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FORUM REVIEW ARTICLE

Human Disorders Affecting the Selenocysteine Incorporation Pathway Cause Systemic Selenoprotein Deficiency

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Abstract

Significance: Generalized selenoprotein deficiency has been associated with mutations in *SECISBP2*, *SEPSECS*, and *TRU-TCA1-1*, 3 factors that are crucial for incorporation of the amino acid selenocysteine (Sec) into at least 25 human selenoproteins. *SECISBP2* and *TRU-TCA1-1* defects are characterized by a multisystem phenotype due to deficiencies of antioxidant and tissue-specific selenoproteins, together with abnormal thyroid hormone levels reflecting impaired hormone metabolism by deiodinase selenoenzymes. *SEPSECS* mutations are associated with a predominantly neurological phenotype with progressive cerebello-cerebral atrophy.
Recent Advances: The recent identification of individuals with defects in genes encoding components of the selenocysteine insertion pathway has delineated complex and multisystem disorders, reflecting a lack of selenoproteins in specific tissues, oxidative damage due to lack of oxidoreductase-active selenoproteins and other pathways whose nature is unclear.
Critical Issues: Abnormal thyroid hormone metabolism in patients can be corrected by triiodothyronine (T3) treatment. No specific therapies for other phenotypes (muscular dystrophy, male infertility, hearing loss, neurodegeneration) exist as yet, but their severity often requires supportive medical intervention.
Future Directions: These disorders provide unique insights into the role of selenoproteins in humans. The long-term consequences of reduced cellular antioxidant capacity remain unknown, and future surveillance of patients may reveal time-dependent phenotypes (*e.g.*, neoplasia, aging) or consequences of deficiency of selenoproteins whose function remains to be elucidated. The role of antioxidant therapies requires evaluation. *Antioxid. Redox Signal.* 33, 481–497.

Keywords: SECISBP2, SEPSECS, TRU-TCA1-1, selenium, selenoprotein deficiency, thyroid hormone metabolism

Introduction

SELENIUM (SE) IS AN ESSENTIAL MICRONUTRIENT that is incorporated as the amino acid selenocysteine (Sec) into human selenoproteins, encoded by 25 separate genes. Most selenoproteins function as oxidoreductases, with the Sec residue involved in catalytic activity. Selenoproteins have diverse functions ranging from maintenance of redox potential; regulation of redox-sensitive biochemical pathways; protection of genetic material, proteins, and membranes from oxidative damage; metabolism of thyroid hormones; regulation of gene expression; and control of protein folding (Table 1). However, the function of several selenoproteins is unknown (57).

Biosynthesis of selenoprotein requires a UGA codon within its messenger RNA (mRNA) to be recoded as the amino acid Sec, preventing its recognition as a premature stop, possibly targeting the transcript for nonsense-mediated decay (57, 79). This process is achieved *via* a unique Sec-insertion machinery, comprising cis-acting SElenium-Cysteine Insertion Sequence (SECIS) elements located in the 3′-UTR (untranslated region) of selenoprotein mRNAs and

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Table 1. Selenoproteins

Selenoprotein	*Main known function*	*Subcellular localization*	*Expression pattern*
GPX1	Oxidoreductase protection against oxidative stress	Cytoplasmic	Most tissues
GPX2	Oxidoreductase protection against oxidative stress	Nuclear and cytoplasmic	Gastrointestinal tract, bone marrow, immune system, liver, gallbladder, kidney, and urinary bladder
GPX3	Oxidoreductase protection against oxidative stress	Secreted	Most tissues, high in kidney, thyroid, adipose
GPX4	Oxidoreductase protection against oxidative stress	Nucleus and mitochondria	Most tissues, high in testis, adipose tissue
GPX6	Oxidoreductase protection against oxidative stress	Predicted secreted	Testis, epididymis, olfactory system
TXNRD1	Oxidoreductase protection against oxidative stress	Nuclear and cytoplasmic	Ubiquitous
TXNRD2	Oxidoreductase protection against oxidative stress	Cytoplasmic and mitochondria	Ubiquitous
TXNRD3	Oxidoreductase protection against oxidative stress	Intracellular	Most tissues, high in testis
DIO1	Oxidoreductase thyroid hormone metabolism	Intracellular membrane-associated	Kidney, liver, and thyroid gland
DIO2	Oxidoreductase thyroid hormone metabolism	Intracellular membrane-associated	Low in several tissues, high in thyroid, esophagus, cervix, ectocervix, pituitary, endometrium, and brain
DIO3	Oxidoreductase thyroid hormone metabolism	Intracellular membrane-associated	Several tissues, high in cervix, uterine, placenta, and urinary bladder
MSRB1	Oxidoreductase, Methionine Sulfoxide Reduction	Nuclear and cytoplasmic	Ubiquitous
SELENOF	Oxidoreductase ER-associated protein folding control	Endoplasmic reticulum	Ubiquitous
SELENOH	Unknown Oxidoreductase dna/rna binding motif	Nuclear	Ubiquitous
SELENOI	Oxidoreductase phospholipid biosynthesis	Transmembrane	Ubiquitous
SELENOK	ER-associated protein folding control	ER, plasma membrane	Ubiquitous
SELENOM	Unknown	Nuclear and perinuclear	Ubiquitous
SELENON	Oxidoreductase redox-related calcium homeostasis	Endoplasmic reticulum	Ubiquitous
SELENOO	Protein AMPylation activity	Mitochondria	Ubiquitous
SELENOP	Transport/oxidoreductase	Secreted, cytoplasmic	Most tissues, high in liver and small intestine
SELENOS	Oxidoreductase ER-associated protein folding control	Endoplasmic reticulum	Ubiquitous
SELENOT	Unknown oxidoreductase	Endoplasmic reticulum	Ubiquitous
SELENOV	Unknown	Intracellular	Thyroid, parathyroid, testis, and brain
SELENOW	Oxidoreductase protection against oxidative stress	Intracellular	Ubiquitous
SEPHS2	Selenophosphate synthesis	Intracellular	Ubiquitous, high in liver kidney

SELENOP, selenoprotein P; SEPHS2, selenophosphate synthetase 2.

the UGA codon, interacting with trans-acting factors (SECIS binding protein 2 [SECISBP2], Sec tRNA [transfer RNA], specific eukaryotic elongation factor [EEFSEC], and Sec-tRNA$^{[Ser]Sec}$) (Fig. 1) (4, 24, 33, 58, 85).

In contrast to other amino acids, selenocysteine does not have an aminoacyl-tRNA synthetase, but is synthesized on its own tRNA, encoded by *TRU-TCA1-1* (Fig. 1) (5). This tRNA was originally named the human opal suppressor gene and a truncated pseudogene that is not expressed also exists (68). Delivery to the ribosome and subsequent cotranslational insertion of Sec is mediated by a multiprotein complex (81), which includes EEFSEC and SECISBP2 as well as other factors. The specialized elongation factor EEFSEC (rather than general elongation factors eEf1a and EF-Tu, which delivers all other aa-tRNAs) delivers Sec-tRNA$^{[Ser]Sec}$ to the ribosome acceptor site (85). SECISBP2 interacts with the SECIS element, a stem–loop structure present in the 3′-UTR of every selenoprotein mRNA (12, 13, 23). The SECIS elements within each selenoprotein mRNA are distinct, but share common structural features, consisting of two helices separated by an internal loop, a GA quartet of non-Watson–Crick base pairs, and an apical loop, resulting in the adoption of a ''kink-turn'' structure (21, 33, 41, 89, 90). All selenoprotein mRNAs contain a single SECIS element, except for selenoprotein P (SELENOP), which has two, tandemly repeated SECIS elements. This configuration coincides with

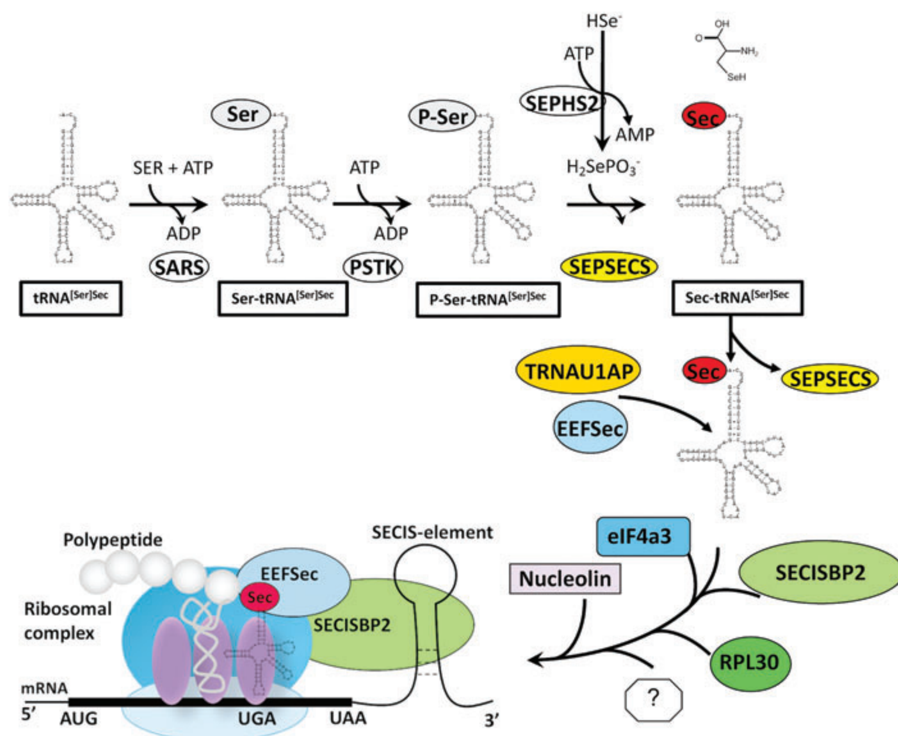


FIG. 1. Pathway of selenocysteine synthesis and its incorporation into selenoproteins. Sec is synthesized on its own tRNA (tRNA[Ser]Sec), which undergoes maturation through sequential modifications, with initial attachment of serine by SARS resulting in Ser-tRNA[Ser]Sec. Subsequent phosphorylation of this serine residue by PSTK generates O-Phosphoseryl-tRNA[Ser]Sec. Finally, SEPSECS catalyzes the acceptance of a selenophosphate, generated from selenide and ATP by SEPHS2, resulting in Sec-tRNA[Ser]Sec. An intermediate complex that includes Sec-tRNA[Ser]Sec, TRNAU1AP, and EEF-SEC is subsequently formed. This complex is guided by an interaction with SECISBP2 to the SECIS-element of selenoprotein mRNA, ready for incorporation into the nascent polypeptide. Other factors (ribosomal protein L30, eukaryotic initiation factor eIF4a3, nucleolin, …) also have regulatory roles and influence the Sec insertion process. EEFSEC, Sec tRNA-specific eukaryotic elongation factor; mRNA, messenger RNA; PSTK, phosphoseryl-tRNA kinase; SARS, seryl-tRNA synthetase; Sec, selenocysteine; SECIS, SEleniumCysteine Insertion Sequence; SECISBP2, SECIS binding protein 2; SEPHS2, selenophosphate synthetase 2; SEPSECS, O-phosphoserine tRNA:Sec tRNA synthase; tRNA, transfer RNA; TRNAU1AP, tRNA selenocysteine 1 associated protein 1. Color images are available online.

human SELENOP being the only selenoprotein that contains more than one selenocysteine residue (57). The SECIS element is required for Sec incorporation, whereas other motifs in some selenoprotein mRNAs (*e.g.*, Sec redefinition element, proximal stem loop element) have been described as contributing to the translation process (15, 22, 47, 48, 62).

The EEFSEC–Sec-tRNA[Ser]Sec–SECISBP2 complex, bound to SECIS in the selenoprotein mRNA, is believed to be in close proximity to the ribosomal complex, thereby preventing recognition of UGA as a translational stop and poised to mediate Sec incorporation into the polypeptide when the UGA codon is presented. Ribosomal protein L30 (RPL30), tRNA selenocysteine 1 associated protein 1 (TRNAU1AP), eukaryotic translation initiation factor 4A3 (EIF4A3), and nucleolin are other factors that regulate the Sec insertion process (Fig. 1) (49, 57, 79). However, three factors, Sec-tRNA[Ser]Sec, EEFSEC, and SECISBP2, have been shown to be essential and sufficient for Sec incorporation *in vitro* (42, 59, 63, 82).

The biological importance of selenoproteins is highlighted by the fact that both Trsp (mouse tRNA[Ser]Sec) and Secisbp2 null mice are embryonically lethal (19, 78). Mutations in individual human selenoproteins and their consequences have recently been reviewed in detail elsewhere (35): selenoprotein N (myopathy), glutathione peroxidase 4 (respiratory failure and skeletal defects), thioredoxin reductase 2 (associated with familial glucocorticoid deficiency and dilated cardiomyopathy), and thioredoxin reductase 1 (generalized epilepsy); two reports describe patients with a complex, hereditary spastic paraplegia and mutations in SELENOI, which catalyzes the transfer of phosphoethanolamine from CDP-ethanolamine to diacylglycerol to produce phosphatidylethanolamine (2, 46). Here, we describe mutations in genes (*SEPSECS*, *SECISBP2*, and *TRU-TCA1-1*) encoding three components of the selenocysteine insertion pathway, affecting general incorporation of selenocysteine into selenoproteins and their clinical consequences.

SECISBP2

SECISBP2 is an obligated limiting factor for selenoprotein synthesis, as first shown by the absence of selenoprotein synthesis in SECISBP2-depleted cell lysates, with restoration of production by repletion with SECISBP2 (23, 24). Human SECISBP2 is a large (854 amino acids, 120-kDa) protein, with the first 400, amino (N-) terminal, residues being dispensable for its function *in vitro* (25, 26) (Fig. 2). In contrast, the carboxy-terminal (C-terminal) region (amino acids 399–784) is both necessary and sufficient for SECIS-

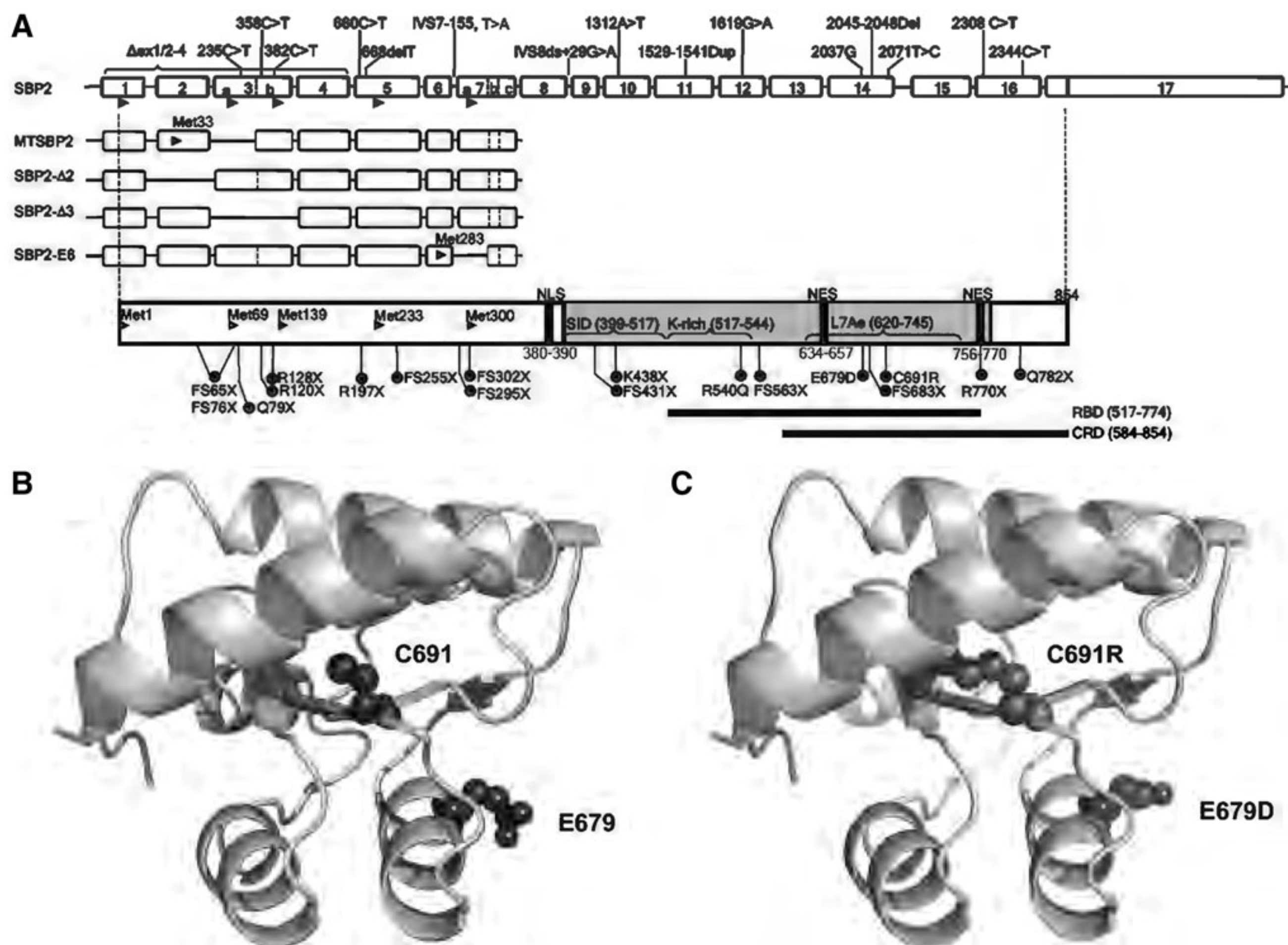


FIG. 2. Genomic organization of *SECISBP2* and functional domains of SECISBP2 with the position of human mutations. (A) The organization of the *SECISBP2* gene (*top*), with naturally occurring aminoterminal splice variants, each containing distal exons 8–17 shown next; the functional domains of SECISBP2 protein with the location of human mutations superimposed is shown (*bottom*). *Arrowheads* denote the location of ATG codons, which could function as alternative sites for the initiation of translation. Functional domains in SECISBP2 protein: N-terminal domain (1–399); minimal functional protein (*shaded gray*, 399–784); SID (399–517); minimal RBD (517–784); Lysine-rich domain involved in RNA specificity and ribosome binding (517–544); L7Ae homology module (620–745); NLS (380–390); redox-sensitive CRD (584–854); and two NES (NES1: 634–657; NES2: 756–770) (69). **(B, C)** Model of the L7ae RBD of SECISBP2. The position of wild-type residues (E679, C691) **(B)** and corresponding mutations (E679D, C691R) at these locations **(C)** is shown. The model was generated by using the phyre2 web portal, which predicts and analyzes protein structures based on homology/analogy to solved protein crystal structures (51). CRD, cysteine-rich domain; NES, nuclear export signals; NLS, nuclear localization signal; N-terminal, amino-terminal; phyre2, protein homology/analogy recognition engine 2; RBD, RNA-binding domain; SID, Sec incorporation domain. The figures were generated with MacPyMOL Molecular Graphics System, Schrödinger, LLC.

binding and Sec incorporation *in vitro* and contains several functional domains. The Sec incorporation domain (SID), located centrally in SECISBP2, is not essential for SECIS binding but required for Sec incorporation. The RNA-binding domain (RBD) contains an L7Ae-type RNA interaction motif identified in a large family of ribosomal proteins (*e.g.*, RPL30, SUP1, eRF-1, and 15.5-kD/Snu13p) (4, 5, 16, 25, 26), which interacts with the ''kink-turn'' structure adopted by the SECIS-element. The RBD mediates interaction with the SECIS element (34, 89) and 28S ribosomal RNA (25, 49, 56, 59). A domain amino-terminal (N-terminal) to the L7Ae module, referred to as either the bipartite, SID, or K-rich region, is involved in specific recognition of SECIS elements and other regulatory RNA motifs, thereby also controlling selenoprotein expression levels (17, 30, 83). SECISBP2 also contains several other functional motifs, as shown in Figure 2 (69).

Alternative splicing events in the 5′-region of human *SECISBP2*, with use of alternative initiation of translation from downstream ATG start codons in exons 2, 3a, 3b, 5, and 7, generates five different protein isoforms, each containing a varying N-terminal protein sequence (Fig. 2) (24, 70). These alternate splicing events alter content of the dispensable N-terminal region, but not the essential C-terminal domain, within protein isoforms. Nevertheless, it is possible that the alternately spliced isoforms do play a role in regulation of SECISBP2-dependent Sec incorporation and selenoprotein expression *in vivo*. During protein synthesis, dynamic interaction

of SECISBP2 with the ribosome and SECIS element is essential for recruitment of the EEFsec/Sec-tRNA$^{[Ser]sec}$ complex to the UGA codon, enabling the incorporation of Sec into the polypeptide.

Homozygous or compound heterozygous mutations in *SECISBP2* have been described in 13 individuals from 11 families (Table 2). Disruption of SECISBP2 function prevents appropriate Sec incorporation into selenoproteins during their biosynthesis, resulting in a multisystem disorder due to deficiency of diverse selenoproteins (Table 3). The biochemical signature that identifies SECISBP2-deficient patients consists of low circulating selenium (reflecting low plasma SELENOP and GPX3) and abnormal thyroid hormone levels due to diminished activity of deiodinases (Table 3) (31, 75). Most cases present in childhood due to growth retardation with raised circulating free thyroxine (FT4), normal to low free triiodothyronine (FT3), and raised reverse triiodothyronine (T3) levels, reflecting deficiency of all three deiodinase enzymes.

Muscle weakness, due to progressive rigid spine muscular dystrophy, affecting axial and proximal limb muscles with raised creatine kinase (CK) levels and fatty infiltration on imaging, is similar to that seen in patients with mutations in selenoprotein N. In one patient (proband E), male azoospermic infertility was described, reflecting loss of testis selenoproteins (mitochondrial GPX4, thioredoxin reductase, and selenoprotein V) required for spermatogenesis (75).

Significantly decreased expression levels of antioxidant selenoenzymes are associated with increased levels of cellular reactive oxygen species (ROS). Clinical consequences of raised cellular ROS include skin photosensitivity, progressive sensorineural hearing loss, and possibly increased total body adipose tissue mass paradoxically associated with enhanced systemic insulin sensitivity (75).

Reduced red blood cell and total lymphocyte counts, with impaired mononuclear cell cytokine secretion and T cell proliferation (similar to findings in T cell-specific Trsp null mice) (80), were recorded in one case, proband E (75). Although other hematological and immune cell phenotypes have not been formally evaluated, immunodeficiency is neither a reported feature in other *SECISBP2* mutation cases nor seen in mouse models of selenoprotein deficiency. Additional

Table 2. Human *SECISBP2* Mutations: Genetics and Effect

Family	*Gene mutation*	*Predicted protein change*	*Alleles affected*	*Suggested mechanism*	*Ethnicity*	*References*
A	c.1619 G>A	R540Q	Homozygous	Predicted to affect SECIS and ribosome binding	Saudi Arabian	(31)
B	c.1312A>T c.IVS8ds +29 G>A	K438X fs431X	Compound heterozygous	Premature stop, no/decreased full-length protein	Irish/ Kenyan	(31)
C	c.382 C>T	R128X	Homozygous	Premature stop, no full-length protein	Ghanaian	(28)
D	c.358 C>T	R120X	Compound heterozygous	Premature stop, no full-length protein	Brazilian	(9)
	c.2308 C>T	R770X		Premature stop, no full-length protein		
E	c.668delT	F223fs255X	Compound heterozygous	Premature stop, no full-length protein	British	(75)
	c.IVS7 -155, T>A	fs295X+fs302X		Premature stop, no or decreased full-length protein		
F	c. 2017T>C	C691R	Compound	Predicted to affect SECIS and ribosome binding, increased degradation	British	(75)
	1–5 Intronic SNPs	fs65X+fs76X	Heterozygous	Premature stop, no full-length protein/splice variants affected		
G	c.1529_1541dup CCAGCGCCCCACT c.235 C>T	M515fs563X Q79X	Compound heterozygous	Premature stop, no full-length protein	Japanese	(43)
H	c.2344 C>T c.2045–2048 delAACA	Q782X K682fs683X	Compound heterozygous	Premature stop, no full-length protein	Turkish	(32)
I	c.660 C>T	R197X	Compound heterozygous	Premature stop, no full-length protein/splice variants affected	Argentinian	(37)
	c.2108 G>T or C	E679D		Predicted to affect SECIS and ribosome binding		
J	c.800_801insA	K267Kfs*2	Homozygous	Premature stop, no full-length protein	Turkish	(20)
K	c.283delT c.589C>T	T95Ifs31* R197X	Compound heterozygous	Premature stop, no full-length protein	N/A	(55)

N/A, not available; SECIS, SEleniumCysteine Insertion Sequence; SNP, single nucleotide polymorphism.

Table 3. Human *SECISBP2* Mutations: A Multisystem Disorder with a Thyroid Signature

Family	*[Se]*	*TT4*	*FT4*	*TT3*	*rT3*	*TSH*	*Growth and skeletal*	*Musculoskeletal*	*Neurocognitive*	*Hearing*	*Other*
A	L	H	H	L	H	H	Short stature, DBA	N/A	Normal mental development	Normal	N/A
B	L	H	H	N/L	H	N	Short stature, DBA	N/A	N/A	Normal	N/A
C	L	H	H	N/L	H	N	Short stature, DBA	N/A	N/A	N/A	
D	L	H	H	N/L	H	H	Short stature, DBA, kyphoscoliosis	Hypotonia, hip girdle weakness, spirometry: reduced expiratory and inspiratory flow, fatty infiltration of muscle	Impaired mental development and motor coordination	Bilateral sensorineural loss	Failure to thrive, bilateral clinodactyly, asymmetric leg length, peripheral sensory neuropathy, and increased fat mass
E	L	N/A	H	N	N/A	N	Short stature, genu valgus	Lumbar spinal rigidity, reduced axial and neck strength, spirometry: reduced vital capacity, nocturnal hypoventila-tion, fatty infiltration of muscle	Developmental delay	Bilateral sensorineural loss, vertigo	Azoopsermia, Raynauds disease, photosensitivity, mild lymphopenia and reduced red cell mass, low insulin and high adiponectin levels, favorable blood lipid profile, and low intrahepatic lipid
F	L	N/A	H	L	N/A	N	Short stature	Proximal and axial myopathy, lumbar rigidity, fatty infiltration of adductor muscles	Developmental delay	Bilateral sensorineural loss	Failure to thrive, eosinophilic colitis, increased fat mass, high adiponectin levels, and hypoglycemia with low fasting insulin
G	L	N/A	H	N/L	N/A	N/H	Short stature, DBA	Fatty infiltration of mus-cles	Delayed motor and intellectual development, IQ 70	Bilateral mild conductive loss rotatory vertigo	Failure to thrive, hypoplastic thyroid gland, no photo-sensitivity, and increased fat mass
H	L	H	H	L	H	N/H	Short stature	N/A	Mental and motor retardation, IQ 50	N/A	N/A
I	N/A	H	H	L	H	N	DBA	N/A	N/A	N/A	Failure to thrive
J	L	H	H	L	H	N/H	Decreased growth velocity from 13 years	Muscle weakness, fatty infiltration of the muscles	Normal early development	Normal	Right-eye ptosis, attention-deficit disorder with poor school performance, impaired growth hormone response, obese (body mass index 29.5), and impaired OGTT
K	N/A	H	H	L	H	N	Short stature, DBA	N/A	Developmental delay	N/A	Failure to thrive
L	L	H	H	L	H	N	Delayed growth	Hypotonia, myopathy with fatty infiltration of muscles, and ataxic gait	Developmental delay	Bilateral sensorineural loss	Dysarthic speech, tapered fingers, seizures, and increased fat mass

DBA, delayed bone age; FT4, free thyroxine; H, high; IQ, intelligence quotient; L, low; N, normal; N/H, high-normal; N/L, low normal; OGTT, oral glucose tolerance test; rT3, reverse triiodothyronine; Se, selenium; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine; TSH, thyroid stimulating; hormone.

age-dependent phenotypes such as neurodegeneration, premature aging, or neoplasia may emerge but have not been described hitherto.

Oral selenium supplementation in some *SECISBP2* patients raised total serum Se levels, but without clinical effect (9, 20, 28) or altering synthesis (circulating GPX's, SELENOP) or action (thyroid hormone metabolism) of selenoproteins (77). Treatment of probands C and F with T3 alone (thyroxine [T4] was not effective in one case) or in combination with growth hormone (proband G) resulted in an improvement in growth, development, and bone maturation. Treatment of proband G with a combination of alpha tocopherol (vitamin E) and T3 resulted in the most promising response, with decreased serum levels of lipid peroxidation products, altered FT4 and FT3 concentrations, and increased circulating white blood cells and neutrophils, all of which were reversed after treatment withdrawal (74). These observations suggest that treatment with antioxidants, to counteract the effects of elevated cellular ROS, is the best available therapeutic option for this disorder.

Most *SECISBP2* mutations identified to date cause premature stop codons, resulting in the absence of full-length SECISBP2 protein. However, elegant minigene experiments have shown that, for premature stops located in the N-terminal part of the protein, initiation of translation from alternative, downstream ATG codons in exons 5 (Met233) and 7 (Met300) permits low-level synthesis of shorter SECISBP2 isoforms (28, 75). Some premature stop mutations are situated downstream of Met300 and may completely eliminate synthesis of functional protein. However, stop mutations (*e.g.*, R770X, Q782X), distal to the RBD, might generate C-terminally truncated proteins whose RNA binding and nuclear localization functions remain, partially, intact (Fig. 2). In a patient with defective mRNA splicing due to an intronic mutation IVS8ds +29G>A (31), it has been shown that levels of normally spliced transcript are only reduced by 50% and if a similar mechanism operates with other splice site mutations, this suggests some preservation of normally spliced SECISBP2 mRNA in such cases.

Three missense *SECISBP2* mutations (R540Q, C691R, and E679D) have been described: The R540Q mutation localizes to the K-rich region within the RBD (Fig. 2), with the R540Q mutant exhibiting reduced binding to SECIS elements in GPX1 and DIO2 mRNAs, correlating with diminished GPX1 and DIO2 enzyme activity in patient-derived primary cells and the mouse model. Detailed analyses suggest that R540Q mutant SECISBP2 fails to bind only a subset of SECIS-elements, consistent with the K-rich region mediating the recognition of specific (type I and type II) SECIS elements (14); a mouse model revealed a possible tissue-specific pattern of SECISBP2 protein stability, correlating with varying loss or preservation of expression of different selenoproteins (14, 31, 96).

The C691R SECISBP2 mutation, also located in its RBD, is expected to affect RNA binding. Homology modeling, based on the crystal structure of the spliceosomal 15.5 kDa protein (87), suggests that the mutation of cysteine to a bulky and charged arginine residue (75) may destabilize its hydrophobic core, disrupting local protein structure (Fig. 2B, C). *In vitro* assays, showing enhanced proteasomal degradation of the C691R mutant SECISBP2 protein, confirmed this (75). A mouse model suggests that the C691R mutant SECISBP2 is unable to bind RNA and is non-functional (96). The E679D SECISBP2 mutation is predicted to be deleterious (PolyPhen-2 algorithm score of 0.998) and also located in the RBD and may, therefore, affect its RNA binding function, but this has not been investigated in detail (37) (Fig. 2).

Knowledge that knockout (KO) of Secisbp2 in mice is embryonic lethal (78), with no evidence for an alternative Sec-incorporation mechanism in humans, suggests that there is some residual SECISBP2 activity in all patients. All patients described to date are expected to harbor at least one allele that directs the synthesis of SECISBP2 at either reduced levels or that is only partially functional, in combination with either a mutant or a shorter form of the protein synthesized from Met300 (Table 2). A limited number of patients, mostly compound heterozygous for different *SECISBP2* mutation combinations, with limited knowledge of phenotypes in heterozygous relatives, have been described, making it difficult to assess the effect of a specific *SECISBP2* mutation or its correlation with the severity of phenotype. However, since SECISBP2 is rate limiting for Sec incorporation, a significant reduction in functional SECISBP2 protein levels will result in diminished but not complete loss of selenoprotein synthesis. A further variable is that differences in the architecture of SECIS elements within different selenoprotein mRNAs may dictate the extent to which reduced SECISBP2 protein limits their biosynthesis and expression levels *in vivo*, as suggested by elegant experiments testing SECISBP2 with luciferase reporter genes containing different SECIS elements (60, 84). Future studies, undertaking ribosomal RNA (96) or selenoprotein expression profiling in secisbp2 mutant mouse models and *in vitro* reconstitution experiments with different mutant SECISBP2 proteins and SECIS element containing luciferase reporter genes (84), may help us better understand the effect of specific SECISBP2 mutations. In turn, greater understanding of how different mutations affect selenoprotein expression may enable better prediction of clinical outcome or targeting of therapy in patients.

TRU-TCA1-1

Selenocysteine is synthesized on its own tRNA (Fig. 1), $tRNA^{[Ser]Sec}$ (encoded by *TRU-TCA1-1*) that has several unique features, being longer (90 nucleotides *vs.* usual 78), with an atypical long acceptor- and D-stem with a few modified bases (57) that distinguishes it from other tRNAs (Fig. 3A). Its promoter region, containing both tRNA and U small nuclear RNA (snRNA) gene regulatory elements (18, 39, 44) forming a new class of RNA polymerase III transcribed genes, also differs from other tRNA genes (Fig. 3B) and consists of four regulatory elements: an activator element, containing an SPH motif, but is octamer independent; the proximal sequence element; an extended TATA-motif, essential for efficient transcription; and an internal B box (64, 65).

Two major isoforms of Sec-$tRNA^{[Ser]Sec}$ have been identified, containing either 5-methoxycarbonyl-methyluridine (mcm^5U) or its methylated form 5-methoxycarbonylmethyl-2′-O-methyluridine (mcm^5Um) at position 34 (Fig. 3C). Uridine 34 is located in the anticodon loop and its methylation may contribute to stabilization of the codon–anticodon interaction (19, 27, 45, 79). Methylation at uridine 34 is

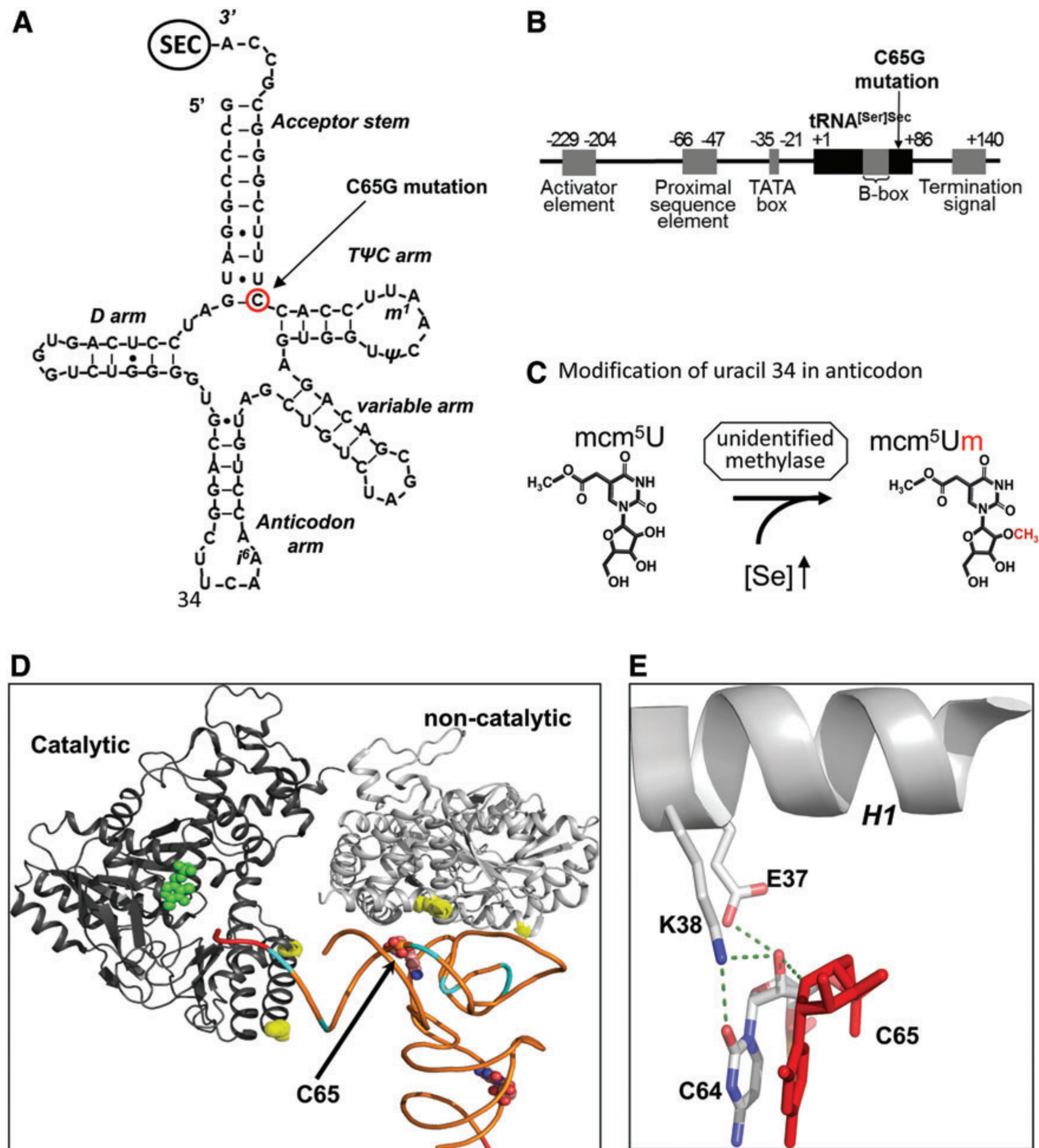


FIG. 3. Genomic and structural organization of Sec-tRNA[Ser]Sec showing the position of human mutation. (A) The primary structure of human Sec-tRNA$^{[Ser]Sec}$ is shown in a cloverleaf model, with the location of C65G *TRU-TCA1–1* mutation identified in the patient indicated (*circled red*). The acceptor stem constitutes paired 5′ and 3′ terminal bases, with the D arm, the anticodon arm, the variable arm, and the TψC arm depicted. Mammalian Sec-tRNA$^{[Ser]Sec}$ undergoes post-transcriptional modification at positions 34 (mcm^{5}U or mcm^{5}Um), 37 (i6A), 55 (ψ), and 58 (m1A). **(B)** Schematic of the *TRU-TCA1-1* gene showing the coding region (*black boxes*) and regulatory elements (*gray boxes*) (43), with the location of C65G mutation identified in the patient. **(C)** The two Sec-tRNA$^{[Ser]Sec}$ isoforms, containing either mcm^{5}U or mcm^{5}Um modifications of the uracil at position 34 in the anticodon arm, differ from each other by a single methyl group on the 2′-O-ribosyl moiety. This reaction is catalyzed by an unknown methylase, and abundance of the mcm^{5}Um isoform increases with selenium concentration (19). **(D)** Crystal structure showing catalytic and non-catalytic dimers of the SEPSECS tetramer bound to tRNA$^{[Ser]Sec}$ (71), with the position of the C65 nucleotide indicated. Other nucleotides in tRNA$^{[Ser]Sec}$ (*cyan*) and amino acids in SEPSECS (*yellow*) involved in RNA–protein interaction are also highlighted, as are the nucleotides (*red*) toward the position of Sec and the pyridoxal-5-phosphate substrate (*green*) within the catalytic domain. **(E)** A close-up of the structure around C65, showing H-bonds (*dashed green lines*) formed between C64 and the C64–C65 backbone with residues (E37 and K38) situated in helix 1 (H1) of the non-catalytic dimer of SEPSECS. mcm^{5}U, 5-methoxycarbonyl-methyluridine; mcm^{5}Um, 5-methoxycarbonylmethyl-2′-O-methyluridine. Color images are available online.

executed by a methylase whose identity is not known and the relative abundance of the two Sec-tRNA$^{[Ser]Sec}$ isoforms is known to be influenced by systemic Se-status, increasing selenium levels resulting in more mcm^{5}Um isoform (19, 27, 45). Each Sec-tRNA$^{[Ser]Sec}$ subtype has a different role, with synthesis of cellular housekeeping selenoproteins (*e.g*., TXNRD1, TXNRD3, GPX4) being dependent on the mcm^{5}U isoform; whereas the expression of cellular, stress-related selenoproteins (*e.g*., GPX1, GPX3, SELENOW) requires the mcm^{5}Um isoform (19, 79). Methylation of mcm^{5}U, the final step in post-transcriptional maturation of Sec-tRNA$^{[Ser]Sec}$, is dependent on correct aminoacylation (53, 54), intact secondary/tertiary structure (54), and other, prior, base modifications (Fig. 3A) of the tRNA (27).

A single patient, homozygous for a single nucleotide change (C65G) in *TRU-TCA1-1* has been identified (Fig. 3A) (76). The proband exhibits a similar phenotype to that seen in *SECISBP2* mutation patients. However, a comparison of cellular selenoprotein expression profiles in the two disorders has revealed differences, with the expression of relative housekeeping selenoproteins (*e.g*., TXNRDs, GPX4) being more preserved than in *SECISBP2* cases. In contrast, the expression of stress-related selenoproteins (*e.g*., GPX1, GPX3) was similarly reduced in both disorders. In primary cells from the *TRU-TCA1-1* mutation patient, lower total tRNA$^{[Ser]Sec}$ expression with a disproportionately greater diminution in Sec-tRNA$^{[Ser]Sec}$ mcm^{5}Um levels was observed, with decreased i^{6}A modification at position 37 suggesting that its post-transcriptional maturation is impaired. The mutation had no effect on tRNA$^{[Ser]Sec}$ aminoacylation with serine or Sec synthesis or its interaction with O-phosphoserine tRNA:Sec tRNA synthase (SEPSECS). Low levels of tRNA$^{[Ser]Sec}$ in the proband were insufficient to direct normal synthesis of stress-related selenoproteins, but they were not rate limiting for adequate synthesis of some housekeeping selenoproteins and similar, differential preservation of selenoprotein synthesis has been observed in murine tRNAsec mutant models (19, 57).

The human SEPSECS–tRNA$^{[Ser]Sec}$ structure shows that C65 is situated in the acceptor arm, next to C64 in the TΨC–arm (Fig. 3D). C64 interacts with Lys38 and Glu37 in helix 1 of the non-catalytic part of the SEPSECS moiety (Fig. 3E), raising the possibility that the C65G mutation could affect the stability of the Sec-tRNA$^{[Ser]Sec}$–SEPSECS complex and the selenoprotein synthesis.

In summary, these observations indicate that reduction in Sec-tRNA$^{[Ser]Sec}$ levels, with particular deficiency of the Sec-tRNA$^{[Ser]Sec}$ mcm^{5}Um subtype, contributes to the selective pattern of selenoprotein deficiency seen in the proband. The precise mechanism mediating the reduction in mutant Sec-tRNA$^{[Ser]Sec}$ presence remains unclear, with defective post-transcriptional modification of mutant Sec-tRNA$^{[Ser]Sec}$ or instability of the mutant Sec-tRNA$^{[Ser]Sec}$–SEPSECS complex being possibilities.

SEPSECS

Human SEPSECS was initially identified as an autoantigen (soluble liver antigen/liver pancreas) in autoimmune hepatitis (52). Subsequent studies in which mammalian cell extracts were treated with autoimmune hepatitis patients' serum showed that SEPSECS co-precipitated with Sec-tRNA$^{[Ser]Sec}$, as part of a ribonucleoprotein complex (40). This led to the identification of SEPSECS as the enzyme that catalyzes the conversion of O-phosphoserine-tRNA$^{[Ser]Sec}$ to Sec-tRNA$^{[Ser]Sec}$, using selenophosphate as a donor substrate (81, 93) (Fig. 1).

Crystal structures of the archaeal and murine Sepsecs apoenzymes as well as human wild type and mutant SEPSECS complexed with Sec-tRNA$^{[Ser]Sec}$ have been solved, suggesting that SEPSECS is a distinct member of the fold type I family of the pyridoxal phosphate-dependent enzyme family (7, 38, 68a, 73). The human structure shows a complex containing an SEPSECS tetramer binding two Sec-tRNA$^{[Ser]Sec}$ molecules through their long acceptor-TΨC arms, with the non-catalytic SEPSECS dimer mediating RNA–protein interactions that stabilize the complex and the CCA end of Ser-tRNA$^{[Ser]Sec}$ residing in the active site of the catalytic SEPSECS dimer (Figs. 3D and 4B). The conversion of Ser-tRNA$^{[Ser]Sec}$ to Sec-tRNA$^{[Ser]Sec}$ by SEPSECS is pyridoxal-5-phosphate (PLP) cofactor dependent, with the proposed mechanism involving a conformational change in SEPSECS on Ser-tRNA$^{[Ser]Sec}$ binding, enabling the phosphoserine of Ser-tRNA$^{[Ser]Sec}$ to be oriented correctly for conversion to occur (68a).

Homozygous and compound heterozygous mutations in *SEPSECS* (Table 4, Figs. 4, and 5) are associated with profound intellectual disability, global developmental delay, spasticity, epilepsy, and hypotonia with progressive microcephaly due to cortical and cerebellar atrophy on magnetic resonance imaging (1). Additional phenotypes described in other patients include axonal neuropathy, optic atrophy, and early onset epileptic encephalopathy with burst suppression (6, 67, 72). The timing of presentation in patients with *SEPSECS* mutations is variable, ranging from severe prenatal onset to delayed postnatal presentation and a mild, late onset phenotype in three patients (50, 86). This disorder is classified as autosomal recessive pontocerebellar hypoplasia type 2D (PCH2D, OMIM No. 613811), also known as progressive cerebellocerebral atrophy (PCCA) (1, 11). Mice homozygous for a *SEPSECS* mutation (Y334C) present with Sedaghatian-type spondylometaphyseal dysplasia and die shortly after birth, in contrast to humans with the same mutation. This divergence in phenotype may be due to species differences, dietary environment, or the genetic background of the mouse line used (36).

The effect of *SEPSECS* mutations on selenoprotein expression has only been studied in four patients (Family E, G, F; 6). In brain tissue levels of TXNRD1, TXNRD2, GPX1, and GPX4 proteins are reduced, correlating with increased cellular oxidative stress. However, selenoprotein deficiency is not generalized with normal TXNRD levels in a patient's fibroblast and muscle cells, suggesting that residual SEPSECS activity preserves selenoprotein synthesis in some tissues or existence of alternative pathway(s). Consistent with findings in human tissues Sepsecs mutant mice exhibit decreased Gpx4 expression in neurons but not hepatocytes (36).

Although the selenium content of brain is not high (95), it is kept very stable (10, 66), exemplified by the fact that in systemic selenium deficiency circulating SELENOP delivers this trace element preferentially to this organ at the expense of other tissues (66). This may explain why a reduction in SEPSECS activity could have a greater effect on brain development and function compared with other tissues.

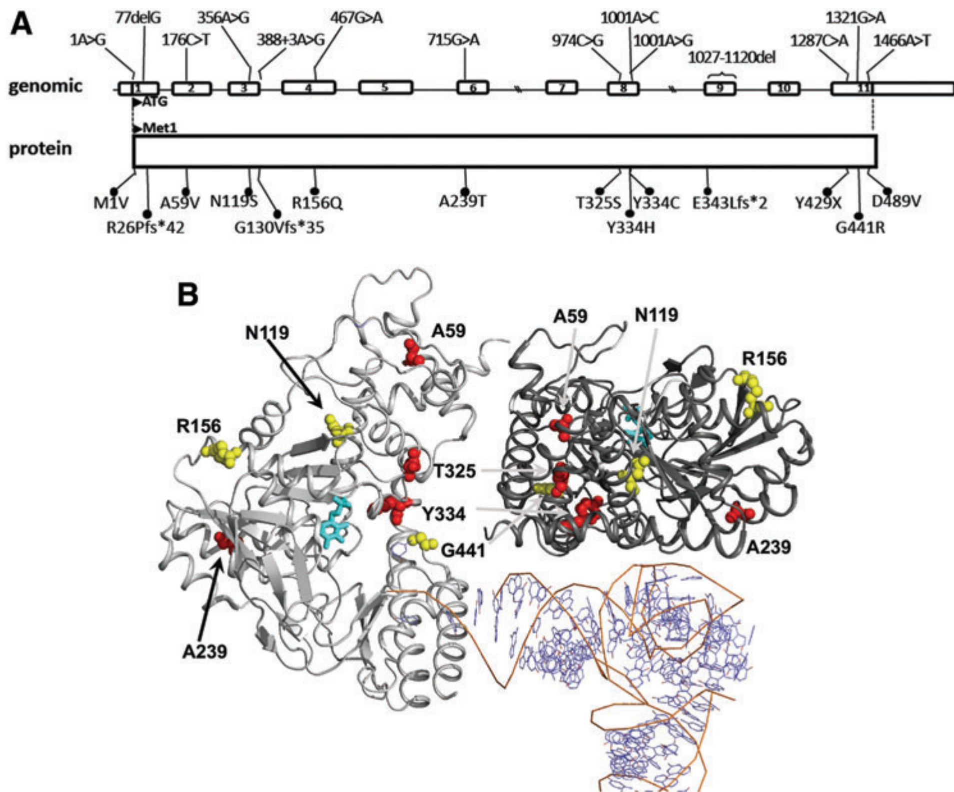


FIG. 4. Genomic and structural organization of SEPSECS with the positions of the human mutations. (A) The organization of human *SEPSECS* gene (*top*) and schematic of SEPSECS protein (*bottom*) with the location of human mutations superimposed. *Arrowheads* denote the location of the ATG start codon. **(B)** Crystal structure showing a single catalytic–non-catalytic dimer from the complex bound to tRNA$^{[Ser]Sec}$, with the position of all the human SEPSECS point mutations superimposed (71). Mutations associated with early onset (*red*) or late onset (*yellow*) disease and the pyridoxal-5-phosphate (*cyan*) substrate are highlighted. Color images are available online.

It is remarkable that an overt central nervous system (CNS) phenotype is not reported in patients with *SECISBP2* and *TRU-TCA1-1* defects, but with most individuals with these mutations being children the possibility of a late onset neurological phenotype cannot be discounted. However, a neurodegenerative phenotype has been described in brain-specific *Gpx4*, *Trsp*, and *Secisbp2* KO mouse models, suggesting that Se deficiency and/or reduced redox capacity has a larger impact on the mouse brain compared with the human brain (78, 91, 92).

Biochemical hallmarks of selenoprotein deficiency in *SECISBP2* and *TRU-TCA1-1* mutation cases include low circulating selenium and abnormal thyroid hormone levels, reflecting deficiency of circulating selenoproteins (SEPP, GPx3) or all three deiodinase enzymes, respectively (31, 75). In *SEPSECS* mutation patients, low serum selenium is not reported to be part of the phenotype and thyroid status has only been partially investigated in four patients, documenting either normal thyroid hormone (Family H; 72) or normal T4 but elevated thyroid stimulating hormone (TSH) levels (Family E, G, F; 6). However, more detailed investigation, with measurement of T3 or reverse T3 levels, which would be abnormal with decreased deiodinase enzyme activity, has not been undertaken in *SEPSECS* mutation cases (6). Myopathic features with raised CK levels, abnormal mitochondria, cytoplasmic bodies, and increased lipid accumulation in muscle have been documented in one *SEPSECS* mutation case (Family H; 72), with broad-based gait and postural instability suggesting muscle weakness in another patient (Family O, 86). Similar findings have been noted in adult *SECISBP2* mutation patients, reflecting deficiency of selenoprotein N and altered redox capacity in skeletal muscle (75). Overall, these observations suggest that some *SEPSECS* mutation patients can exhibit phenotypes associated with more global deficiency of selenoproteins. It is also possible that severity of neurological problems in patients has precluded detailed investigation and ascertainment of non-neurological phenotypes.

The availability of the crystal structure of human SEPSECS–tRNA$^{[Ser]Sec}$ (68a, 73) (Figs. 4 and 5), together with an SEPSECS activity assay using an *Escherichia coli* strain lacking endogenous Sec-synthase (SelA) activity (94), has enabled detailed studies of some *SEPSECS* (A239T, Y334C, T325S, and Y429X) mutations (1, 6, 73). These pathogenic variants were found to be less soluble than WT protein *in vitro*, with loss of functional activity. The A239T mutant SEPSECS failed to form stable tetramers, possibly as a result of a steric clash destabilizing two helices (H8–H9) within the enzyme's core. Y334C and T325S SEPSECS mutants are predicted to fold similar to wild-type SEPSECS in the crystal structure and retain binding to tRNA$^{[Ser]Sec}$ (73) but affect its catalytic pocket, reducing enzyme activity (1, 6, 73). Mutation of Tyr334 to Histidine is also recorded (6), and *in silico* analysis predicts that this variant is likely to have a similar deleterious effect as the Y334C mutation (Fig. 5). The premature stop SEPSECS mutant (Y429X) is insoluble and inactive (6, 73), and three other premature stop mutants (Table 4) can be expected to have a similar effect.

In silico analysis predicts that the A59V SEPSECS mutation results in steric hindrance destabilizing helix 2 and helix 3, possibly affecting its catalytic function and/or dimerization (Fig. 5). The N119S SEPSECS mutation is a conservative amino acid substitution with a probable small effect, weakening an H-bond network in the non-catalytic dimer and possibly affecting RNA interaction. Likewise, the mutation of Arg156 to Glutamine in SEPSECS is a

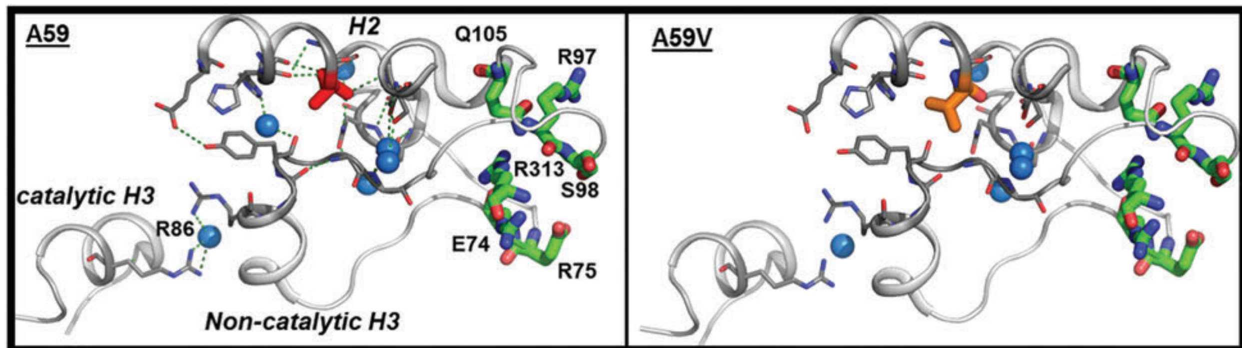
TABLE 4. HUMAN *SEPSECS* MUTATIONS: GENETICS AND EFFECT

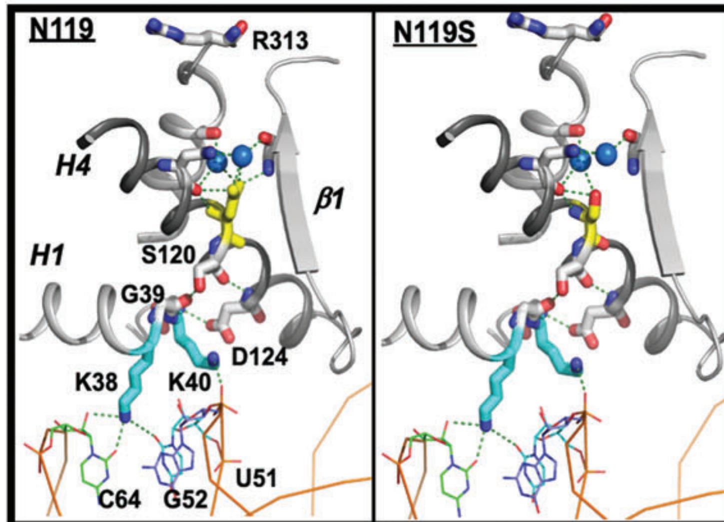
Family	*Gene mutation*	*Predicted protein change*	*Alleles affected*	*Suggested mechanism*	*Ethnicity*	*Phenotype*	*References*
A, B	c.1001A>G	Y334C	Homozygous	Affects folding and reduced catalytic activity	Jewish/ Iraqi	PCH2D	(1)
C, D	c.715G>A c.1001A>G	A239T Y334C	Compound heterozygous	Affects folding and reduced catalytic activity	Iraqi/ Moroccan	PCH2D	(1)
E, F, G	c.974C>G	T325S	Compound heterozygous	Affects folding and reduced catalytic activity	Finnish	PCH2D	(6)
	c.1287C>A	Y429X		Premature stop, no full-length protein			
H	c.1001A>C	Y334H	Homozygous	Predicted to affect folding and reduce catalytic activity	Arabian	PCH2D	(72)
I	c.77delG	R26Pfs*42	Compound heterozygous	Premature stop, no full-length protein	Japanese	Late onset PCH2D	(50)
	c.356A>G	N119S		Predicted reduced catalytic activity/ RNA binding			
J	c.356A>G	N119S	Compound heterozygous	Predicted reduced catalytic activity/ RNA binding	Japanese	Late onset PCH2D	(50)
	c.467G>A	R156Q		Predicted reduced catalytic activity			
K	c.1A>G	M1V	Compound heterozygous	No ATG start, absence of full-length protein,	N/A	PCH2D	(97)
	c.388+3A>G	G130Vfs*35		premature stop, no full-length protein			
L	c.1466A>T	D489V	Homozygous	Likely pathogenic	Jordan	Developmental Delay/ intellectual Disability	(61)
M	c.1027–1120del	E343Lfs*2	Homozygous	Premature stop, no full-length protein	N/A	Neurodegenerative disease	(3)
N	c.176C>T	A59V	Homozygous	Predicted to affect folding, dimerization and reduce activity	N/A	EOEE-BS; PCH2D	(67)
O	c. 1321G>A	G441R	Homozygous	Predicted to reduce catalytic activity	N/A	Late onset PCH2D	(86)
P	N/A	N/A	N/A	N/A	N/A	PCH2D	(8)

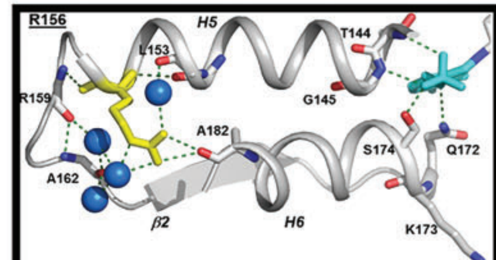
EOEE-BS, early onset epileptic encephalopathy with burst suppression; PCH2D, pontocerebellar hypoplasia type 2D.

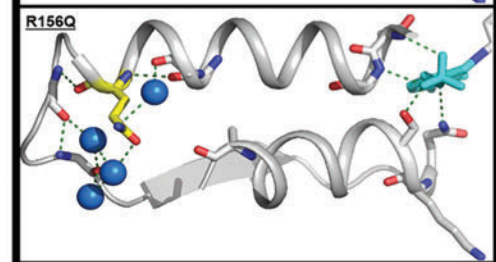
conservative change, predicted to perturb local structure minimally and possibly reducing activity of the catalytic site (Fig. 5). The G441R SEPSECS mutation, situated close to the catalytic site, changes the side chain of this amino acid from small and neutral to large and polar, but as Gly441 is situated within a loop, its possible effect on catalytic activity might be limited. Absence of two SEPSECS mutations (M1V, D489V) from crystal structures precludes *in silico* analyses: M1V affects the first methionine in SEPSECS and unless an alternative start codon (*e.g.*, position 61) is used, no protein will

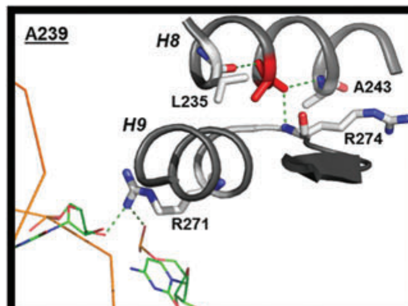
FIG. 5. Detailed views comparing wild-type SEPSECS crystal structure and mutated amino acids modeled in the SEPSECS crystal structure that cause either early onset (*red*) or late onset (*yellow*) disease (71). Hydrogen bonds (*dotted green lines*), pyridoxal-5-phosphate substrate (*cyan*), tRNA (*orange*), and H_2O (*blue*) are shown. The helices (H), beta-sheets (β), amino acids, and nucleotides involved in hydrogen bond networks or that are part of the active catalytic domain are labeled. Crystal structures for T325S and Y334C SEPSECS mutants are available and in the panels with these mutations an overlay of wild type (*gray*) and mutant (*cyan*) is shown (73). The effect of these mutations is described in detail in section SEPSECS in main text. The model was generated by using the phyre2 web portal, which predicts and analyzes protein structures based on homology/analogy recognition to solve protein crystal structures (51). The figures were generated with MacPyMOL Molecular Graphics System, Schrödinger, LLC. Color images are available online.

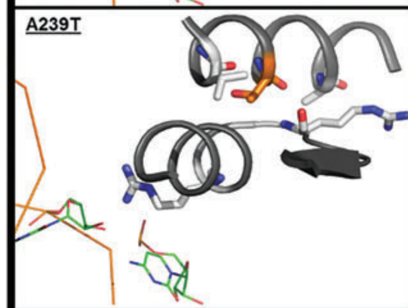

A59
H2
Q105
R97
catalytic H3
R86
R313
S98
E74
R75
Non-catalytic H3
A59V

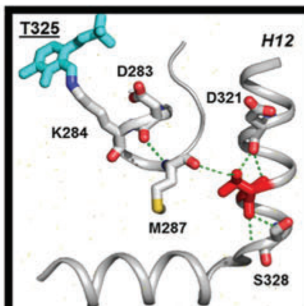

N119
R313
H4
β1
H1
S120
G39
D124
K38
K40
C64
G52
U51
N119S

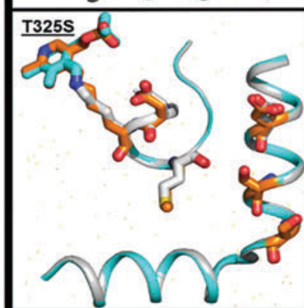

R156
H5
T144
L153
R159
G145
A182
S174
Q172
A162
β2
H6
K173

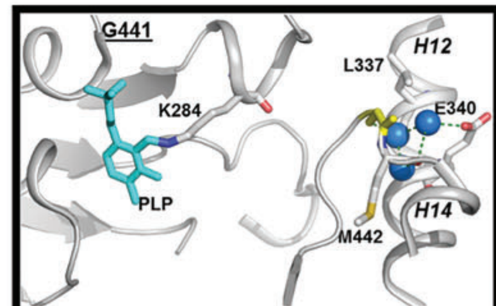

R156Q

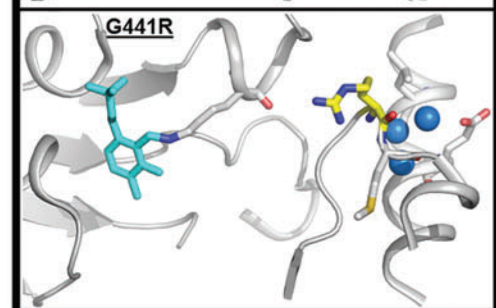

A239
H8
L235
A243
H9
R274
R271

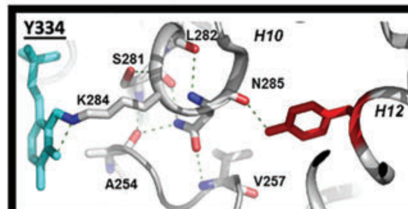

A239T


T325
H12
D283
D321
K284
M287
S328


T325S


G441
H12
L337
K284
E340
PLP
H14
M442


G441R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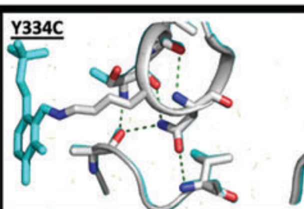
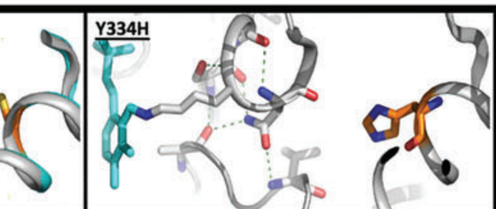
H10
S281
N285
K284
H12
A254
V257


Y334C


Y334H

be generated; the D489V mutation changes the size and charge of this amino acid and can, therefore, be expected to have a major impact on protein function/stability.

Three SEPSECS mutation patients (patient I [R26Pfs*42–N119S], J [N119S–R156Q], and O [G441R]) presented with late onset PCH2D, with a progressive but milder degree of CNS atrophy (50, 86). *In silico* analyses suggest that these mutations have a less deleterious effect on SEPSECS function (Fig. 5), but it is also conceivable that environmental factors or patients' genetic background may have modulated their phenotype. Future studies need to investigate the relationship between mutations, their effect on SEPSECS protein function, and general expression of selenoproteins in different tissues and patient phenotypes in more detail.

Conclusions

SECISBP2, Sec-tRNA$^{[Ser]Sec}$, and SEPSECS are essential components of the selenoprotein biosynthesis pathway. Unsurprisingly, in patients, harboring mutations in any of these genes, the expression of most members of the selenoproteome is affected, sometimes in a tissue-specific manner, resulting in a complex, multisystem phenotype. The combination of the nature of the gene defect, genetic/ethnic background of individuals, and environmental factors (*e.g.*, selenium and/or iodine status) might also contribute to inter-individual differences in phenotypes. Further complexity is due to the fact that most patients harbor compound heterozygous mutations with monoallelic mutations in individuals having no reported phenotype. This makes it difficult to assess the effect of a particular mutation on the Sec-incorporation pathway. Although a substantial body of knowledge regarding the individual functions of SECISBP2, Sec-tRNA$^{[Ser]Sec}$, and SEPSECS exists, we do not have a comprehensive understanding of the Sec-insertion pathway. Nevertheless, most observations in patients with gene defects accord with our current knowledge of this pathway. However, it is interesting that mutations in *SECISBP2* and *TRU-TCA1-1* present with similar phenotypes (growth retardation and myopathy together with abnormal thyroid function), whereas the dominant phenotype in *SEPSECS* mutation cases is PCCA.

In all patients, some phenotypes (*e.g.*, photosensitivity, age-dependent hearing loss, and neurodegeneration) are clearly progressive, perhaps reflecting the absence of antioxidant selenoenzymes and resulting in cumulative oxidative damage to DNA, proteins, and membrane lipids and dysfunction of redox-dependent signaling pathways. Some phenotypes can clearly be linked to deficiency of specific selenoproteins (*e.g.*, abnormal thyroid function and DIO1,2,3; low plasma Se and SELENOP, GPX3; azoospermia and SELENOV, GPX4, and TXRND3; myopathy; and SELENON).

However, the precise role of many selenoproteins in human biological processes is unknown, making the identification of causal links between altered expression of specific selenoproteins and human disease a particular challenge. Further, the role of individual selenoproteins needs to be analyzed in the context of a complex cellular biochemical environment, where antagonistic, additive, and synergistic effects can occur. Recent advances, including analyzing selenoprotein expression in mouse models, RNA ribosome profiling (96) *in vitro* technologies such as CRISPR-Cas9-VLP (88), dissection of SECIS and other functional RNA elements (22, 62) using luciferase-based reporter assays (84), and modeling using crystal structures (*e.g.*, SEPSECS–Sec-tRNA$^{[Ser]Sec}$, EEFSEC) (29), can help us better understand the complex Sec-insertion pathway in general and more specifically the effect of mutations in genes within this pathway and their consequences on selenoprotein expression. This knowledge will provide the essential basis for understanding the pathogenesis of human disease due to generalized or specific selenoprotein deficiencies and may enable the identification of therapies targeted at specific processes (*e.g.*, oxidative stress) in which selenoproteins play a key role.

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Abbreviations Used

CK = creatine kinase
CNS = central nervous system
C-terminal = carboxy-terminal
EEFSEC = Sec tRNA specific eukaryotic elongation factor
FT3 = free triiodothyronine
FT4 = free thyroxine
KO = knockout
mcm^5U = 5-methoxycarbonyl-methyluridine
mcm^5Um = 5-methoxycarbonylmethyl-2′-O-methyluridine
mRNA = messenger RNA
NMD = nonsense-mediated decay
N-terminal = amino-terminal
PCCA = progressive cerebellocerebral atrophy
PCH2D = pontocerebellar hypoplasia type 2D
RBD = RNA-binding domain
ROS = reactive oxygen species
RPL30 = ribosomal protein L30
Se = selenium
Sec = selenocysteine
SECIS = SEleniumCysteine Insertion Sequence
SECISBP2 = SECIS binding protein 2
SELENOP = selenoprotein P
SEPSECS = O-phosphoserine tRNA:Sec tRNA synthase
SID = Sec incorporation domain
T3 = triiodothyronine
T4 = thyroxine
tRNA = transfer RNA
UTR = untranslated region