, Squires *et al.* (85) also tested the R540 SBP2 mutation identified in family A for its ability to immunoprecipitate selenoprotein mRNAs. The R540Q mutant exhibited significant decrease in RNA binding relative to wild-type SBP2 for most selenoproteins tested. In particular, Sel15 and GPx4 mRNAs showed the highest crossing point changes, in the range of ~ 4, equivalent to 8-fold lower binding affinity for the mutant SBP2, while the changes for most selenoprotein mRNAs were lower, in the range of 0 to 2. Surprisingly, the mRNA level of a selenoprotein with a noncanonical SECIS element, SelO, exhibited a greater increase in crossing point (*i.e.*, increased binding) with the R540Q mutant than in the wild-type SBP2 immunoprecipitation.

In support of these observations, a recent study by Takeuchi *et al.* (86) identified in human SBP2 a lysine (K) rich domain between K516 and K544, essential for SECIS and 60S ribosomal subunit binding. This domain contains the R540 amino acid which is conserved across species. Using deletion and alanine scanning mutagenesis, the amino acid sequence 526–540 was demonstrated to be essential for binding form 1 SECIS RNAs but its mutation did not affect form 2 SECIS recognition (86). Altogether these results showed that the R540Q mutation alters the binding specificity of SBP2. Because SBP2 is a limiting factor in cells (56), the relative ability of the mutant SBP2 to bind with relatively higher affinity to some SECIS elements than others would determine which members of the selenoprotein family are expressed, thus creating an imbalance in the relative amounts of selenoproteins synthesized even in selenium-adequate conditions.

The proband of family C was homozygous for a nonsense gene mutation that produces an early stop codon (R128X). Both parents and a sister were heterozygous but showed no growth or thyroid test abnormalities (25). Despite the severity of the defect, the patient had a relatively mild phenotype, similar to that associated with partial SBP2 deficiency demonstrated in families A and B. The recent finding that SBP2 has several mRNA and protein isoforms (68) prompted us to explore the possibility that the mutation produced alternative splicing and/or the synthesis of a partial SBP2 molecule with possible functional activity that proceeded normally (25).

Exon 3, where the mutation R128X is located, is subject to alternative splicing, resulting in three different SBP2 isoforms: a) the intact isoforms containing the entire exon 3 (intact Ex3); b) an isoform lacking 121 bp of the 5'-segment of exon 3 (partial Ex3); c) an isoform lacking the entire exon 3 (absent Ex3) (68). Using quantitative PCR (qPCR) and primers sequences crossing specific exons, we tested for the relative proportion of the three isoforms of SBP2 mRNA described above in the RNA extracted from leukocytes of all family members (25). In order to quantify all three isoforms in combination, qPCR of exon 4 to 5 was also performed. Compared to the sister expressing only the WT alleles (100%), the expression of the intact Ex3, partial Ex3, absent Ex3, and exons 4 to 5 in the heterozygote parents were 74, 89, 62, and 76%, respectively, while the homozygote proband expressed 67, 79, 64, and 69% (25).

The abundance in mRNA harboring the mutant and WT sequence were quantified using cDNA derived from leukocytes of the heterozygote parents (25). The mean ratios of mutant relative to WT sequences were: 0.64/1 for intact Ex3 isoform, 0.62/1 for partial Ex3 isoform, and 0.60/1 for all isoforms combined. This suggests a modest allele-preferential expression due to nonsense-mediated decay in heterozygote subjects (13).

To further characterize the consequence of the SBP2 mutation R128X on protein synthesis, the transcription and translation of the mutant R128X and WT SBP2 were analyzed using minigenes as previously described (68). Two colonies of plasmids containing the minigene with the mutation (R128X) were studied along with two controls, the minigene that produces a stop at the nearby codon 126 (K126X) and the WT (68). As shown in Fig. 8, minigenes with stop codons at 126 and 128 lacked the full length protein (25). However, they synthesized the smaller fragments from downstream AUGs at codons 139, 233, and 300, also produced by the WT minigene (25). Minigenes K126X and R128X synthesized 2- to 3-fold more SBP2 molecules from AUG 139 than the WT and those from AUG 233 were 2-fold more abundant in R128X but not K126X (25). From these data it can be concluded that although mutant SBP2 mRNA in leukocytes may undergo some nonsense mediated decay, SBP2 isoforms ATG 130 and ATG 233 showed 2–3-fold increase in truncated R128X minigene, indicating that a compensatory effect might exist (25). These smaller molecules contain the functional domains, including those required for selenocysteine insertion and RNA binding and ribosome interaction (residues present in exons 12 through 16) (22).

Selenium Supplementation and TH 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 TH, were tested.

The phenotype produced by SBP2 gene defects manifests as growth delay and abnormal thyroid function tests caused by reduced synthesis of selenocysteine-containing enzymes. As the affected individuals had decreased serum Se compared to unaffected siblings, we considered Se supplementation as a means to improve the overall Se availability and possibly also selenoprotein synthesis. Two different studies were done, one

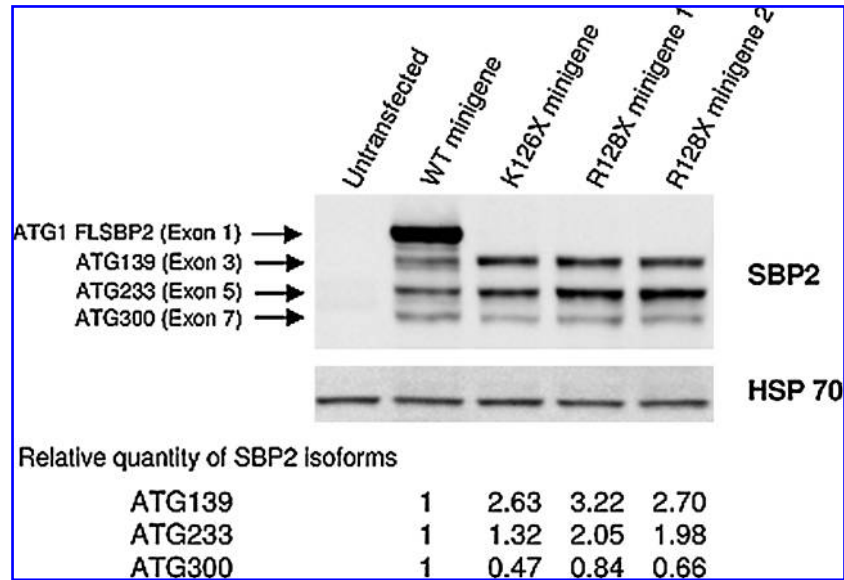


FIG. 8. Western blot of SBP2 synthesized by minigenes expressing the mutation identified in family C. Minigenes constructs, identified above each lane, were transiently transfected into HEK293T cells and products were electrophoresed, blotted, and developed with antibodies to SBP2. Bands generated from different ATGs are identified as described previously (68). Their relative proportion was quantified by density measurement using Image J and expressed relative to the corresponding band generated by the WT full length (FL) minigene and corrected for HSP 70; HSP, heat shock protein.

in the children from family A (two unaffected siblings serving as controls) (79), and another in the affected child of family C.

It is known from clinical studies that a high Se status confers reduced health risks and protects against some chronic diseases (70). Therefore, improving the Se status might still be of benefit to the affected individuals. Two different forms of Se supplementation were used, Se-rich yeast and selenite. Selenomethionine is the main compound in Se-rich yeast that can be incorporated nonspecifically into all circulating serum proteins (10), whereas selenite is metabolized and inserted as selenocysteine into the growing peptide chain of selenoproteins (96), therefore resulting in different Se bioavailability.

The three affected subjects of family A II-2, II-6, and II-7, ages 18, 11, and 9, respectively, and normal subjects II-3 and II-5, ages 17 and 14, respectively, were given Se supplementation (79). Initially, three incremental doses of Se-rich yeast (100, 200, and 400 μ g) were given daily for one month. After a wash-out period of 3 months, 400 rig sodium selenite was given daily for another month. As shown in Fig. 9A, all subjects, irrespective of genotype, responded to treatment by increase in total serum Se concentrations after Se-rich yeast supplementation. Therefore, not only the normal siblings but also the SBP2 deficient children metabolized this Se form successfully. This positive supplementation effect was in contrast to the effect of selenite, as the affected subjects failed to show similarly strong supplementation success as the normal siblings, and displayed only slight increase in SePP concentrations (Fig. 9C) without a positive effect on circulating GPx3 activity (79) (Fig. 9B).

These studies indicate that Se-rich yeast might be the compound of choice, because it readily increased the Se concentrations in serum, and this might serve as an important Se reservoir during times of need, such as in severe illness. However, the thyroid function test abnormalities, including elevated T4 and rT3 and low T3 concentrations, persisted in

the SBP2-deficient individuals with both Se preparations and at all doses (79). This study showed that the SBP2-deficient individuals do not profit from surplus Se supplementation as judged by those parameters that are readily measurable and directly related to the impairment (79). It is of note that the subjects were not residing in a Se-deplete area since the unaffected siblings displayed normal circulating Se levels.

When the proband of the family C was evaluated for short stature, the only positive findings were a low serum Se and abnormal thyroid function tests consisting of high FT4, low FT3, and normal TSH. Based on these results, a defect in the conversion of T4 to T3 caused by deficiency in the Se-containing deiodinases was suspected. Thus, selenium replacement at a dose of 50 μ g/day in the form of selenite (for 6 months) and later selenomethionine (for 4 months) was given. Despite this Se supplementation regimen, no changes in serum Se, thyroid function tests, or growth were observed during the initial 4 months of supplementation with selenite only, before the TH supplementation was started (Fig. 10). Of note is that this lower dose of Se was insufficient to increase serum Se levels (Fig. 10).

Since Se supplementation failed to correct the abnormal thyroid function tests or to improve growth (Fig. 11), the pediatrician initiated treatment with L-T3. The hormone was first given in a dose of 20 μ g daily as divided doses and later, because of TSH suppression, the dose was reduced to 10 μ g/day (Fig.10). During this period, in addition to an increase in height velocity (Fig. 11), there was advancement in bone age with narrowing of the gap between chronological and bone age (Table 1) (25). Although treatment with 20 μ g of L-T3 reduced the serum FT4 levels and suppressed TSH below the lower limit of normal, no alterations in cardiac parameters were observed and the serum cholesterol concentration did not change. Discontinuation of L-T3 administration for only 5 days resulted in a return of thyroid tests to

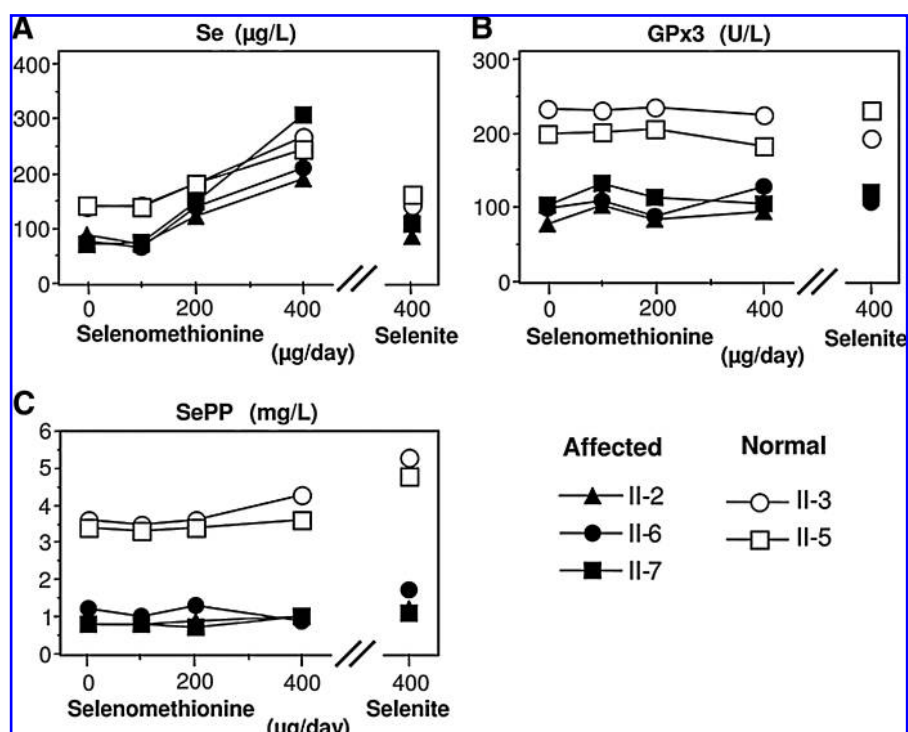


FIG. 9. Effects of selenium (Se) supplementation in the form of Se-rich yeast (primarily selenomethionine) and selenite for SBP2-deficient individuals of family A and their normal siblings. (A) Serum Se concentration; (B) glutathione peroxidase (GPx) 3 enzymatic activity in serum; (C) serum selenoprotein P (SePP) concentration. Affected and normal individuals are identified in the legend and described in detail in Fig. 3A.

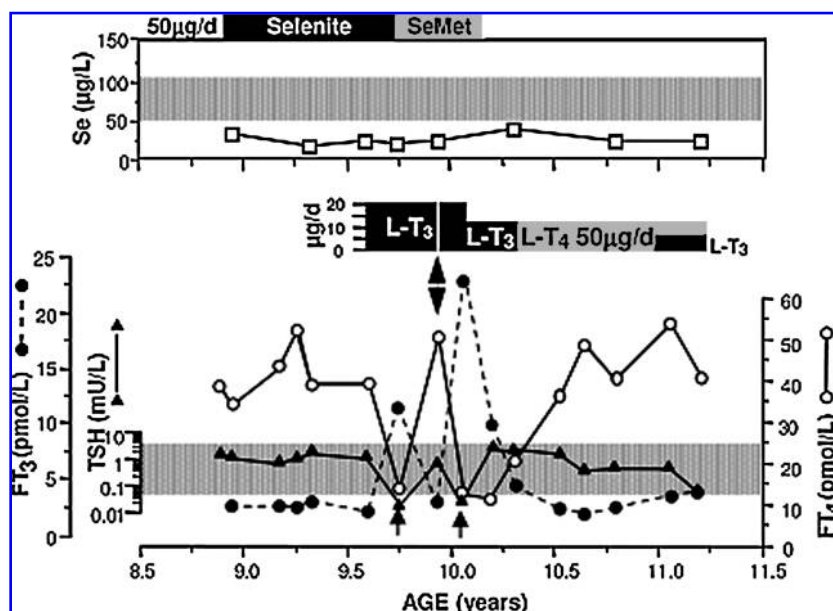


FIG. 10. Clinical course of the proband of family C (subject II-3, Fig. 3C). Note the low serum Se concentration that did not respond to Se treatment in the form of selenite or selenomethionine (SeM). Normal range is for children and adults living in England and Wales as provided by the Trace Elements Laboratory Department of Clinical Biochemistry, City Hospital, Birmingham, UK. The thyroid function test abnormalities (high serum T4 and low T3 concentrations in the presence of a normal serum TSH level), characteristic of SBP2 defects, were persistent during the initial 7-month period of observation. Treatment with 20 µg L-T3, reduced the serum T4 levels but also suppressed the serum TSH concentration (arrows). Variations in serum FT3 concentrations are due to different interval of time between blood sampling and the last dose of L-T3. The double head arrow indicates cessation of L-T3 treatment for 5 days which promptly returned the baseline thyroid test abnormalities.

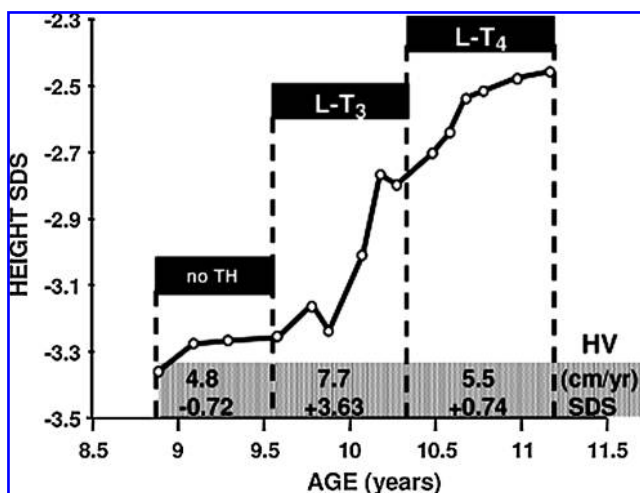


FIG. 11. Effect of thyroid hormone treatment on linear growth of the proband of family C (subject II-3, Fig. 3C). Height is expressed as standard deviation score in the ordinate and as a function of chronological age (31). Height velocities (HV) are changes in height over a >6-month interval and expressed in cm/year (87) and as SDS. In this plot, they are averaged for the three periods defined by the treatment given.

near pre-treatment values (Fig. 10). Treatment with 50 μ g/day of L-T4 brought the serum T4 levels to baseline values and slowed down the increase in height velocity (Fig. 11), though the gap between chronological and bone age continued to narrow (Table 1). Finally, L-T3 at a lower dose was reinstated but L-T4 was continued in order to maintain the basal serum T4 level (25).

It is of note that, in contrast to the results described above, 20 μ g of L-T3 daily given to the proband of family A, for 8 months, did not increase his growth rate. This treatment was initiated at the age of 13.5 years. Evidence for compliance is the reduction of his TSH from a baseline of 1.8 to 0.19 mU/L.

Clinical Course of Subjects with SBP2 Defects

The long-term clinical evolution of partial SBP2 defects, as those described in this review, remains largely unknown. Postnatal growth retardation appears to be common. Yet the magnitude varies, as well as the age of onset and of catch up growth. Heights of the probands for each of the three families (A, B, and C), at chronological ages of 11.5, 6, and 8.9 years, were in the 1st, 97th, and <1st centile, respectively, with bone ages of 7.5, 6, and 3.1 years. Of note is that the proband of

family B reached a normal stature by the age of 6 years, when the first accurate measurement became available. Longitudinal measurements available for members of family A showed that the height of three affected individuals was from <1 to 3rd centile, resulting in adult heights from 1 to 10th centile. This contrasts with that of the unaffected four siblings ranging from the 3rd to 25th centile during growth and reaching adult heights in the 10th to 50th centiles. Thus, in terms of linear and bone growth during childhood, the impairment was most severe in the subject homozygous for an early stop codon in the SBP2 gene from family C, followed by the proband of family A with the hypomorphic missense mutation. The least and transient effect on growth was observed in the child from family B, expressing 24% of the WT SBP2.

Information about the clinical progress during adulthood is currently limited to one case, that of the 32-year-old male who presented with azoospermia and bilateral high frequency sensorineural hearing loss (59). In part, this outcome was predicted earlier (25, 28). Infertility was suspected based on the knowledge that selenoprotein P is required for the development of sperm (12, 63) and the dual role of PhGPx in sperm maturation (89). PhGPx exists as a soluble peroxidase in spermatids and as structural protein, enzymatically inactive in mature spermatozoa, representing at least 50% of the capsule material that embeds the helix of mitochondria. Selenium deficiency was found to cause mechanical instability of the mitochondrial midpiece (95). With regard to hearing impairment, D2-deficient mice were shown to have defective auditory function, retarded differentiation of the cochlear inner sulcus and sensory epithelium, and deformity of the tectorial membrane (62), an auditory phenotype similar to that caused by systemic hypothyroidism or TH receptor deletions (30). However, affected members of family A had normal audiograms, in agreement with their demonstrated partial D2 deficiency.

Similarly, it can be hypothesized that, due to impaired antioxidative protection, affected subjects may be prone to the development of malignancies (18). Recent studies by Squires *et al.* (84) have shown that attenuated expression of SBP2 using siRNA in MSTO and SY5Y cell lines causes increased frequency of oxidative damage-induced lesions in the telomeric DNA, without affecting telomerase, implying that selenoproteins may help protect telomeric reserve in mammalian cells. Thus, 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.

Unfortunately, results of early treatment experience with Se and TH are insufficient in predicting whether these agents could be favorable to the long-term outcome.

Other TH Metabolism Defects in Humans and Mice

Until recently, only the acquired forms of TH metabolism defects in humans were known to exist. Frequently encountered is the "low T3" syndrome in nonthyroidal illness that is the most common thyroid function test abnormality in patients with acute illness (88). Low serum T3 can be detected within 2 h after the onset of severe physical stress. As the severity of the illness progresses, there is a gradual development of a more complex syndrome associated with low levels

TABLE 1. BONE AGE AND LINEAR GROWTH IN PROBAND OF FAMILY C

Chronological Age (years)	Bone age ¹ (years)	Difference ² years	Height SDS ³	Treatment (months)
8.9	3.1	5.8	-3.36	None
10.2	5.2	5.0	-2.77	LT3 (7) ⁴
11.1	6.4	4.7	-2.48	L-T4 (9) ⁴

¹Tanner Whitehouse (TW20) (87); ²Chronological minus bone age; ³Standard deviation scores; ⁴Months of treatment in parenthesis.

of T3 and T4. Altered TH levels have been reported in starvation (36), acute and chronic medical illnesses (69, 73, 93), bone marrow transplantation (54, 92), surgery (19, 100), trauma (16), and can be seen in any severe systemic illness (58, 83). Decreased 5'-monodeiodination, reducing both the conversion of T4 to T3 and the degradation of rT3, is the principal mechanism responsible for the decrease in circulating T3 and increase in rT3 levels in severe illness (46–48, 99). With more prolonged illness, increased turnover of T3 and T4 and an alteration in TSH secretion play secondary roles. Some inflammatory cytokines such as TNF α , IL1, and IL6 have been recently implicated at both central and peripheral levels. Exogenous administration of TNF α and IL1 in humans and animals replicates the thyroid function tests changes reported in the syndrome (24, 27, 34, 39, 65, 91). Certain pharmacologic agents (dopamine, amiodarone, corticosteroids) may also alter the pattern of thyroid function tests in a similar way (14, 64, 74, 90) and this should be taken in consideration when evaluating patients with nonthyroidal illness. Multiple tests at different time points are recommended.

Another form of acquired abnormality in TH metabolism is that caused by increased D3 content in hemangiomas (44). The phenotype resolves with tumor involution or resection. Several cases in infants (1, 5, 37, 41, 44, 51), and one in an adult (43), have been reported. Hemangiomas are the most common tumors in infancy, with a prevalence of 5%–10% among one-year olds. Paraneoplastic D3 enzymatic activity was also recently demonstrated in a nonvascular tumor, in a 54-year-old man with a large malignant solitary fibrous tumor (75). The phenotype is that of consumptive hypothyroidism, with increased TSH and marked elevation of rT3 in the context of normal T3 and T4. The high level of D3 produced by the tumor inactivates T4 by conversion to rT3 at rates that exceed the synthetic capability of the thyroid gland. D3 activity of the tumor was found to be 3- to 7-fold higher than that of term placenta, the human tissue with the highest D3 activity (42).

Several mouse models of deiodinase deficiencies have been reported. A naturally occurring D1 deficiency was identified in C3H mouse strain (78) caused by a 21-base pair insert in the promoter of Dio1 locus in the C3H strain found to cosegregate with low D1 activity in four other mouse strains (57). The D1 KO (77), D2KO (76), D3KO (40), and combined D1/D2KO (35) mice were generated by homologous recombination targeting of the respective genes. In addition, the C3H/D2KO (17) mouse was generated as well, by breeding D2KO mice in the C3H genetic background.

There is no doubt that the thyroid phenotype in subjects deficient in SBP2 is secondary to the defect in iodothyronine metabolism due to a reduced amount of iodothyronine deiodinases. Yet the serum thyroid abnormalities of high T4, low T3, and high rT3, do not perfectly match any of the mouse models of deiodinase deficiencies (4, 17, 35, 40, 76, 77). All have increased serum T4 and normal T3 concentrations except for the D3KO mouse. The latter has low T4 and T3 concentrations. Serum rT3 is high in the D1 KO (77) and double D1/D2KO (35) and TSH is increased in the D2KO (76), C3H/D2KO (17), and D1/D2KO (35). As supported by our previous findings of reduced but not absent D2 activity in subjects from family A (Fig. 5A), it is possible that the phenotype in humans with SBP2 deficiency represents the combined but partial deficiency in all three deiodinases, not fully reproduced in any of the available mouse models of total but

selective deiodinase deficiencies. The finding of a predominant thyroid phenotype in SBP2 defects highlights the importance of selenoproteins for thyroid feedback regulation.

Conclusions and Speculations

The phenotype produced by *SBP2* gene mutations is characterized by low T3, high T4, and high rT3 concentrations in serum, and variable but transient growth delay without other obvious abnormalities when presenting during childhood. In the families we investigated, the observed phenotype seems to be the consequence of selective reduction in selenoprotein synthesis (25, 28).

SBP2 is believed to be the major determinant of Sec incorporation as its *in vitro* addition increases selenoprotein synthesis by 20-fold, whereas its immunodepletion eliminates Sec incorporation (20). The absence of more prominent and generalized symptoms in the patients described above is due to the partial loss of SBP2 function and selectivity of selenoprotein deficiency.

Growth impairment is greatly variable and a genotype–phenotype correlation is not yet apparent. Similarly, the degree of impairment of thyroid function tests does not show such correlation. Serum concentrations in affected subjects of families A, B, and C were, respectively, for T4 15.9 ± 1.9 (mean \pm SD for the three affected), 14.9, and 14.0 μ g/dl; for T3 84 ± 11 , 86, and 98 ng/dl. Similarly, no quantitative differences were observed in the reduction of the serum GPx3 activity which was 9.9 ± 6.4 (mean \pm SD for the three affected), 7.7, and 9.2-fold below the normal mean in affected subjects of families A, B, and C, respectively. Unfortunately, no other selenoproteins, including deiodinases could be measured in the members of families B and C because of the limited amount of leukocytes and their low level of deiodinase expression. In addition, family C refused to provide skin samples for culture of fibroblasts.

The exact cause of early growth retardation in individuals with *SBP2* gene mutations remains unknown. While the growth retardation observed in Se deficient rodents (60, 81) is associated with a reduction in GH and IGF1 (60), this was not found in two of the affected individuals in which it was measured. Treatment with Se had no effect on the affected child from family C nor had the higher Se doses given to subjects of family A (79). Based on the accelerated growth during L-T3 treatment in the proband of family C, it appears that the growth retardation might be due to reduced thyroid hormone action on peripheral tissues. Yet there was no overall correlation between the magnitude of serum T3 reduction and growth delay. Furthermore, the growth rate of the proband of family A was not affected by L-T3 treatment and some catch-up growth was observed with the passage of time. Affected subjects reached normal adult height, though possibly lower than their genetic growth potential. It is uncertain whether delayed puberty is a characteristic for males with the syndrome or a coincidental occurrence.

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Abbreviations Used

BW = body weight
C3H = mouse strain
Cys = cysteine
db-cAMP = dibutyl-*l*-cAMP
Ds, D1, D2, D3 or Dios = deiodinases 1, 2, 3, protein or gene, respectively
eEF^{Sec} = Sec-specific elongation factor
FTI = free T4 index
GPx = glutathione peroxidases
IGF = insulin-like growth factor
IL1, IL6 = interleukin 1 and 6
KO = knock-out
L-T3 = levo-triiodothyronine
L-T4 = levo-thyroxine
NES = nuclear export signal
NL = normal
NLS = nuclear localization signal
PhGPx = phospholipid hydroperoxide glutathione peroxidase
qPCR = quantitative PCR
REMSA = RNA electrophoretic mobility shift assays
RTH = resistance to thyroid hormone
SBP2 = selenocysteine insertion sequence (SECIS) binding protein 2
SD = standard deviation
SDS = standard deviation score
Se = selenium
Sec = selenocysteine
SECIS = selenocysteine insertion sequence
Secp43 = 43 kDa RNA binding protein
SeM = selenomethionine
SLA = the soluble liver antigen protein
T2 = 3,3'-diiodothyronine
T3 = liothyronine or 3,3',5-triiodothyronine
rT3 = reverse T3 or 3,3',5'-triiodothyronine
T4 = thyroxine or 3,3',5,5'-tetraiodothyronine
TH = thyroid hormone
TNF α = tumor necrosis factor alpha
TR1 = thioredoxin reductases
TRH = thyrotropin releasing hormone
tRNA^{Sec} = Sec-specific tRNA
TSH = thyroid stimulating hormone or thyrotropin
UbE2G1, UbE2G2, UbE2L3 = ubiquitin-conjugating enzyme E2G 1, E2G2, and E2L3 respectively
UTR = untranslated region
VDU1, VDU2 = von Hippel-Lindau protein interacting de-ubiquitinating enzyme 1 and 2
WT = wild-type, normal

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