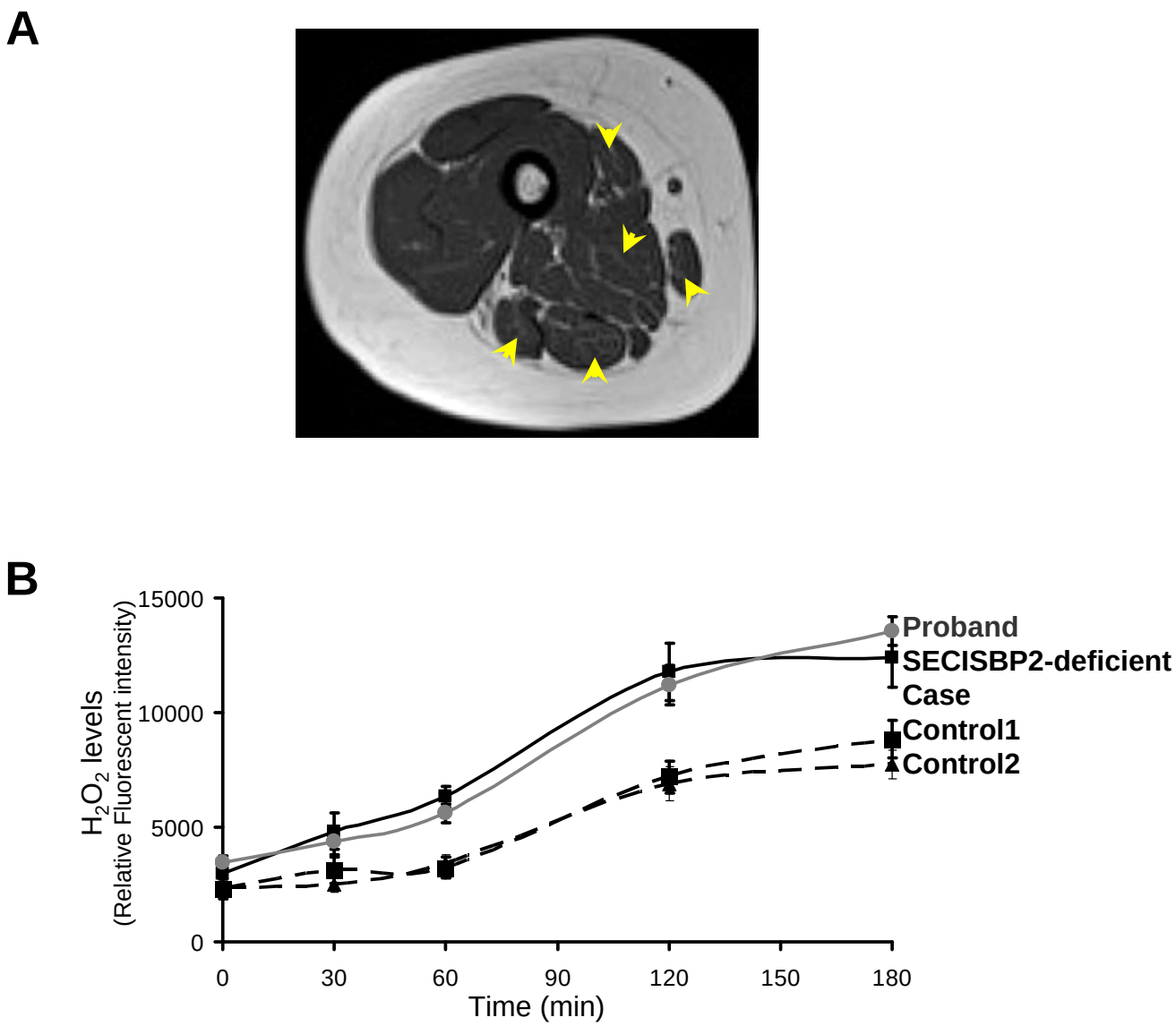
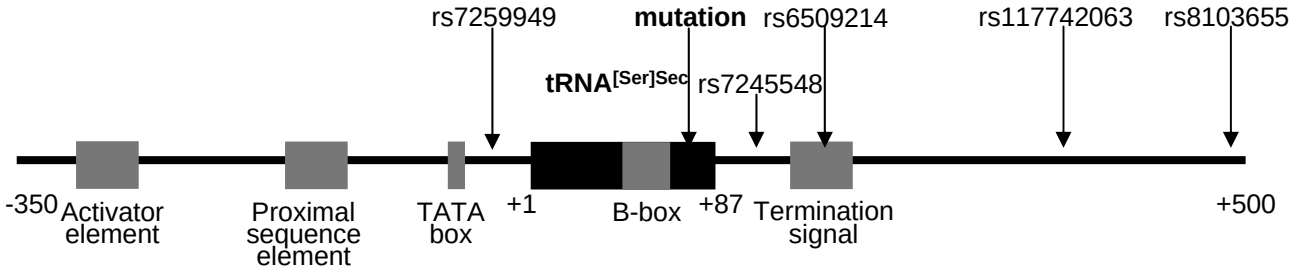
Supplementary data

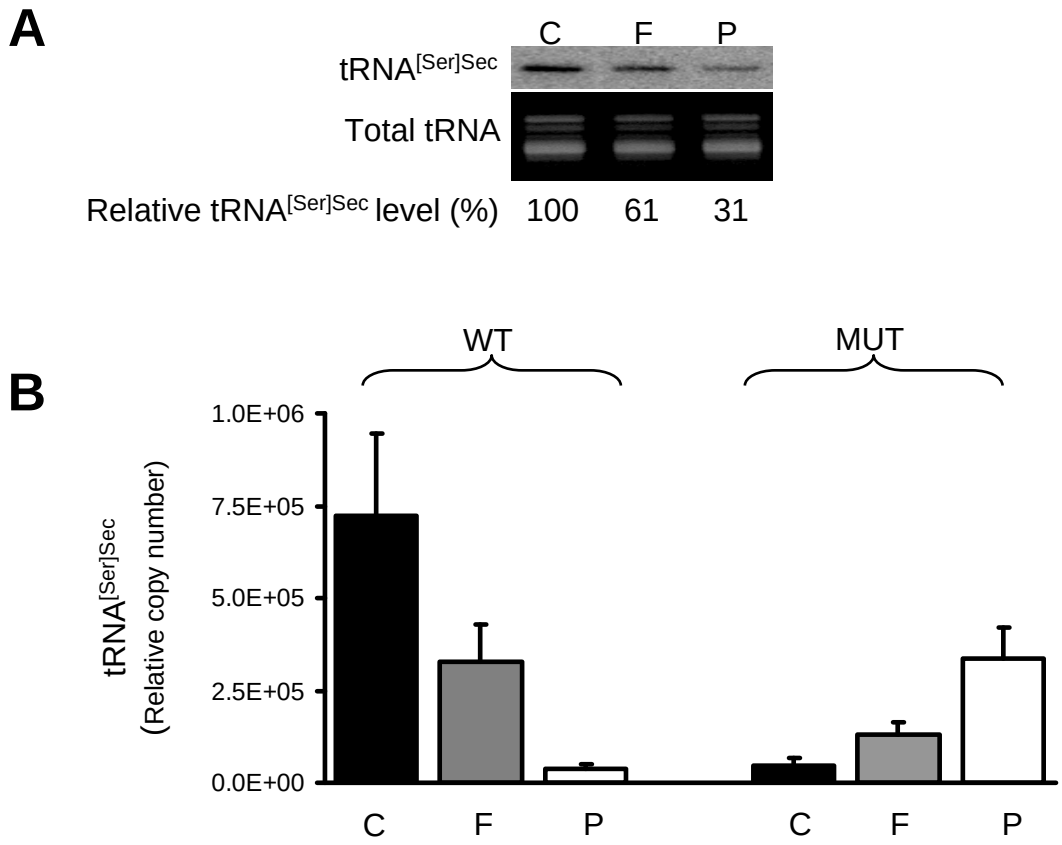


Supplementary Figure 1. Muscle imaging and cellular ROS in patient.
A. T1-weighted magnetic resonance imaging (MRI) scan showing mild signal change in adductor, sartorius, gracilis and semitendinosus muscles.
B. Endogenous H_2O_2 levels in dermal fibroblasts from proband, a patient with SECISBP2-deficiency and controls. Data represent mean ± SEM of at least 3 independent experiments, $P<0.05$ at all time points (2-tailed Student's *t* test).

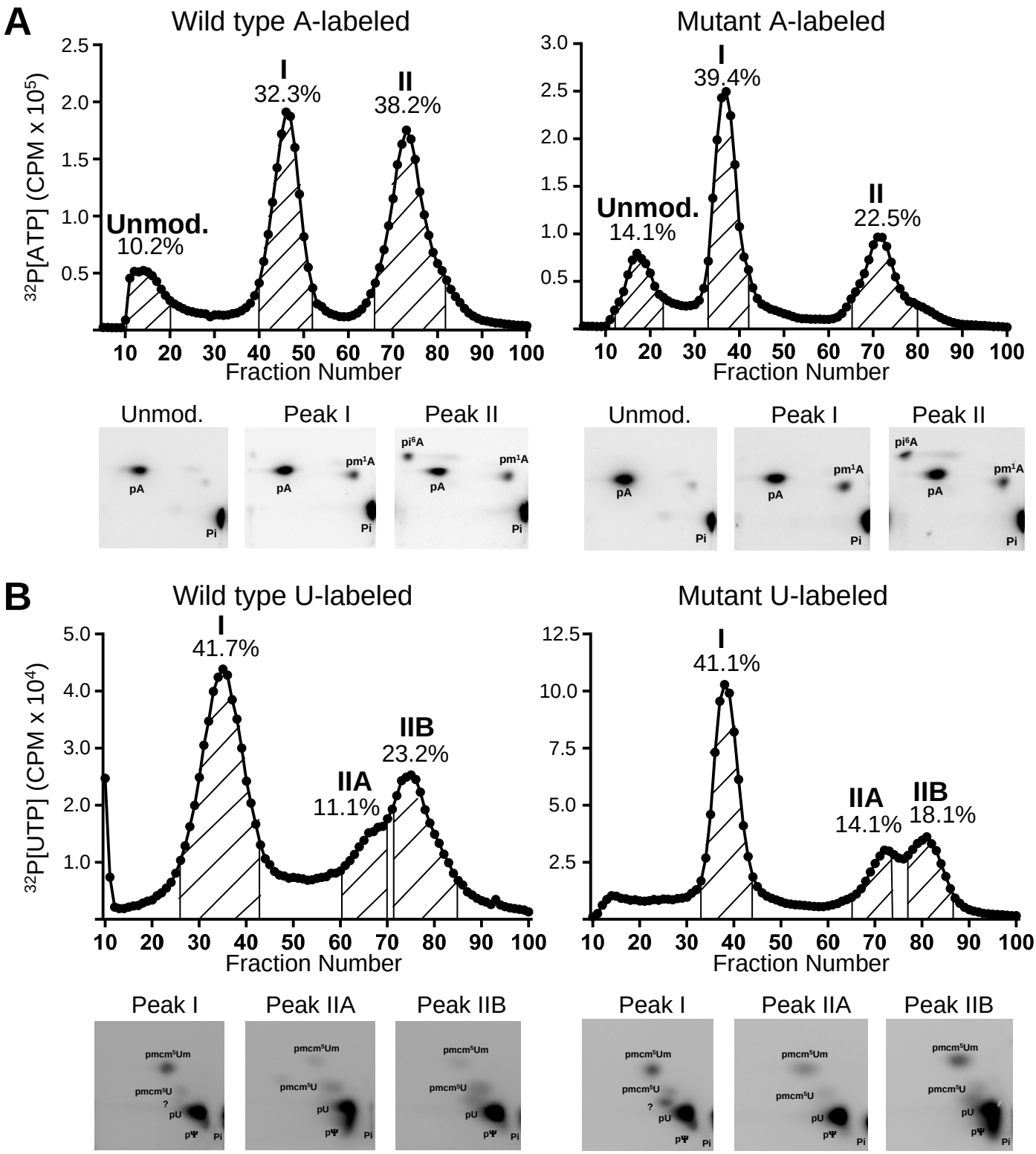


Position	dbSNP	Proband	Father	Mother	Sibling 1	Sibling 2	Sibling 3
-12	rs7259949	C/C	C/G	C/C	C/G	C/G	C/G
78	mutation	G/G	C/G	C/G	C/C	C/C	C/G
106	rs7245548	G/G	G/G	G/G	G/G	G/G	G/G
131	rs6509214	A/A	A/A	A/A	A/A	A/A	A/A
321	rs117742063	C/C	T/C	C/C	T/C	T/C	T/C
493	rs8103655	G/G	A/G	A/G	A/A	A/A	A/G

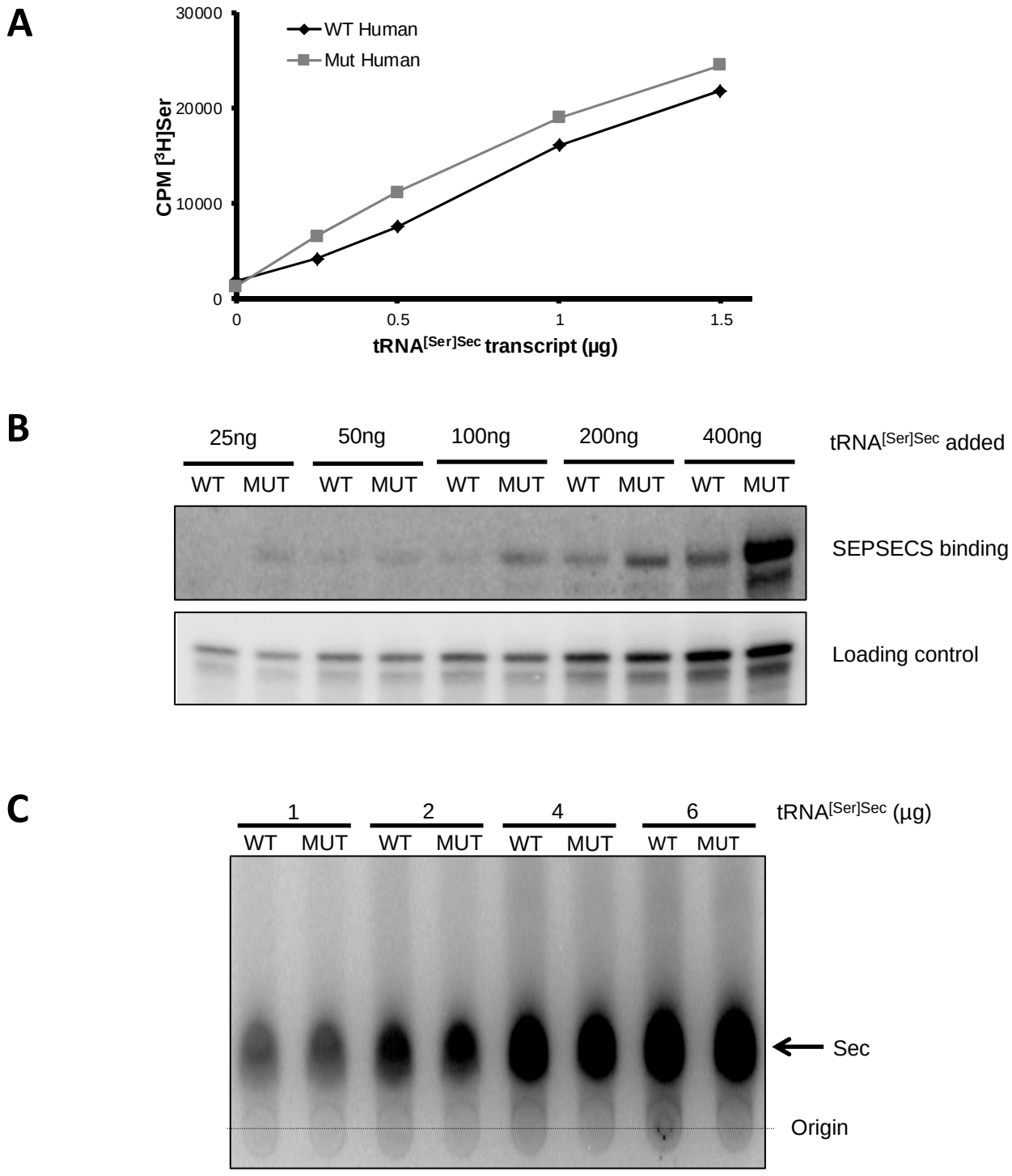
Supplementary Figure 2. The *TRU-TCA1-1* SNP genotypes in proband and family members. A schematic of the *TRU-TCA1-1* gene showing the coding region (black box) and regulatory elements (grey boxes) (27) with the location of nucleotide changes identified in the proband (arrowed). The genotypes of proband and family members at these locations are tabulated below.



Supplementary Figure 3. tRNA[Ser]Sec expression in patient-derived cells.
A. Northern blot showing levels of total and tRNA[Ser]Sec in primary cells from control (C), father (F) and proband (P). Experiment performed once.
B. Allele-specific qPCR measuring wild type (WT) or mutant (MUT) transcripts in primary cells from control (C), father (F) and proband (P). Data represent mean ± SEM of at least three independent experiments with $P<0.05$ between different individuals for WT or MUT tRNA[Ser]Sec (2-tailed Student's *t* test). Experimental details are given in Online Methods and References therein.



Supplementary Figure 4. Analyses of wild type and mutant tRNA$^{[Ser]Sec}$ expressed in *Xenopus* oocytes. Elution profiles of radiolabelled tRNA$^{[Ser]Sec}$ isoforms isolated from *Xenopus* oocytes expressing wild type or mutant tRNA$^{[Ser]Sec}$, labelled either with ATP (**A**) or UTP (**B**). 2D chromatograms of modified bases in tRNAs from each peak are shown in lower panels. The percent of each peak relative to the total counts recovered from the column runs are given in the figures. Experimental details are given in Online Methods and references therein, and a detailed account of the sequence of base modifications is found in reference 27. "Unmod." indicates unmodified radiolabelled tRNA$^{[Ser]Sec}$ transcript. Experiment performed once.



Supplementary Figure 5. Functional assays with wild type and mutant tRNA[Ser]Sec.
Assays of serine aminoacylation (**A**), interaction with Sec synthase (SEPSECS) (**B**) and selenocysteine synthesis (**C**) of either wild type (WT) or mutant (MUT) tRNA[Ser]Sec templates. Experimental details are given in Online Methods and references therein. Experiments performed once.

Supplementary Table 1: Oligonucleotides used to sequence, quantify and express tRNA $^{[Ser]Sec}$.

Primer	Sequence (5' to 3')
Sequencing Forward1 tRNA$^{[Ser]Sec}$	CCTGCTCAGAGCCTTTGG
Sequencing Forward2 tRNA$^{[Ser]Sec2}$	CTGGGCCCTTTAACAGCTT
Sequencing Forward3 tRNA$^{[Ser]Sec}$	CACGATAAATGAGTGGGGAGA
Sequencing Reverse1 tRNA$^{[Ser]Sec}$	TCCCCAAACCGTCTCATTTA
Sequencing Reverse2 tRNA$^{[Ser]Sec}$	AGTTATGGTCGCGTCCTTTG
Sequencing Reverse3 tRNA$^{[Ser]Sec}$	CGGGCAACAAGCAAAATAAG
RT tRNA$^{[Ser]Sec}$ WT	CCCGAAAG
RT tRNA$^{[Ser]Sec}$ MUT	CCCGAAAC
qRCR tRNA$^{[Ser]Sec}$ Forward	GATGATCCTCAGTGGTCTGGGGTGC
qRCR tRNA$^{[Ser]Sec}$ Reverse	TGGAATTGAACCACTCTGTCGC
Cloning Forward tRNA$^{[Ser]Sec}$	AGTTCTAGACTCGAGGGGCCAGGGTGAATCAGACTC
Cloning Reverse tRNA$^{[Ser]Sec}$	AGTAGCGGCCGCACGCGTCCGGAGGGGGAAATAAGTAACG

SUPPLEMENTAL METHODS

Characterisation of patients

All investigations were part of an ethically approved protocol (Cambridgeshire LREC 98/154) and/or clinically indicated being undertaken with prior informed patient consent. Serum free T4, free T3, and TSH were measured using an automated immunoassay system (Advia Centaur, Siemens) and reverse T3 by competitive RIA (Quest Diagnostics). Plasma selenium was measured by inductively coupled plasma mass spectrometry (ICPMS; Thermo Elemental). Red cell and plasma GPx were assayed by enzymatic analysis (Ransel) as described previously (12).

Selenoprotein and tRNA$^{[Ser]Sec}$ expression in patient-derived cells

Radiolabeled selenoprotein studies. Peripheral blood mononuclear cells, isolated by Ficoll gradient centrifugation, were cultured either in standard medium (DMEM plus 10% FBS) supplemented with 10 μCi ^{75}Se (University of Missouri research reactor facility) or in methionine-free medium supplemented with 50 μCi ^{35}S-Methionine (Perkin Elmer), harvested after 48 h, and analyzed by SDS-PAGE and autoradiography as described previously (4).

Western blotting. Dermal fibroblast cells, lysed in RIPA buffer containing a protease inhibitor cocktail, or blood plasma were analyzed by SDS-PAGE and Western blotting using specific antibodies as follows: Skin fibroblasts: SECISBP2 (rabbit polyclonal (13)); GPx4 (goat polyclonal EB07316, Everest Biotech); GPx1 (goat polyclonal ab50427, Abcam); SEPN1 (14) (customised rabbit polyclonal ab137); SELS (rabbit polyclonal

V6639, Sigma); SELT (15) and SELH (16) Blood plasma: SEPP1 (mouse monoclonal, LF-MA0141, AbFrontier) and GPx3 (mouse monoclonal, sc-58361, Santa, Cruz).

Quantitative real-time PCR (qPCR) assays. Selenoprotein transcripts were measured by qPCR as previously described (4). The comparative Ct method was used to quantify transcripts and normalize to cyclophilin A expression levels. Additional software was used to normalize data to expression levels in control cells (GeneSpring 7.2 software, Agilent) and generate heatmap (PARTEK genomics suite, Partek).

$tRNA^{[Ser]Sec}$ transcripts were quantified using the same system but with an adjusted method: reverse transcription was performed using specific primers for either wild type or mutant alleles (Supplemental Table 1), and incubated with template (1h at 37°C) before starting the reaction.

H_2O_2 generation in patient fibroblasts

Extracellular H_2O_2 accumulation in dermal fibroblasts (cultured in HAM's F12 plus 20% FBS), was assayed using Amplex Red (Invitrogen) and measurement of fluorescence according to the manufacturer's protocol. Values were adjusted for viable cell numbers quantified by the Cell TiterGLO assay (Promega).

Homozygosity mapping and *TRU-TCA1-1* sequencing

Homozygosity mapping. High-density single nucleotide polymorphism (SNP) genotyping of the proband and unaffected family members was undertaken by AROS Applied Biotechnology A/S (www.arosab.com), using the Affymetrix SNP 6.0 microarray

platform. The presence of potentially pathogenic copy number variants (CNVs) in the proband was excluded using the Chromosome Analysis Suite (ChAS) 2.1 analysis software (Affymetrix). Genotypic data was extracted using the Genotyping console (Affymetrix) and used for homozygosity mapping. Briefly, the haplotypes of affected and unaffected individuals within the family were compared to identify continuous runs of homozygosity (ROH) present only in the proband, applying the web-based HomozygosityMapper software tool (17) (http://www.homozygositymapper.org). The chromosomal locations of candidate genes involved in selenoprotein biosynthesis (Figure 3A) were superimposed on the regions of homozygosity identified in the proband.

The *SECISBP2* gene was sequenced as described (4) and $tRNA^{[Ser]Sec}$ 850 bp of the gene region were amplified using specific primers (Supplemental Table 1, Supplemental Figure 2) and sequenced using Big Dye Terminator V3.1 sequencing kit and the 3100 AVANT analyser (Applied Biosystems).

Mutant $tRNA^{[Ser]Sec}$ characterization

Assay of $tRNA^{[Ser]Sec}$ base modification in patient-derived cells. Total tRNA was isolated from control, father and proband EBV cells, aminoacylated (18) and fractionated on a RPC-5 column as described (19-21). The amounts of seryl-$tRNA^{[Ser]Sec}$ relative to the total seryl-$tRNA^{Ser}$ population, and of the distributions of the two $tRNA^{[Ser]Sec}$ isoforms, containing either mcm^5U or mcm^5Um at the wobble position, were determined as described previously (20, 21).

Assay of base modification of $tRNA^{[Ser]Sec}$ synthesised in vitro. [α-^{32}P]ATP and [α-^{32}P]UTP

(specific activity, 3000 Ci/mmol) were purchased from Perkin Elmer. [α-^{32}P]ATP and [α-^{32}P]UTP-labelled wild type and mutant tRNA$^{[Ser]Sec}$ transcripts were prepared as described (8, 22) using a Riboprobe in vitro transcription system (Promega) according to manufacturer's instructions. ^{32}P-labeled wild type and mutant tRNA$^{[Ser]Sec}$ transcripts were extracted using phenol:chloroform:isoamyl alcohol (125:24:1, pH 4.5) and tRNA was precipitated with 0.1 volumes of 3 M ammonium acetate and 3 volumes of ethanol. Transfer RNA was collected by centrifugation, washed with 70% ethanol, dried and dissolved in H_2O. Defolliculated *Xenopus* oocytes were obtained from Xenopus 1 (Dexter, MI) and maintained in OR-2 medium (5 mM HEPES, pH 7.8, 82.5 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $NaHCO_3$) at 25°C. Forty nanoliters of a mixture containing ~2 x 10^5 CPM of either [α-^{32}P]ATP or [α-^{32}P]UTP-labelled wild type or mutant tRNAs were injected into the cytoplasm of oocytes and the oocytes incubated at 25°C for 16 h. After incubation, tRNAs were extracted as described (23, 24) and chromatographed on an RPC-5 column (19) in a 0.525–0.675 M NaCl gradient as given (22). Column fractions were counted in a liquid scintillation counter, the peaks of tRNA isoforms pooled and used for minor base analysis by two-dimensional chromatography as described (7, 9, 22, 25).

Aminoacylation, interaction with SEPSECS and Sec synthesis with wild type and mutant tRNA$^{[Ser]Sec}$ Increasing amounts of wild type and mutant Sec tRNA transcripts were incubated with [^{3}H]Ser and seryl-tRNA synthetase (SARS), quantifying aminoacylation efficiency as described previously (18, 20, 21) .

Wild type and mutant tRNA$^{[Ser]Sec}$ transcripts were incubated with His-tagged SEPSECS,

complexes isolated with anti-His agarose and analysed by gel electrophoresis, hybridized with [^{32}P]-labelled tRNA oligonucleotide and autoradiography as described previously (26).

Sec synthesis reactions, containing necessary components (SARS, PSTK, SEPSECS and SEPHS2), were programmed with either wild type or mutant tRNA$^{[Ser]Sec}$ transcripts. The ability of transcripts to direct [^{75}Se]Sec formation was analysed by thin layer chromatography as described previously (26).

tRNA$^{[Ser]Sec}$ expression in fibroblasts. Wild type or mutant tRNA$^{[Ser]Sec}$ genes (-420 bp to +257 bp) were PCR amplified (primers listed in Supplemental Table 1), cloned into pUC19 and verified by sequencing. Dermal skin fibroblasts were transiently transfected (Neon Transfection System, Life Technologies) as described in their protocol "Guidelines for reprogramming fibroblast" (part number: A14702PIS). In short, 5 x 10^6 cells (75-90% confluent, DMEM/F12+20%FBS+PSF) were trypsinised, pelleted and resuspended in 100 µl buffer R. Cells were electroporated (pulse voltage: 1650V; pulse width:10 msec; pulse number: 3) with 8 µg of expression vector, cultured in six-well plates and radiolabelled with ^{75}Se and ^{35}S-methionine as described.

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