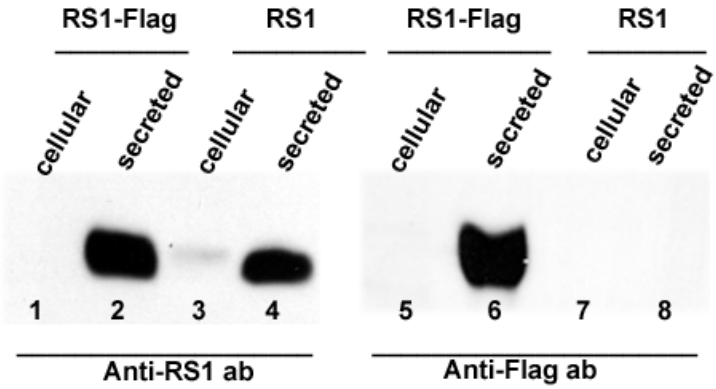
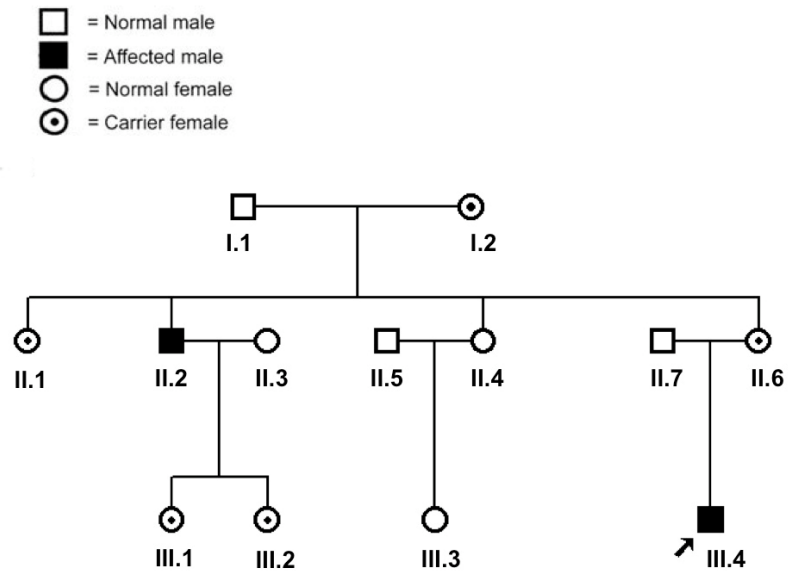
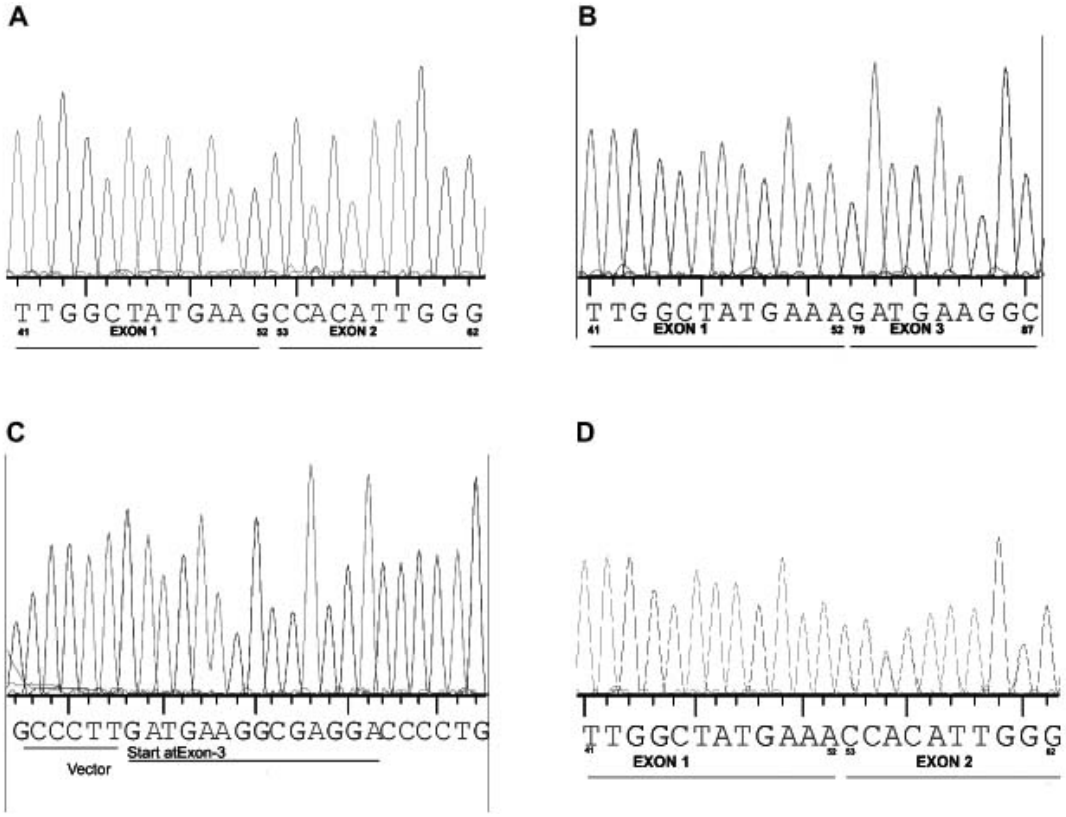


Supp. Figure S1. Expression analysis of RS1 in secreted and cellular fractions derived from COS-7 cells transiently transfected with either *RS1* or *RS1*-Flag cDNA constructs. At 72 h post-transfection cultures were harvested, and cellular and secreted fractions were analyzed for RS1 protein by immunoblotting using anti-RS1 rabbit polyclonal antibody raised against the N terminus amino-acid residues 24–37 of RS1 or with an anti-Flag monoclonal antibody (Stratagene). Results indicate that the addition of Flag epitope to RS1 C-terminus did not alter the secretory nature of RS1, and both RS1 (lane 4) and RS1-Flag (lane 2) were secreted into the medium. While anti-RS1 antibody detected both forms of RS1 (RS1-Flag and RS1; lanes 2, 4) anti-Flag antibody selectively identified only the epitope tagged RS1-Flag (lane 6), but not RS1 (lanes 7, 8). These findings confirm the specificity of the Flag antibody and utility of the epitope tag system in selective identification of a particular variant such as a Mut *RS1* in cells co-expressing both the WT and *RS1* mutants.



Supp. Figure S2. Pedigree of the XLRS family harboring the c.52G>A mutation of *RS1*. The proband is indicated by an arrow. All female carriers were genetically identified.



Supp. Figure S3. The c.52G>A mutation causes differential RNA splicing. Sequence traces of the subcloned full length RT-PCR products: full length RT-PCR products were purified and subcloned into Zero Blunt TOPO PCR vector. Plasmids were prepared from colonies and sequenced. **(A)** Sequence trace of WT control transcript showing normal splicing between exons 1 and 2 and **(B–D)** sequence traces of c.52G>A variant transcripts: **B**, exon-2 skipping; **C**, exons-1 and -2 skipping; **D**, normal splicing.

Supp. Table S1. List of mutations found in XLRS patients with primer sequences used in *RS1* cloning and mutagenesis

	DNA Level	Protein Level	Sense Primers Used in mutagenesis **
1.	c.1A>T	p. Met1Leu	5'-**T**tgtcacgcaagatagaaggcttt-3'
2.	c.35T>A	p.Leu12His	5'-gaaggctttttgttattac**A**tctctttggctatgaagccaca-3'
3.	c.37C>T	p.Leu13Phe	5'-gaaggctttttgttattactt**T**tctttggctatgaagccaca-3'
4.	c.38T>C	p.Leu13Pro	5'-gaaggctttttgttattacttc**C**ctttggctatgaagccaca -3'
5.	c.214G>A	p. Glu 72 Lys	5'-cccatatcacaagcctctgggtttc**A**agtcaggggaggtcacaccggac-3'
6.	c.276G>C	p. Trp 92 Cys	5'-ccggagcagtatgtgggctg**C**tattcttcgtggactgcaaac-3'
7.	c.286G>C	p.Trp 96Cys	5'-gtgggctggtattcttcgtg**C**actgcaaacaaggcccggctcaac-3'
8.	c.304C>T	p.Arg102Trp	5'-tcttcgtggactgcaaacaaggcc**T**ggctcaacagtcaaggctttgggtgt-3'
9.	c.535A>G	p.Asn179Asp	5'-aacaaccgggtcttctatggc**G**actcggaccgcacctccacggtt-3'
10.	c.574C>T	p.Pro192Ser	5'-tccacggttcagaacctgctgcgg**T**cccccatcatctcccgcttcatccgc-3'
11.	c.598C>T	p.Arg200Cys	5'-ccatcatctcccgcttcatc**T**gcctcatcccgctgggctggcacg-3'
12.	c.673C>T	p.Arg213Trp	5'-ctgggctggcacgtccgcattgccatc**T**ggatggagctgctggagtgcgtc-3'

List of Primers used in cloning

13.	Not1 primer (sense)	5' -ggc ggc cgc gcc acc atg tca cgc aag ata gaa ggc tt-3'
14.	Xho1 primer (antisense)	5'-ctcgag tca ggc aca ctt gct gac gcac-3'
15.	Xho1 primer (antisense without stop codon)	5'-ctcgag ggc aca ctt gct gac gcac-3')
16.	Internal Sense (nt 206–228)	5'-tgggtttcgagtcaggggaggtc-3'
17.	Internal Antisense (nt 553–531)	5'-gat gaa ggc ga g gac ccc tgg t-3'
18.	Minigene: Not1-EcoR1 sense*	5'-gag gcg gcc gcg cca cca tgt cac gca aga tag aag gc tt-3'
19.	Minigene: EcoR-Not1 antisense*	5'-acg aat tct tat tag att cca gtg gta ctg ggt at-3'
20.	Minigene: EcoR-BamH1sense+	5'-agg aat tca ctg cta ggg tta aat gaa agg gaa ag-3'
21.	Minigene: EcoR-BamH1antisense+	5'-gtg gat cct cgc ctt cat cct gca-3'

** Mutant residues are shown in upper case. *RS1* gene: The numbering follows GenBank NCBI Reference Sequence: NM_000330.3. Nucleotide 1 is A of the ATG initiation codon (CDS 36–710). RS1 protein: NP_000321.1.

Primers-18-20: PCR amplification of human genomic DNA from a normal control with primers based in exon1, intron1, exon 2, intron 2 and exon 3. *0.56 kb genomic fragment containing the exon 1 and the first 507 bp of intron 1 with Not1 and EcoR1 flanking sites; +1.7 kb second genomic fragment containing 3' sequences of intron 1 (the terminal 764 bp) in frame with exon 2, intron 2, and the first 17 nucleotides of exon 3 with EcoR1 and BamH1 flanking sites.