Missense *SLC25A38* variations play an important role in autosomal recessive inherited sideroblastic anemia

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Supplementary Design and Methods

Isolation of DNA and mutation analysis

Cardiff (UHW)

Genomic DNA was extracted from peripheral blood white cells using salt extraction.[1] Primer pairs (*Online Supplementary Table S1*) were designed using the Primer3 program (*http://frodo.wi.mit.edu/primer3*)[2] with a subsequent specificity check using NCBI Blast, to amplify all seven exons, exon-intron boundaries, 500 bp upstream of the initiation codon and 700 bp downstream of the chain termination codon of the gene (ENSG00000144659) in nine polymerase chain reactions (PCR) using AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). All primers were synthesized by Applied Biosystems (Cheshire, UK) with one of two additional 17 bp M13-derived 5' tails identical for forward or for reverse primers. The PCR products were examined by 2% agarose gel electrophoresis, purified using spin columns (High Pure Product Purification Kit, Roche Diagnostics, Mannheim, Germany) and sequenced with universal primers for each direction using Big Dye Terminator Cycle Sequencing Kit v 3.1 (Applied Biosystems, Foster City, CA, USA). Terminated sequences were purified using DyeEx 2.0 spin kit (Qiagen, CA, USA) and analyzed on the 3130xl sequence analyzer (Applied Biosystems, Foster City, CA, USA).

Paris (APHP)

Genomic DNA was extracted from peripheral blood using the QIAamp DNA blood Mini Kit (Qiagen, CA, USA). Analysis of the *SLC25A38* gene (Genbank mRNA: NM_017875, Genbank protein: NP_060345) was performed by direct sequencing. The seven exons of *SLC25A38* and the exon-intron junctions were amplified by PCR using previously published primer sequences[3] except for exon 7 (*Online Supplementary Table S1*). All exon primers were synthesized by Eurogentech (Belgium). PCR products were examined by gel electrophoresis and purified by treating the reaction mixture with exosap (GE Healthcare, Piscataway, NJ, USA). After purification of PCR products both strands were sequenced using a Big Dye Terminator Cycle Sequencing kit (Applied Biosystems Life Technologies, Carlsbad, CA, USA), purified (Sephadex G50, GE Healthcare, Piscataway, NJ, USA) and sequencing products were analyzed using a 3130xl DNA sequencer (Applied Biosystems Life Technologies, Carlsbad, CA, USA) and the Seqscape analysis software (v2.6).

Barcelona (IMPPC)

Genomic DNA was extracted from 5 mL of peripheral whole blood using the salting out method.[1] The exons and exon-intron boundaries of *SLC25A38* were amplified in nine PCR reactions with primers pairs (*Online Supplementary Table S1*) designed with the Primer3 programme (*http://frodo.wi.mit.edu/primer3*)[2] and synthesized by Invitrogen (Carlsbad, CA, USA). The PCR products were examined by electrophoresis in a 2% agarose gel and purified using a spin column purification kit (Illustra™ GFX™ PCR DNA and Gel Band Purification Kit, GE Healthcare). Sequencing was conducted under Bigdye™ terminator cycling conditions and the reacted products were analyzed on an Automatic Sequencer 23 ABI 3730XL (Applied Biosystems, Foster City, CA, USA). Sequence traces were analyzed using the DNA variant analysis software Mutation Surveyor™ (Softgenetics, State College, PA, USA).

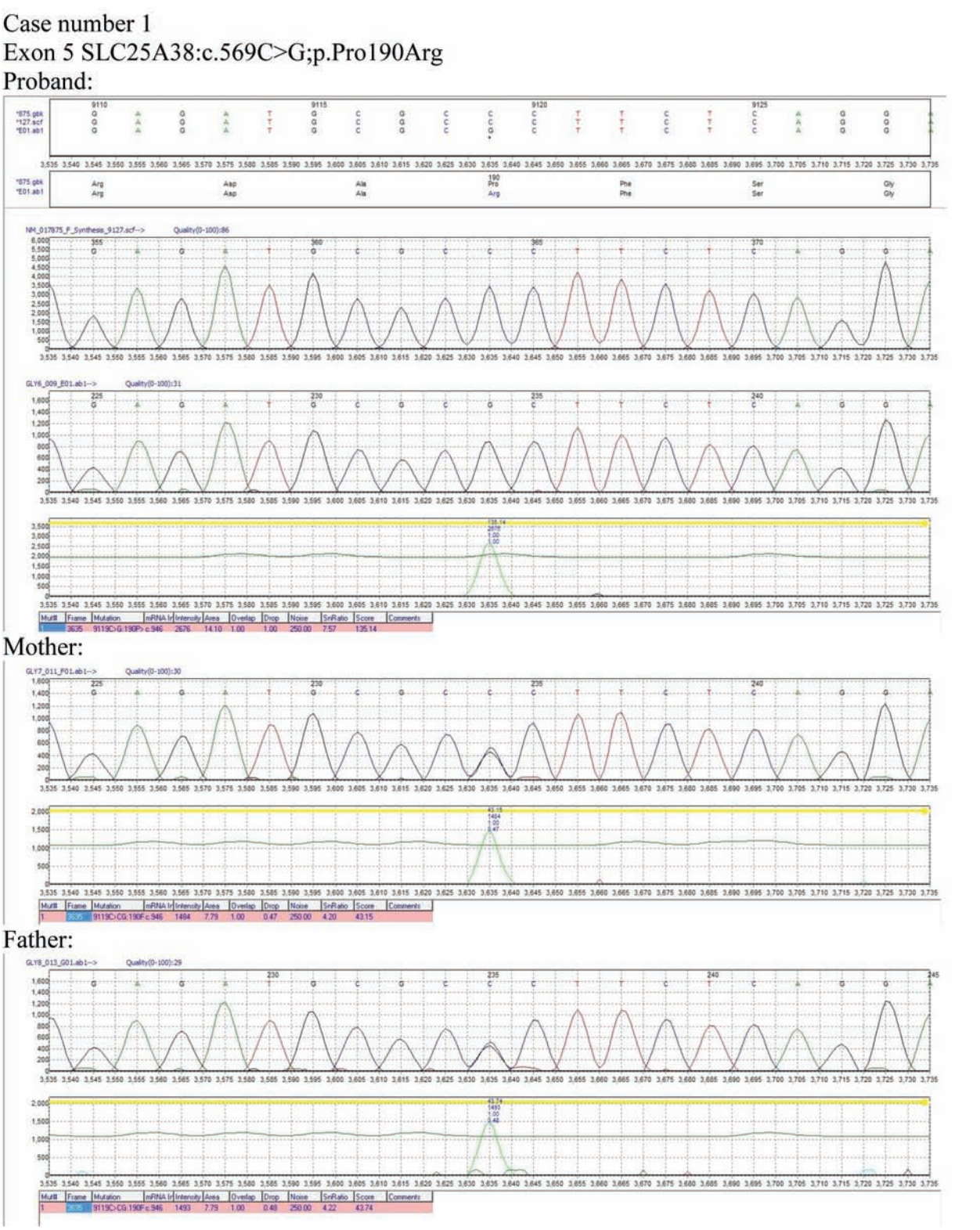
References

1. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.
2. Rozen S, Skaletsky HJ. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol. 2000;132:365-86.
3. Guernsey DL, Jiang H, Campagna DR, Evans SC, Ferguson M, Kellogg MD, et al. Mutations in mitochondrial carrier family gene SLC25A38 cause nonsyndromic autosomal recessive congenital sideroblastic anemia. Nat Genet. 2009;41(6):651-3.

Online Supplementary Table S1. SLC25A38 primers used for PCR and sequence analysis.

Exon	Forward primer	Reverse primer	Product size (bp)
UHW			
exon 1a	GTAGCGCGACGGCCAGTGTTGGCTAGTGGCTGCCTTACT	CAGGGCGCAGCGATGACCTCTCTGACGTCTGCGCCTATAA	477
exon 1b	GTAGCGCGACGGCCAGTGTAATTCCCGCAGCAAGATTGT	CAGGGCGCAGCGATGACCCCCTTCTCAATTCCCTTAGTCA	490
exon 2	GTAGCGCGACGGCCAGTAGGCACCACCAGGTAAGTGTCTA	CAGGGCGCAGCGATGACATGGCCTGTGTTTCTCTCTGAAC	390
exon 3	GTAGCGCGACGGCCAGTGTGTGCCAACCTACGAACACATA	CAGGGCGCAGCGATGACACATATCCCTGAGCCTTCAAACA	457
exon 4	GTAGCGCGACGGCCAGTTATTGGTGTTCCTTCCACACCTT	CAGGGCGCAGCGATGACTCACATATGGCTTGTCTGACCAC	481
exon 5	GTAGCGCGACGGCCAGTCTGATGTGGTCAGACAAGCCATA	CAGGGCGCAGCGATGACATCCCAGTTCCTGACACAATCAT	473
exon 6	GTAGCGCGACGGCCAGTCGTAGCCTCATTTGAATCCAGAC	CAGGGCGCAGCGATGACCCTAGATTTTAACCTGGGCATGG	457
exon 7a	GTAGCGCGACGGCCAGTCCCATTATTCTTACTTTGGGCA	CAGGGCGCAGCGATGACGGGAAAATGCCTTTCCAAGT	579
exon 7b	GTAGCGCGACGGCCAGTCAGCCTTCAGAATCTCCAAAAGA	CAGGGCGCAGCGATGACAGAAAAGGGTTTGGTCCATTTTT	634
APHP			
exon 1	TCTACAGAGTTCCTCCGGC	AAAGGGTAGCCGAGCCTTAG	520
exon 2	GCTGGTCAGGTATAGAGAAAGG	CATCCAACAGAATGGAAGTTG	289
exon 3	TTGAGTGGGGAATTGTTTTATG	TCTCACATATCCTTAAGAGCTGG	255
exon 4	TTAAAGTGTTTGGTCTTTGATTTTC	TTCACATATGGCTTGTCTGACC	362
exon 5	CTGCAGTCTGCTTGTTCAGTG	TCATATCCCAGAGAAAATGGTG	315
exon 6	GGAAGAATTGGTGGGCAAC	GAGTGAAGGGTAAGAACTACTGCTC	329
exon 7	AACAGAGACCCTCACTGTGGTA	CATCTTACTGCAGAATAGTAAGAAGC	298
IMPPC			
exon 1	GTTCCACGCAAAAGCAAAGT	AAAGGGTAGCCGAGCCTTAG	670
exon 2	AAAGGAATTTGCTGGTCAGG	CATCCAACAGAATGGAAGTTGA	299
exon 3	CCAAGGTGCATTGTAGAGATTG	AAGAGCTGGTAAGGTAGATGAGAAA	299
exon 4	CACTTGCATGCGAATCATCT	CAGGAGTTGACATCGGTGGT	367
exon 5	GGTCAGACAAGCCATATGTGAA	TCATATCCCAGAGAAAATGGTG	364
exon 6	GGAAGAATTGGTGGGCAACT	AAGAGATCCTTAAACACCACAAGAA	300
exon 7a	ACAGAGACCCTCACTGTGGT	TGGAGATTCTGAAGGCTGAAA	400
exon 7b	CAAGGGCTGCTGCTTCTTAC	GAGCCTCTGAGAAGTTAACTGAGAA	488
exon 7c	ACCAGGGAAGACTGGATGTG	TGGTTGCATCATTCAGTAAAGC	460

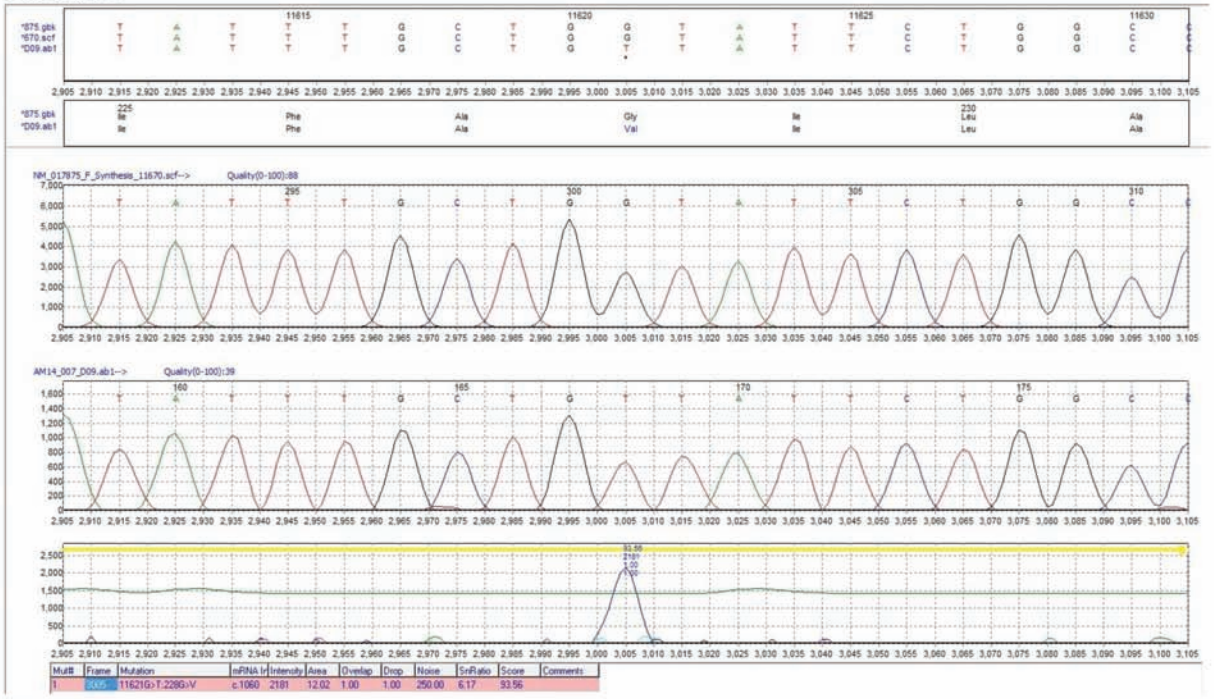
Online Supplementary Figure S1. This shows relevant portions of the sequence files from the cases 1 to 11 with *SLC25A38* variations probably responsible for their congenital sideroblastic anemia. These are visualized using Mutation software Surveyor V3.25 (SoftGenetics LLC, PA USA; distributed by BioGene Ltd., Cambridge, UK).



Case number 2

Exon 6 SLC25A38:c.683G>T,p.228Gly>Val

Proband:



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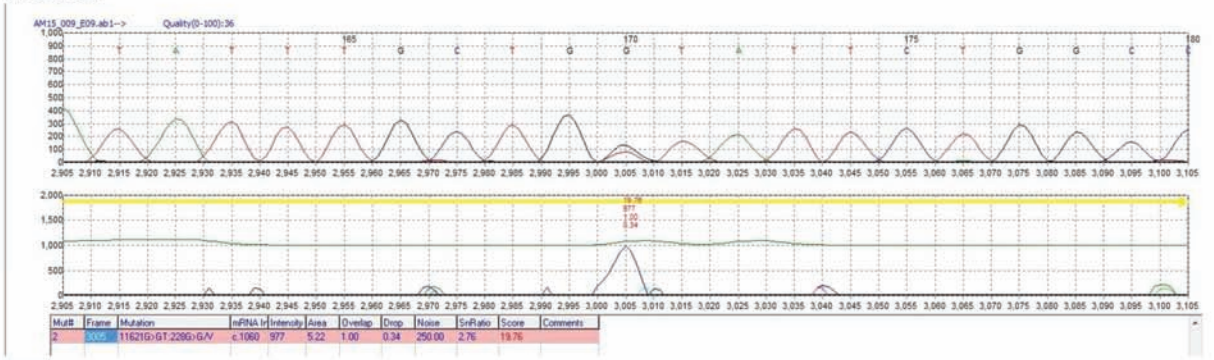

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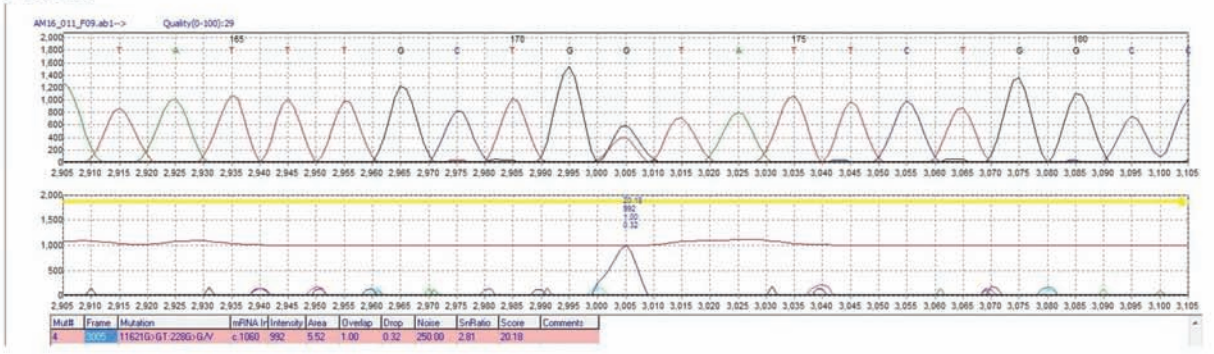
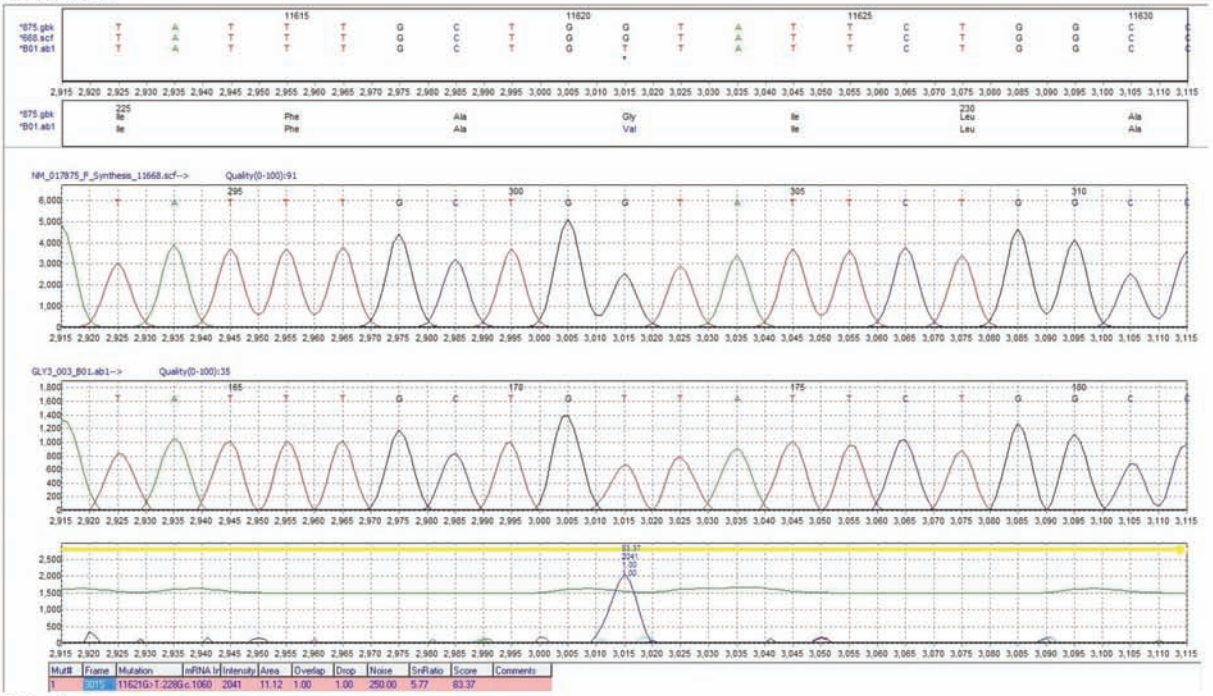


Case number 3

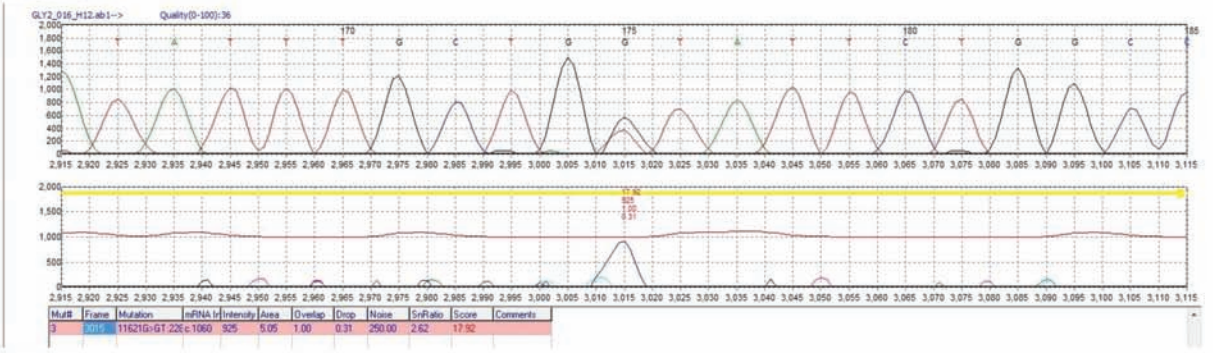
Exon 6 SLC25A38:c.683G>T;p.Gly228Val

Proband:



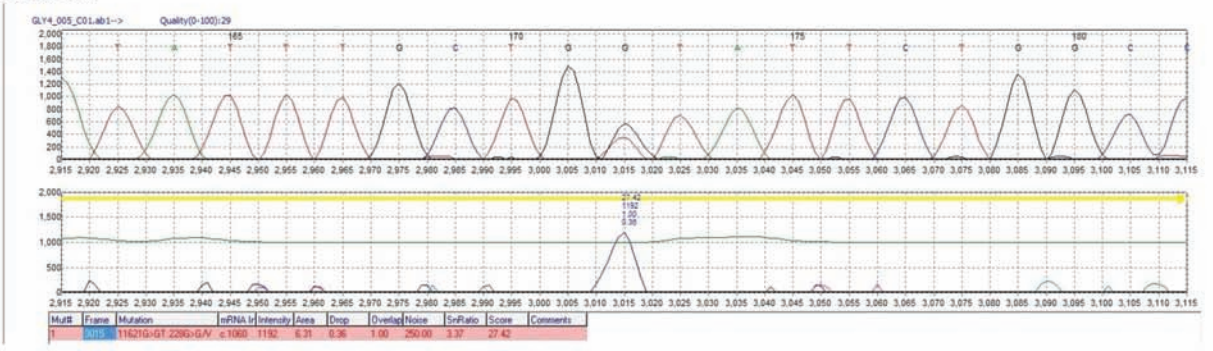
Mut#	Frame	Mutation	mRNA Ir	Intensity	Area	Overlap	Drop	Noise	SnRatio	Score	Comments
1	3015	11621G>T:228G:c.1060	2041	11.12	1.00	1.00	250.00	5.77	83.37		

Mother:



Mut#	Frame	Mutation	mRNA Ir	Intensity	Area	Overlap	Drop	Noise	SnRatio	Score	Comments
3	3015	11621G>GT:228:c.1060	925	5.05	1.00	0.31	250.00	2.62	17.92		

Father:



Mut#	Frame	Mutation	mRNA Ir	Intensity	Area	Drop	Overlap	Noise	SnRatio	Score	Comments
1	3015	11621G>GT:228G>G/V	c.1060	1192	6.31	0.36	1.00	250.00	3.37	27.42	

Case number 4 (compound heterozygote)

Exon 5 SLC24A38:c.[560G>A]+[625G>C];p.[Arg187Gln]+ [His209Asp]

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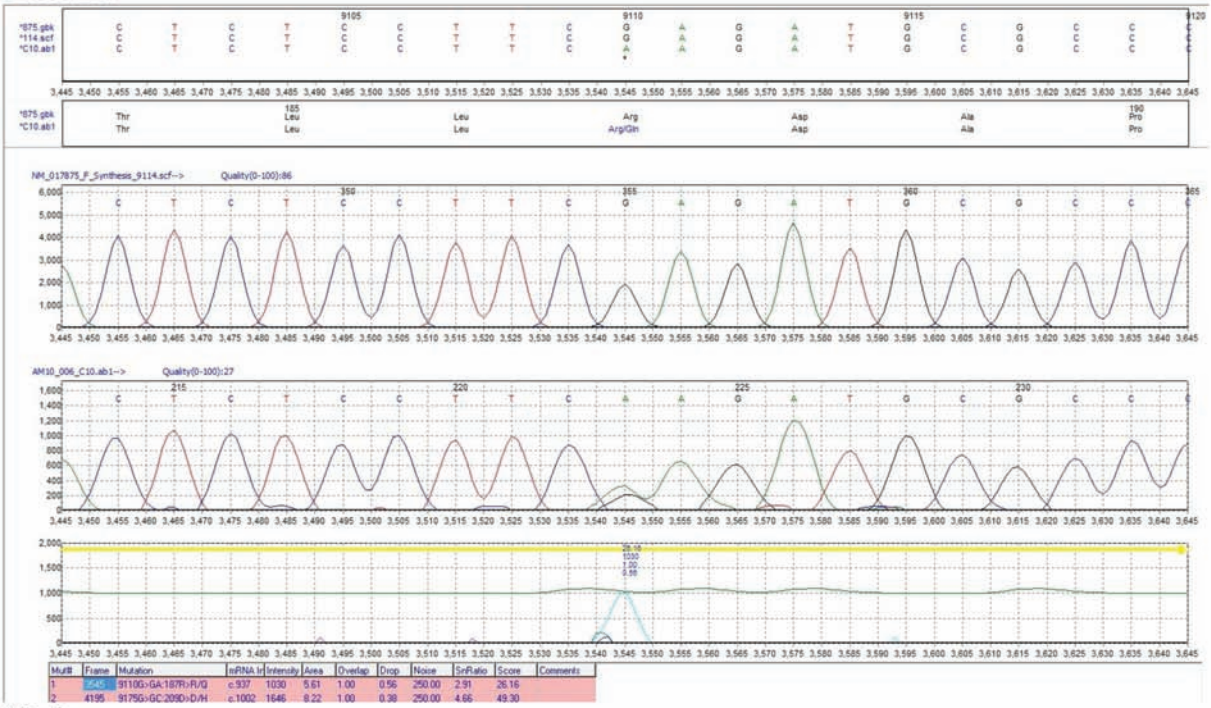
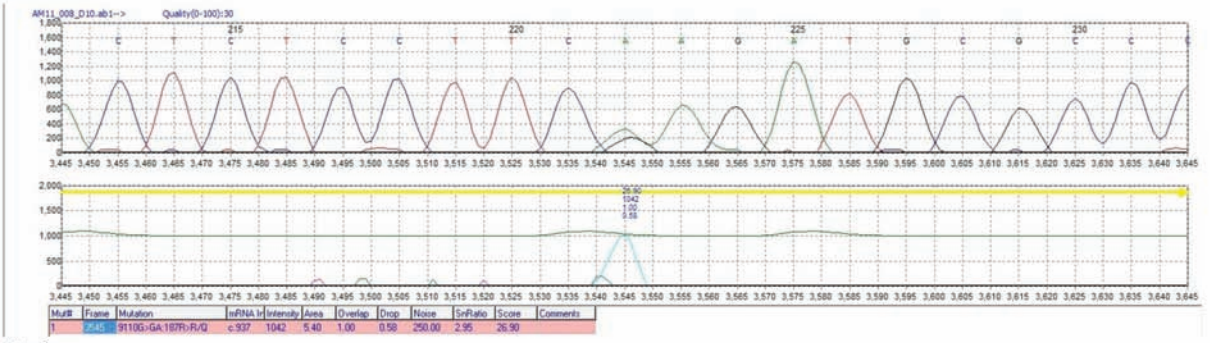


Mut#	Frame	Mutation	mRNA Irr	Intensity	Area	Overlap	Drop	Noise	SnRatio	Score	Comments
1	2645	9110G>GA:187R>R/Q	c.937	1030	5.61	1.00	0.56	250.00	2.91	26.16	
2	4195	9175G>GC:209D>D/H	c.1002	1646	8.22	1.00	0.38	250.00	4.66	49.30	

Mother:



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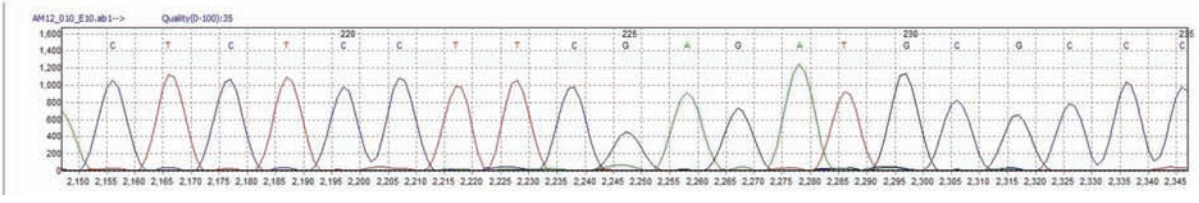
Father:



Exon 5 SLC25A38:c.[560G>A]+[625G>C];p.[Arg187Gln]+[His209Asp]

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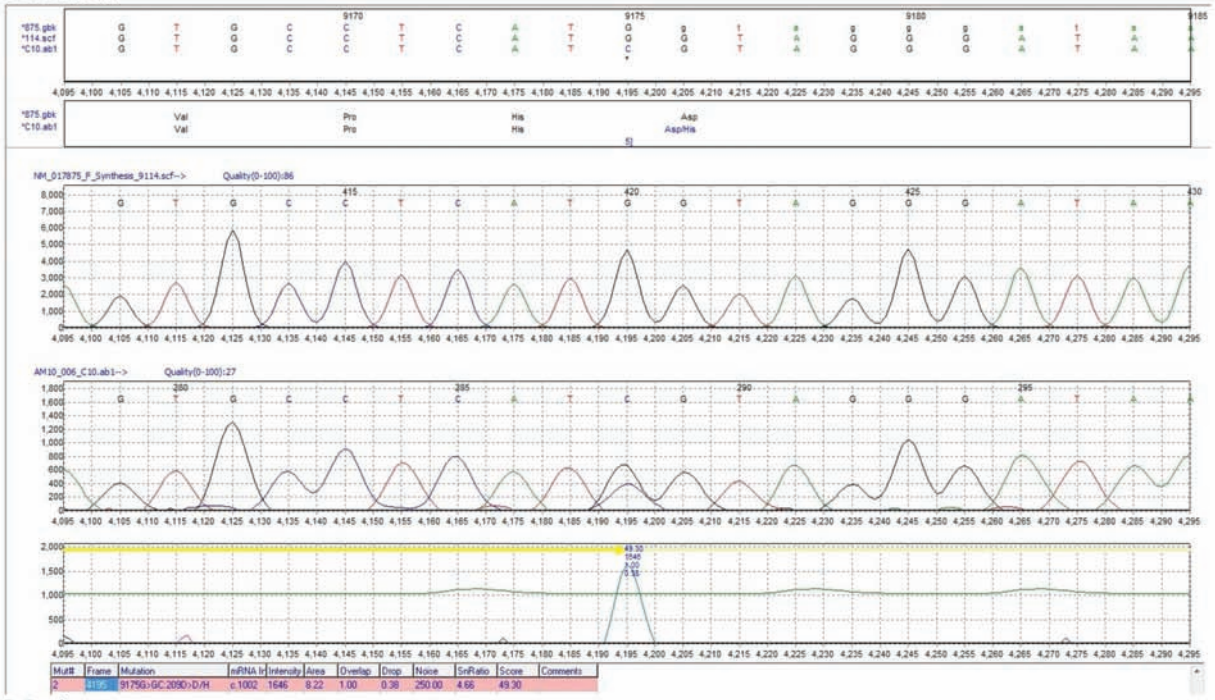

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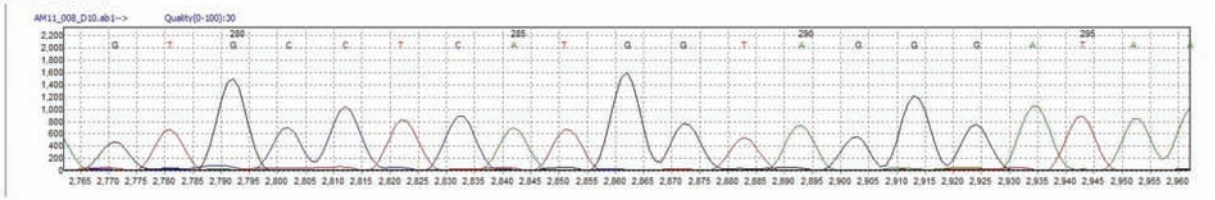

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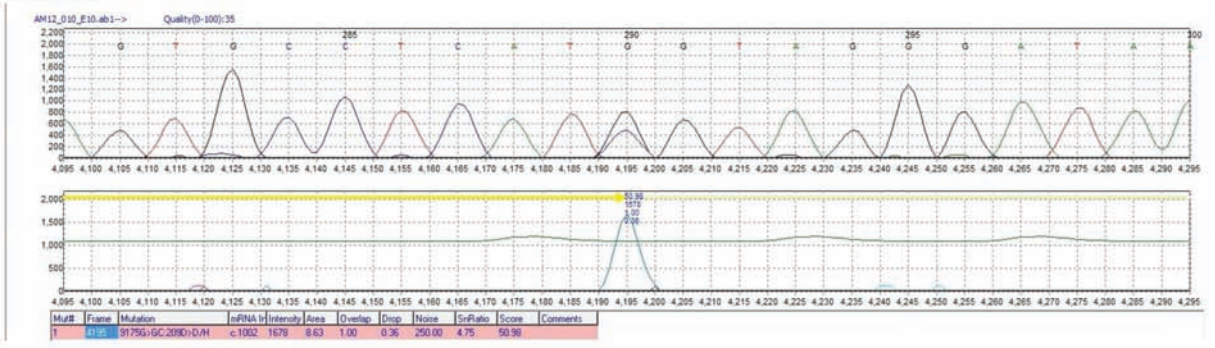
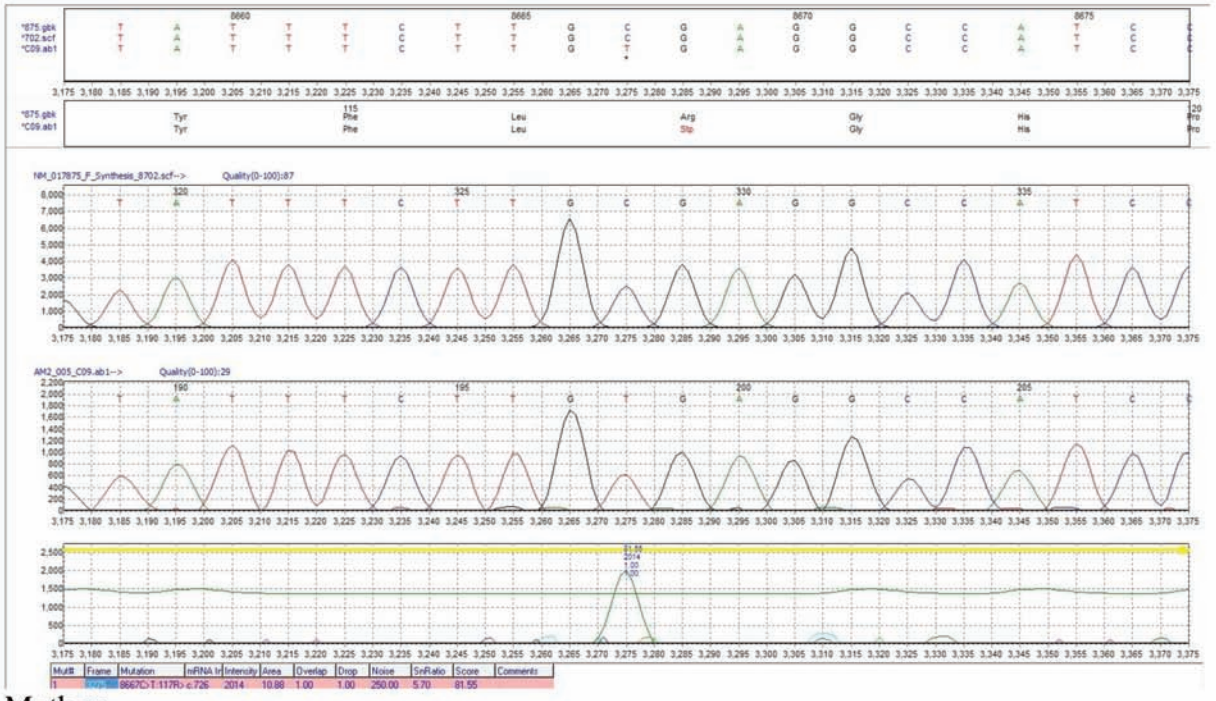


Case number 5

Exon 4 SLC25A38:c.349C>T;p.Arg117Stop

Proband:



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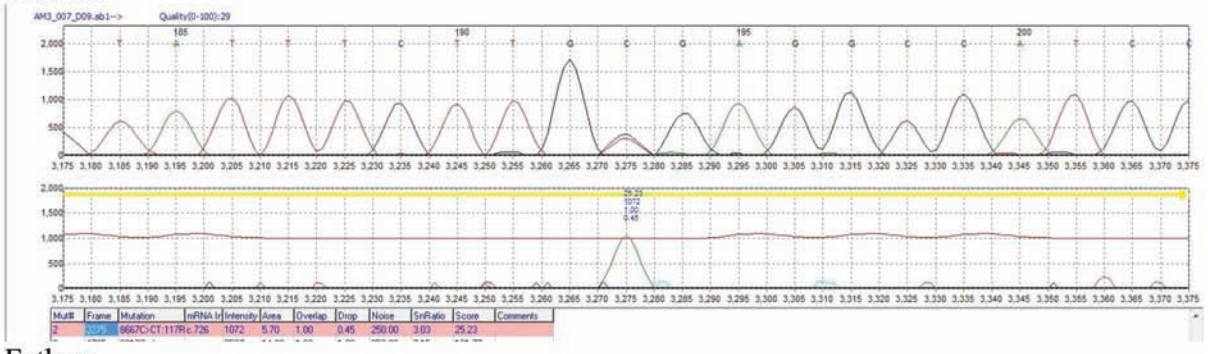

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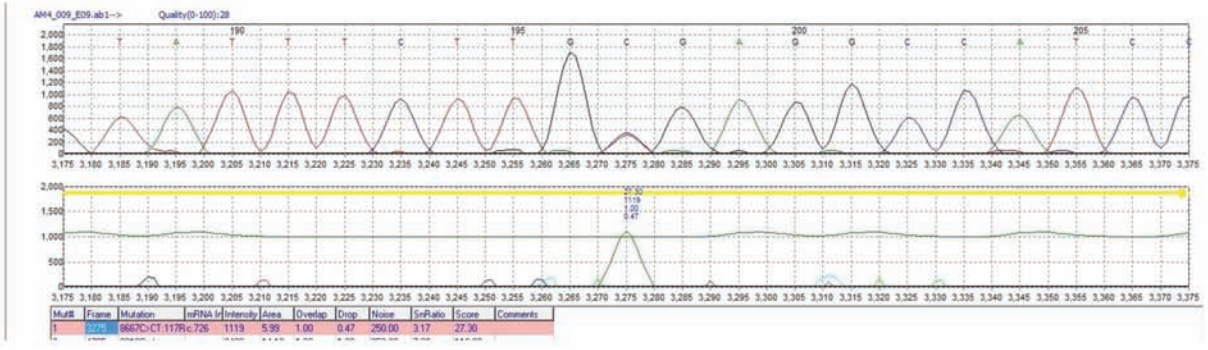
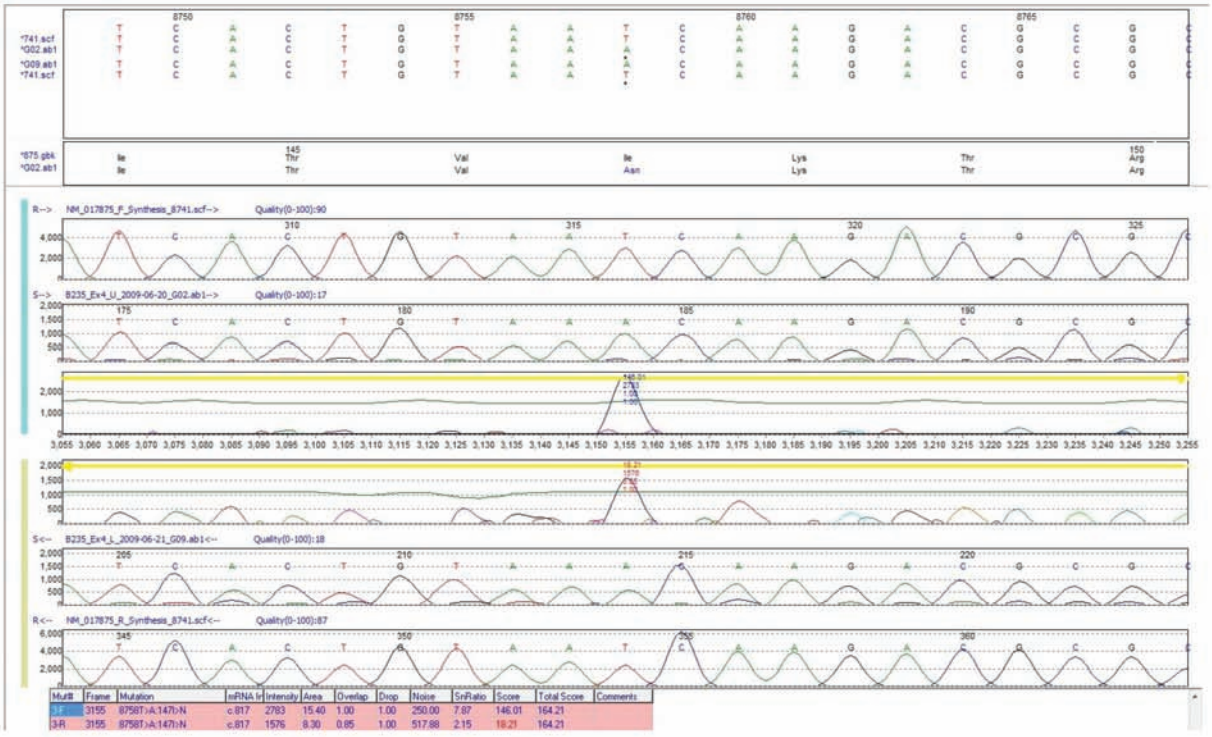
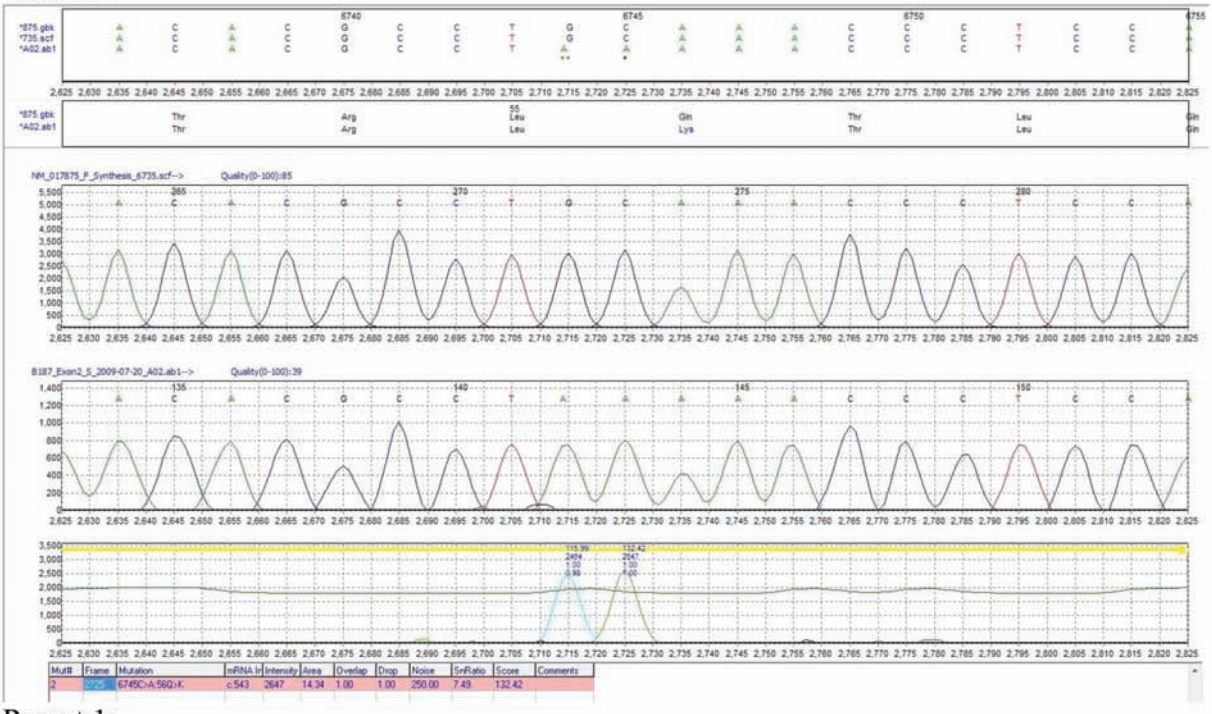


Case number 6:
Exon 4 SLC25A38:c.440T>A;p.Ile147Asn
Proband:



Case number 7:
Exon 2 SLC25A38:c.166C>A;p.Gln56Lys (please note c.165G>A is a common synonymous coding SNP)
Proband:



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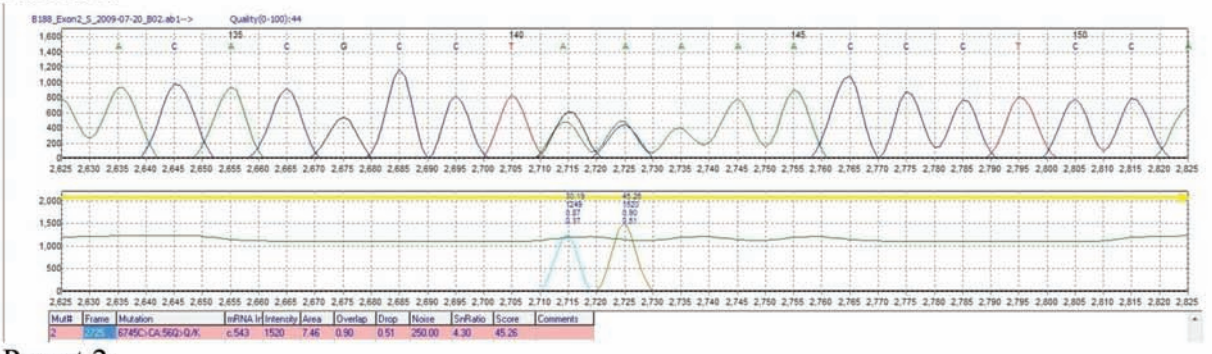

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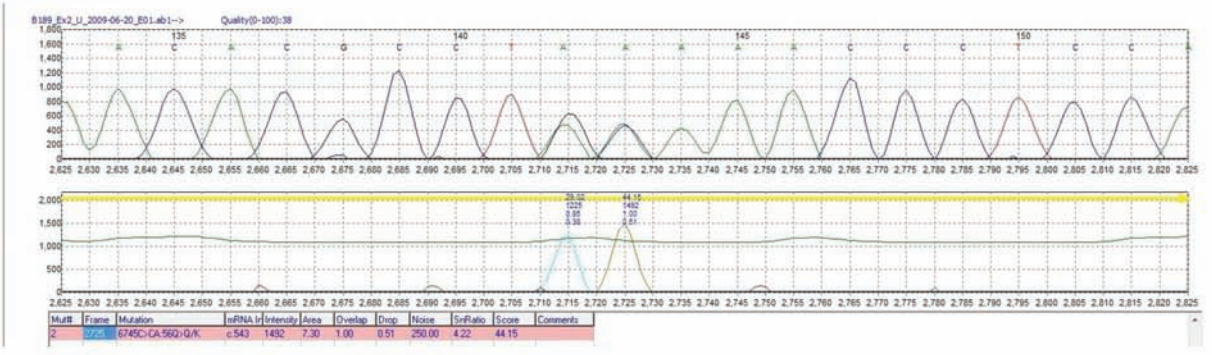
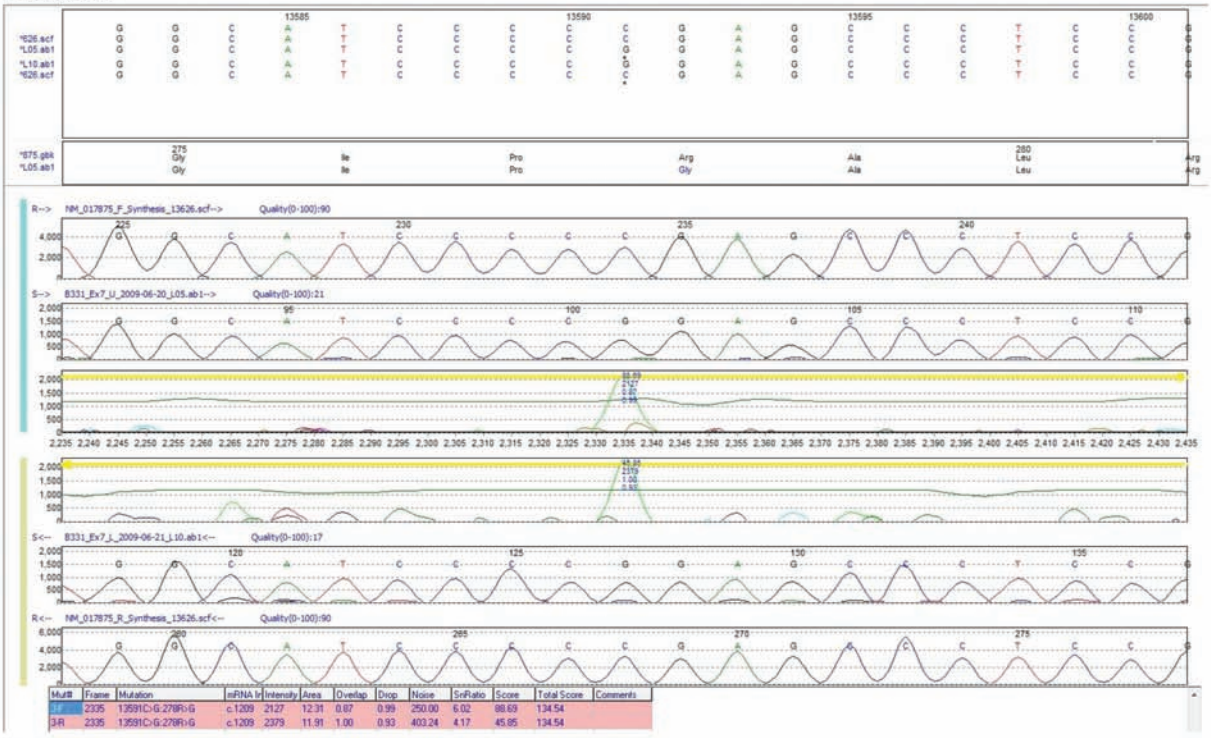


Case number 8:
Exon 7 SLC25A38:c.832C>G;p.Arg278Gly
Proband:



Mut#	Frame	Mutation	mRNA Nr	Intensity	Area	Overlap	Drop	Noise	SnRatio	Score	Total Score	Comments
1F	2335	13591C>G:278R>G	c.1209	2127	12.31	0.87	0.99	250.00	6.02	88.69	134.54	
2R	2335	13591C>G:278R>G	c.1209	2379	11.91	1.00	0.93	403.24	4.17	45.85	134.54	

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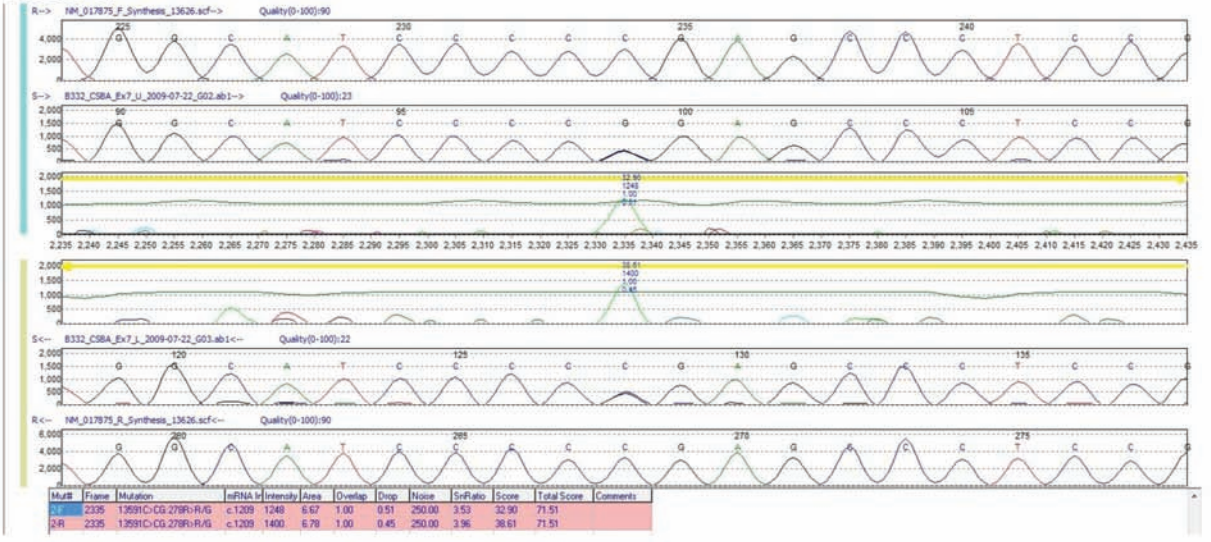

Mut#	Frame	Mutation	mRNA Nr	Intensity	Area	Overlap	Drop	Noise	SnRatio	Score	Total Score	Comments
1F	2335	13591C>CG:278R>R/G	c.1209	1248	6.67	1.00	0.51	250.00	3.53	32.90	71.51	
2R	2335	13591C>CG:278R>R/G	c.1209	1400	6.78	1.00	0.45	250.00	3.96	38.61	71.51	

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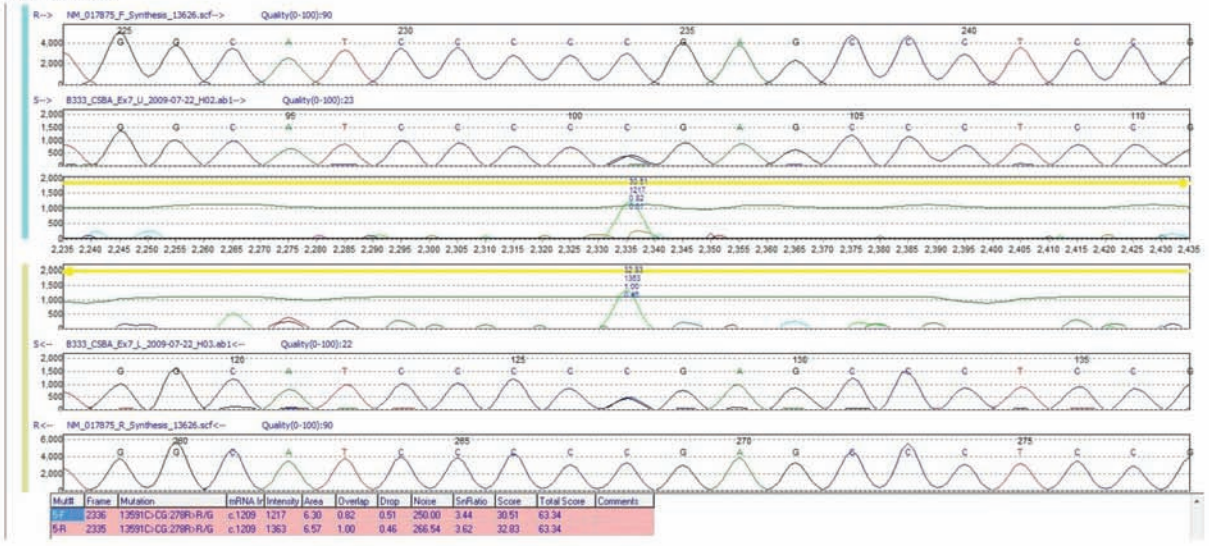
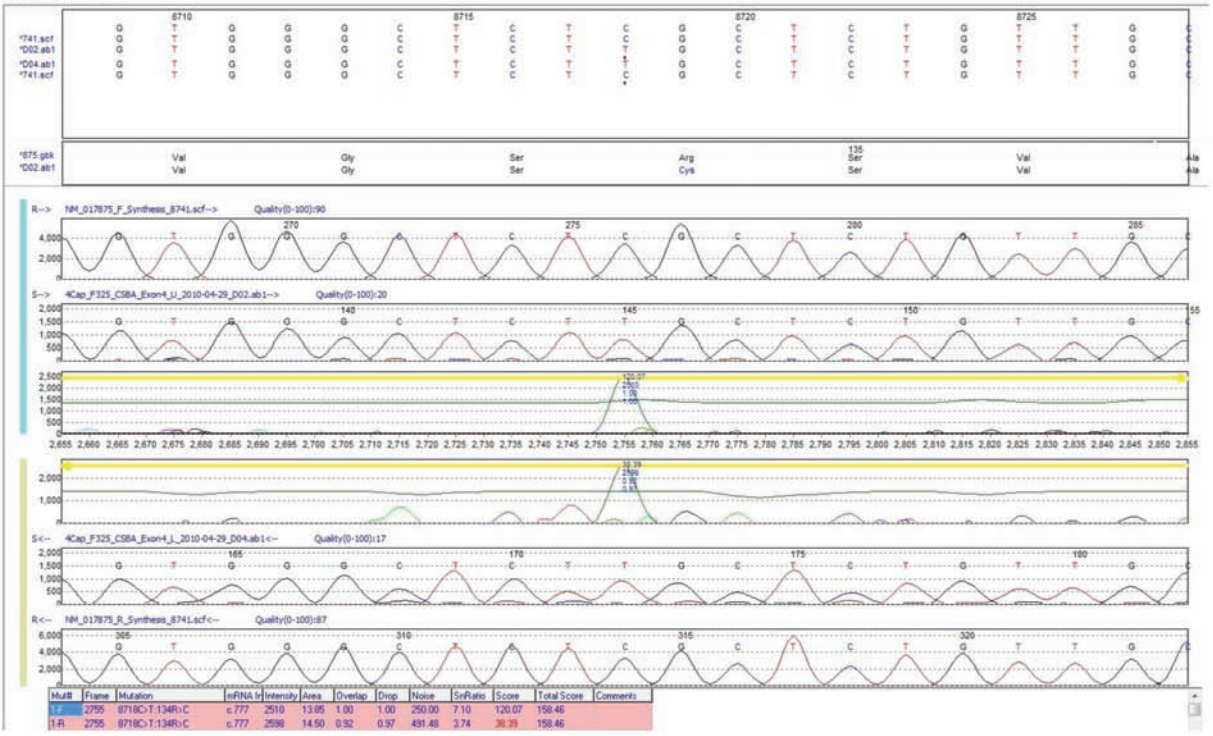


MutR	Frame	Mutation	mRNA Nr	Intensity	Area	Overlap	Drop	Noise	SnRatio	Score	Total Score	Comments
5-F	2336	13591C>CG:278R>R/G	c.1209	1217	6.30	0.82	0.51	250.00	3.44	30.51	63.34	
5-R	2335	13591C>CG:278R>R/G	c.1209	1363	6.57	1.00	0.46	266.54	3.62	32.83	63.34	

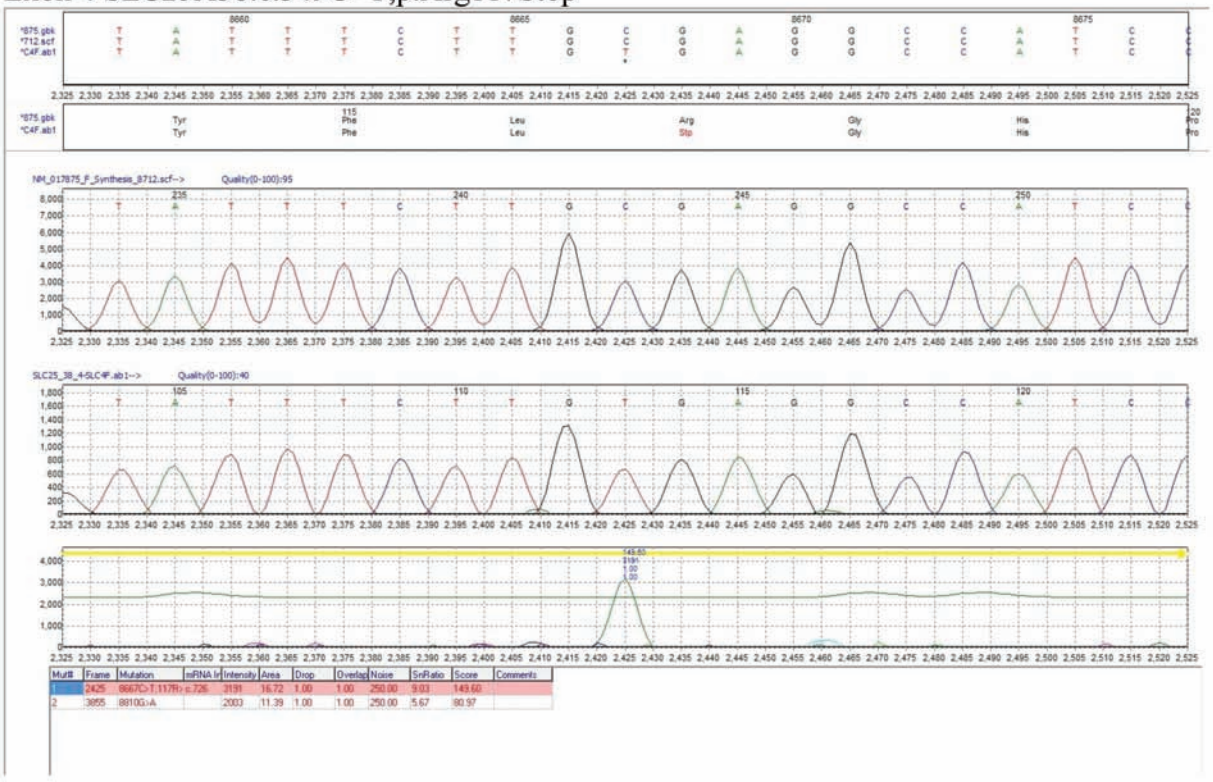
Case number 9:
Exon 4 SLC25A38:c.400C>T;p.Arg134Cys
Proband:



MutR	Frame	Mutation	mRNA Nr	Intensity	Area	Overlap	Drop	Noise	SnRatio	Score	Total Score	Comments
1-F	2755	8718C>T:134R>C	c.777	2510	13.85	1.00	1.00	250.00	7.10	120.07	158.46	
1-R	2755	8718C>T:134R>C	c.777	2598	14.50	0.92	0.97	491.48	3.74	38.39	158.46	

Case number 10

Exon 4 SLC25A38:c.349C>T;p.Arg117Stop



Mut#	Frame	Mutation	mRNA Nr	Intensity	Area	Drop	Overlap	Noise	SnRatio	Score	Comments
1	2425	8667C>T,117R>	c.726	3191	16.72	1.00	1.00	250.00	9.03	149.60	
2	3955	8810G>A		2003	11.39	1.00	1.00	250.00	5.67	80.97	

Case number 11: Exon 4

SLC25A38:[c.324_325delCT]+[349C>T];p.[Tyr109LeufsX43]+[Arg117Stop]

compound heterozygote

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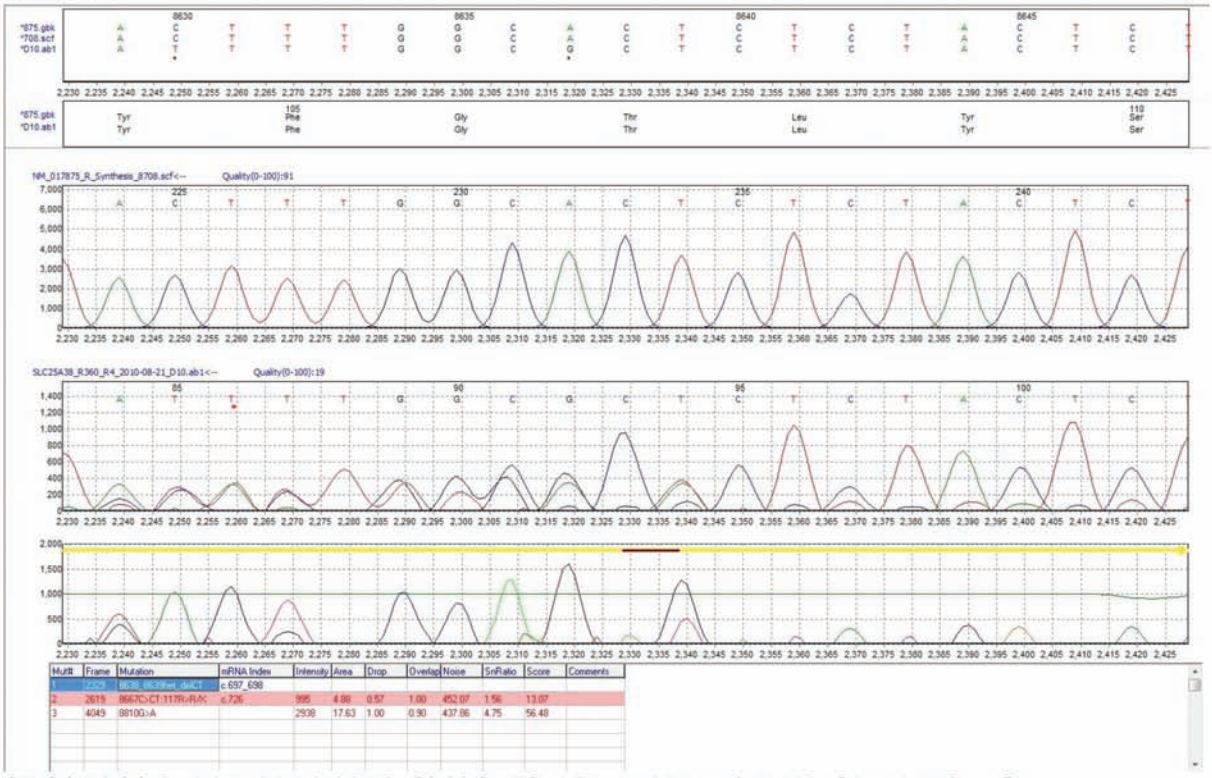
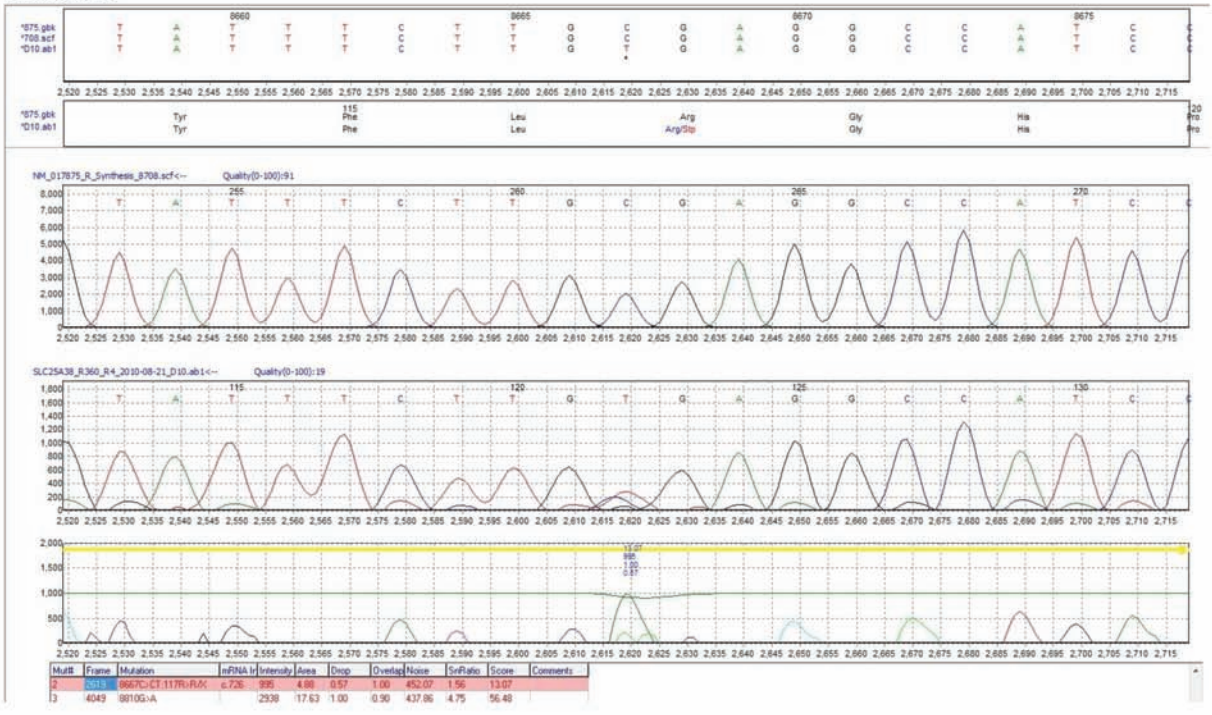
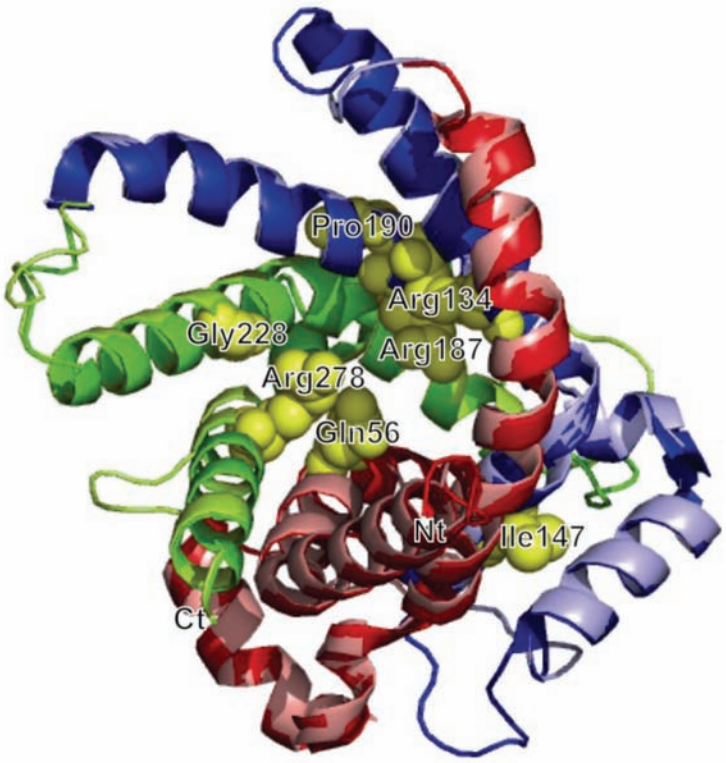


SLC25A38:[c.324_325delCT]+[349C>T];p.[Tyr109LeufsX43]+[Arg117Stop]

compound heterozygote

Proband:





Online Supplementary Figure S2. Predicted structural model of SLC25A38 visualized with PyMol. Superimposed structure of PDB 2c3e (bovine mitochondrial ADP/ATP carrier) together with the predicted model of SLC25A38 by SWISS-MODEL. The C-terminus and N-terminus predicted to lie within the mitochondrial intermembrane space are indicated. Amino acids reported to be new mutations are represented as yellow spheres. The three mitochondrial carrier domains are colored in red, blue or green in the 2c3e structure and in lighter similar colors in the SLC25A38 model.