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

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Abstract

Background

Congenital sideroblastic anemias are rare disorders with several genetic causes; they are characterized by erythroblast mitochondrial iron overload, differ greatly in severity and some occur within a syndrome. The most common cause of non-syndromic, microcytic sideroblastic anemia is a defect in the X-linked ALA Synthase 2 gene but this is not always present. Recently, variations in gene for the mitochondrial carrier SLC25A38 were reported to cause a non-syndromic, severe type of autosomal-recessive sideroblastic anemia. Further evaluation of the importance of this gene was required to estimate the proportion of patients affected and to gain further insight into the range and types of variations involved.

Design and Methods

In three European diagnostic laboratories sequence analysis of *SLC25A38* was performed on DNA from patients affected by congenital sideroblastic anemia of a non-syndromic nature not caused by variations in the ALA synthase 2 gene.

Results

Eleven patients whose ancestral origins spread across several continents were homozygous or compound heterozygous for 10 different *SLC25A38* variations causing premature termination of translation (p.Arg117X, p.Tyr109LeufsX43), predicted splicing alteration (c.625G>C; p.Asp209His) or missense substitution (p.Gln56Lys, p.Arg134Cys, p.Ile147Asn, p.Arg187Gln, p.Pro190Arg, p.Gly228Val, p.Arg278Gly). Only three of these variations have been described previously (p.Arg117X, p.Tyr109LeufsX43 and p.Asp209His). All new variants reported here are missense and affect conserved amino acids. Structure modelling suggests that these variants may influence different aspects of transport as described for mutations in other mitochondrial carrier disorders.

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Conclusions

Mutations in the SLC25A38 gene cause severe, non-syndromic, microcytic/hypochromic sideroblastic anemia in many populations. Missense mutations are shown to be of importance as well as those that affect protein production and their further investigation should shed light on structure-function relationship in this protein.

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Introduction

Congenital sideroblastic anemias (CSA) are heterogeneous disorders of rare occurrence characterized by aregenerative anemia of varying severity, hypochromic peripheral erythrocytes and decreased haem synthesis. In the bone marrow an increased percentage of ringed sideroblasts formed by iron-loaded mitochondria clustered around the erythroblast nucleus is visualised by Perls' staining. Systemic iron overload secondary to chronic ineffective erythropoiesis is an important feature of most CSA. Different forms of CSA are defined at the molecular level, each of which has provided insight into cellular pathways associated with dysfunctional mitochondrial iron metabolism. Some CSA are syndromic while in others microcytic anemia and subsequent iron overload are the sole manifestations.

X-linked sideroblastic anemia (XLSA, MIM# 300751), is the most common non-syndromic genetic form and results from gene mutations in the erythroid-specific gene encoding 5-aminolevulinate synthase 2 (ALAS2, EC 2.3.1.37). ALAS2 is the initial enzyme of haem biosynthesis pathway in erythroid cells and catalyzes the condensation of glycine and succinyl-coenzyme A into 5-aminolevulinic acid (ALA), using pyridoxal phosphate as an essential cofactor. The onset of symptoms in XLSA patients range from birth to the eighth decade and generally the anemia is mild to moderate and often responds to pyridoxine.[1]

Two recent reports indicate that variations in the newly-described *SLC25A38* gene can be responsible for severe pyridoxine-refractory CSA. This anemia was markedly microcytic and inherited in an autosomal recessive manner.[2,3] The patients could present in the first few months of life and were often dependent on regular blood transfusions for normal development. Bone marrow transplantation was therefore sought as a cure and has been successful in two of four published cases. *SLC25A38* is located on chromosome 3p22.1 and encodes a mitochondrial carrier protein required for erythropoiesis. This protein is thought to

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be located in the inner mitochondrial membrane and may act by importing glycine into mitochondria or by exchanging glycine for ALA. Most of the variations reported affect protein levels (nonsense, frameshift or splice site variations) but three missense variations were also found (p.Gly130Glu, p.Arg134His, p.Arg187Pro).[2, 3] To gain further insight into the nature of this type of disorder and the prevalence and nature of *SLC25A38* variations we report here the genetic diagnosis performed on patients with CSA negative for *ALAS2* mutations.

Design and Methods

Three laboratories or clinical centres offering diagnosis for congenital sideroblastic anemia had analysed the *SLC25A38* genomic sequence in twenty four patients. Eleven patients were studied in Cardiff, 12 in Paris and 1 in Barcelona. None was included in the studies already published.[2,3] The criteria for inclusion differed slightly for the three laboratories however shared minimal criteria were non-syndromic CSA (i.e. ring sideroblasts in the bone marrow with no known secondary cause, an absence of features linked to known syndromic types of CSA and an absence of indication in favour of a diagnosis of myelodysplastic syndrome), anemia refractory to pyridoxine and folic acid and an absence of *ALAS2* mutation.

The conditions of DNA extraction, PCR reaction and sequence analysis for the three laboratories were standard and are contained in the Online Supplementary Appendix. All exons, exon-intron boundaries and a varying amount of the 5' and 3' flanking sequence of the SLC25A38 gene were examined using fluorescent chain-terminator cycle sequencing. Newly-designed or previously-published[2] primers were employed (Online Supplementary Table S1).

All participants or their parents as appropriate, had given informed consent for genetic diagnosis, in keeping with the respective regulations from the countries in which the diagnosis

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was performed. In one case, the diagnosis was performed as part of a broader research study (HMA-IRON ERARE-155) approved by the local research ethics committee.

Except for patients 9 and 11 (see Table 1), blood samples from both parents were available. Samples were also studied from 4 additional family members of three families.

Multiple sequence alignment of SLC25A38 proteins from 14 different species was performed using Clustalw2 (*http://www.ebi.ac.uk/Tools/clustalw2/*).[4] Mitochondrial carrier protein domains (PF00153) are shown as defined by Pfam (*http://pfam.sanger.ac.uk/)* on sequence S2538 HUMAN.[5] Potential transmembrane helical regions of human SLC25A38 were obtained from Uniprot (*http://www.uniprot.org*) and from the secondary structure prediction of the 3D protein modelling. The protein 3D-modelling was achieved with Swiss-model web-server (*http://swissmodel.expasy.org/*) in automated mode using the PDB structure of carboxyatractyloside-inhibited bovine mitochondrial ADP/ATP carrier (2c3e) as template.[6,7] The structures' superimposition and the visualization of mutations were performed using the open-source software PyMOL™ 0.99rc6 (*http://www.pymol.org/*).[8]

Results

We studied 24 probands with manifest CSA symptoms, ring sideroblasts in the bone marrow, anemia refractory to treatment with pyridoxine and folic acid, and no *ALAS2* mutations. Of these, 11 were found to have inherited 10 different *SLC25A38* variations that are likely causative of their CSA. These eleven patients, with 15-73% ring sideroblasts in the bone marrow erythroblasts, were of several different ancestral origins (4 Spanish, 1 Portuguese, 1 Algerian, 2 Moroccan, 1 Egyptian, 1 French and 1 Sri Lankan); three were male and eight were female. Additional relevant features of the patients and details of the mutations are presented in Table 1. The anemia was of early onset although not necessarily present at birth. All patients required blood transfusions, sometimes occasional but inevitably becoming

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regular, usually in the first few years of life. This dependence appears delayed in two patients (numbers 8 and 9, Table 1) one of whom did not receive regular blood transfusions until he was 20 years old (patient number 9, Table 1). Increased transferrin saturation was observed in five patients (patients 4,6,7,10 & 11), accompanied in three by increased serum ferritin (patients 6,7 & 10), before significant amounts of blood had been received. Iron overload remains a problem in two patients (patients 4 & 6) despite iron chelation.

Nine patients were homozygous and two (patient 4 and patient 11) were compound heterozygotes for *SLC25A38* variations found in 5 (exons 2 & 4-7) of the 7 exons (Online Supplementary Figure S1). In all instances studied, the parents were found to be heterozygous for the same variation as found in the respective proband. Unaffected siblings or relatives tested were found to have inherited only the usual alleles or were heterozygous for the variation found. None of these variations was identified as a polymorphism in the Ensembl (www.ensembl.org/Homo_sapiens/Info/Index) or NCBI (www.ncbi.nlm.nih.gov/) SNP databases last searched 14.02.2011; the p.Gly228Val variant has not been detected in 90 North European control alleles studied.

Two mutations (p.Arg117X and p.Gly228Val) were found in the homozygous state in two unrelated families. All but two variations described here are missense mutations while one is a chain termination variation (p.Arg117X) and one is a deletion leading to a frame shift mutation p.Tyr109LeufsX43 (Figure 1). The involvement of p.Arg117X, p.Tyr109LeufsX43 and of p.Asp509His variations in CSA has been described previously[2] but seven variations are reported here for the first time. Two new mutations (p.Arg134Cys and p.Arg187Gln) involve amino acids already known to be affected by a different substitution (p.Arg134His and p.Arg187Pro) in patients with CSA[2]. The previously-described missense variation p.Asp209His, formed by a G to C transversion at the end of exon 5, is predicted to abolish a splice donor site[2], thereby leading to a truncated protein and a possibly rapidly degraded

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mRNA by nonsense-mediated mRNA decay. All seven new mutations result in substitutions of conserved amino acids (Figure 1) situated in predicted transmembrane regions forming the three mitochondrial carrier domains (Figures 1 and 2A). Structure analysis shows that five of the seven substitutions affect amino acids with side chains oriented toward or extending into the central transport channel, consistent with a plausible mechanistic role of the newly-described mutations in transport activity (Figure 2B; additional detail can be found in Online Supplementary Figure S2).

Discussion

Our observations confirm and significantly extend the findings in the previous reports regarding *SLC25A38* variations in CSA.[2,3] In Paris, out of all 34 total CSA proband referrals (syndromic and non-syndromic), 15 (44%) had been found to have *ALAS2* variations and, as reported here, *SLC25A38* variations were found in 5 (15%). These percentages are similar to those found by Bergmann et al.[3] (37% *ALAS2*, 15% *SLC25A38*) in a cohort of 83 probands. In Cardiff the percentages were lower probably because of different referral acceptance criteria. Out of 71 CSA proband referrals to Cardiff, 17 (24%) had *ALAS2* variations and *SLC25A38* variations have been found in 5 (7%). Despite the diagnosis of additional genetic causes[3] in the Paris and Cardiff laboratories, and a significant number of cases in which *SLC25A38* was not investigated, the *SLC25A38*-associated CSA currently represents the second most common genetically-defined type referred to our laboratories as was previously reported[3].

None of the heterozygous carriers was affected in any discernible way. In one family (patient no.5) the mother and the proband were heterozygous for haemoglobin Lepore without any noticeable interaction between the two unusual genotypes. All patients described here were severely anemic with markedly microcytic and hypochromic red blood cells. The severity and refractory nature of the anemia led to a dependence on regular blood transfusion

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from an early age, and for some to seek compatible bone marrow donors for transplantation. Two patients in this series who have already undergone this procedure (patients 2 & 5) have been successfully engrafted with a follow-up time of 3yr and 4 months respectively. Patient 10 is currently undergoing this procedure with no significant toxicities so far. These successes strengthen the case for offering this procedure as a possible cure to those severely affected with this type of CSA who have matched donors.

As with all types of dyserythropoietic disorders, in several patients studied here, there was evidence of increased iron absorption prior to blood transfusion. It is thought that the cause of this iron overload is repression of hepcidin expression produced by an expanded but ineffective erythropoiesis.[9,10] In addition, all patients became rapidly transfusion-dependent so that regular monitoring of iron levels and iron chelation therapy to prevent complications of secondary iron overload are essential.

The correct molecular diagnosis in these patients was of great importance providing reassurance that the sideroblastic anemia is non-syndromic and enabling accurate genetic counselling on recurrence risk within the family with the offer of prenatal diagnosis and preimplantation genetic diagnosis if required. It also allowed *SLC25A38* genotyping of potential bone marrow donors.

This study shows that more than half (55%) of the 18 implicated *SLC25A38* variations are missense as opposed to 27% prior to this report which may provide further stimulus for the development of an *in vitro* drug discovery assay to correct the defect. It is also interesting to note that some patients appear to have a milder course of the disease (homozygous p.Arg134Cys and homozygous p.Arg278Gly in this report; homozygous p.Arg117X, compound heterozygotes p.[Arg134His]+[Tyr293X] and p.[X305ArgextX28]+[Asp209His] in a previous report [2]) for reasons apparently unrelated to the variations inherited, suggesting that additional environmental or genetic factors could play a role in disease penetrance.

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The SLC25A38 protein is one member of a large family of mitochondrial carrier proteins[11]. Some of these carriers transport in only one direction, but most exchange one substrate for another. In the case of SLC25A38, the arginine-aspartic acid (RD) dipeptide at 187-188 putatively identifies it as an amino acid transporter. Yeast and zebrafish studies have suggested that SLC25A38 transports glycine into the mitochondrial matrix and exports aminolevulinic acid out of the mitochondrion to the cytosol. Although 3D structure analysis has been reported on only one member of the mitochondrial carrier family, the bovine mitochondrial ATP/ADP carrier (PDB: 2c3e), protein homology modelling and multiple sequence alignment of all mitochondrial carriers indicate they share certain important structural features.[11] These proteins consist of three tandem repeats of mitochondrial carrier domains. Each domain contains two transmembrane helices separated by a stretch of residues including the mitochondrial carrier signature motif encompassing a loop and an additional helical region (Figure 2A & Online Supplementary Figure S2).[11] The six transmembrane helices arrange themselves around a central cavity through which the solutes pass. Key amino acid residues, many of which are charged, have side chains that stretch into this cavity and play a critical role in the opening and closing of the channel gates or in the specific binding, transport and release of the substrates. In addition, certain conserved prolines and glycines are appropriately placed to allow the kinking and swivelling of the transmembrane helices required for transport to occur.[7,11]

By structural similarity here we hypothesize that the new reported missense mutations affect the transport function of SLC25A38 in different ways: mutations Gln56Lys and Arg278Gly will affect the gate to the matrix, mutations Arg134Cys and Arg187Gln will affect substrate binding or specificity and mutations Gly228Val, Pro190Arg and the already described Gly130Glu will affect the pivotal position for substrate binding and gate opening to the intermembrane space. The Ile147 does not appear to have a side chain that stretches into

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the channel but is a conserved hydrophobic residue that lies within a highly-conserved motif at the matrix gate.

Overall, by similarity with mutations present in other mitochondrial carrier disorders, we believe the described findings here represent a step forward in the understanding of the nature of this type of CSA.[11] Further investigation of these natural variants will enhance our knowledge of the function of this particular protein and its role in haem synthesis of developing red cells.

Online Supplementary Appendix

Further details of DNA extraction and mutation analysis methodology are provided in a Design and Methods supplement; Table S1 contains primer sequences; Figure S1 contains the sequence analysis results for each of the mutations found visualized by Mutation Surveyor software; Figure S2 is a colour picture visualizing the position of the substituted amino acids on the modelled 3D structure.

Authorship and Disclosures

BK, CK, GH, AM, EM, MSa and MSw performed molecular diagnosis. JLFS, KM, TM, CO, CP, AL, JSN and SV are involved in the clinical care of the patients and made the conditional diagnoses. CB, BG, AM and MSa designed methodology. BG, AM and MSa performed structural studies and drafted the manuscript. MSa provided the structural model shown here and the visualization of the primary sequence alignment. All authors revised and completed the manuscript. Financial and other disclosures are provided by the authors using the ICMJE (*www.icmje.irg*) Uniform Format for Disclosure of Competing Interests.

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Table 1. Mutations of the *SLC25A38* gene and main features of the patients.

Patient number	Age of presentation	Haemoglobin g/dL diagnosis (age)	Mean corpuscular volume fL at diagnosis	Treatment	Transferrin saturation (age)	Ferritin µg/L (age)	Lowest haemoglobin during treatment g/dL (age)	Mean corpuscular volume fL at lowest haemoglobin	[1]HGVS names of mutations found in SLC25A38 gene ENSG00000144659; Genbank mRNA: NM_017875; Genbank protein: NP_060345
1	5 month	6.2	61.6	Blood transfusion occasional then regular.	not available	not available	2.2	not available	c.[569 C>G]+[569C>G] p.[Pro190Arg]+[Pro190Arg]
2	4 month	7.2	62	Blood transfusion, Bone marrow transplant.	not available	371 (<1year)	6.2	60.4	c.[683G>T]+[683G>T] p.[Gly228Val]+[Gly228Val]
3	2 month	7.2	76	Blood transfusion regular.	not available	577 (<1year)	3.9	76	c.[683G>T]+[683G>T] p.[Gly228Val]+[Gly228Val]
4	birth	6.9 (7month)	61.3	Blood transfusion regular.	84% (7month) 81% (3year)	136 (7month) 1340 (3year)	6.7 (18 month)	73	c.[560G>A]+[625G>C] p.[Arg187Gln]+[Asp209His]
5	birth	6.5 (at birth)	65.3	Blood transfusion occasional then regular. Bone marrow transplant.	100% (2-3year)	548 (2-3year)	5.8 (20 month)	74	c.[349C>T]+[349C>T] p.[Arg117X]+[Arg117X]
6	2 day	10.3	71	Blood transfusion from 2year.	71% (2day) 71% (15year)	398 (2day) 1492 (15year)	5.9 (2year)	65	c.[440T>A]+[440T>A] p.[Ile147Asn]+[Ile147Asn]
7	15 day	9	55	Blood transfusion from 2year.	81% (2year)	740 (2year)	5.5 (1year)	62	c.[166C>A]+[166C>A] p[.Gln56Lys]+[Gln56Lys]
8	3 year	7	50	Blood transfusion occasional.	95% (12year)	537 (12year)	5.5 (12year)	48.5	c.[832C>G]+[832C>G] p.[Arg278Gly]+[Arg278Gly]
9	2 year	not available	not available	Blood transfusion occasional then regular.	72% (22year)	1000 (22year)	4.5 (21year)	55	c.[400C>T]+[400C>T] p.[Arg134Cys]+[Arg134Cys]
10	1 month	5.1	80	Blood transfusion regular. Bone marrow transplant.	56.2% (1month) 91% (3year)	618 (1month) 997 (3year)	4.1 (3year)	67	c.[349C>T]+[349C>T] p.[Arg117X]+[Arg117X]
11	14 month	6.5	55	Blood transfusion regular from 14 month	99% (14 month)	not available	not available	not available	c.[324_325delCT]+[349C>T] p.[Tyr109LeufsX43]+[Arg117X]

[1]HGVS: Human Genome Variation Society recommended mutation descriptions; c refers to the coding region nucleotide(s) affected with A of the initiation ATG codon as 1; p refers to the protein amino acid(s) affected with the initiation methionine as 1.

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Table and Figure Legends

Table 1. Mutations of the SLC25A38 gene and main features of the patients

Figure 1. Multiple amino acid sequence alignment obtained using Clustalw2 for SLC25A38 from 14 species (7 mammalian, 1 bird, 1 frog, 1 fish and 4 yeast/fungi). Amino acids reported in this work to be substituted in congenital sideroblastic anemia patients are marked with a vertical arrow. Shaded bars represent the transmembrane regions as reported by Uniprot and are extended slightly by the structure modelling as shown in the Figure 2. The mitochondrial carrier domains (Pfam) are shown as black boxes. Below the alignment a star indicates that the amino acid at this position is identical for all 14 species, dots indicate amino acids with similar but not identical properties.

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Figure 2. A. The position of the substituted amino acids in predicted secondary structure of human SLC25A38. Predicted helices are shown as blocks and predicted loops are shown as lines. The positions of the amino acid substitutions reported here are shown as white dots with respect to key glycine (black dot or half black-half white dot) and proline (grey dot or half grey-half white dot) residues. The latter were identified by their position in the amino acid sequence relative to the PX[D/E]XX[K/R]X[K/R] and [D/E]GXXXX[W/Y/F][K/R]G parts of the signature sequence for this class of proteins (shown in hashed line here) as discussed in reference 14. The position of the previously-reported substituted amino acid Gly130 (grey text) is also shown. **B.** Predicted structural model by SWISS-MODEL of SLC25A38 visualized with PyMol. The backbone helices and loops are shown in light grey and the side chains of the substituted amino acids are shown as dark grey spheres. The side chains of five of the seven substituted amino acids can be seen to point into the central channel. The side chains of the 190 proline and the 147 isoleucine can be seen as the uppermost and the furthest right of the side chains respectively. The view is from the mitochondrial inter-membrane space down the central channel but somewhat at an angle to show the 147 isoleucine side chain stretching away from the channel toward a short helical section that lies at the matrix side of the inner mitochondrial membrane. Please also refer to the *Online Supplementary Figure S2*.

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Figure 1

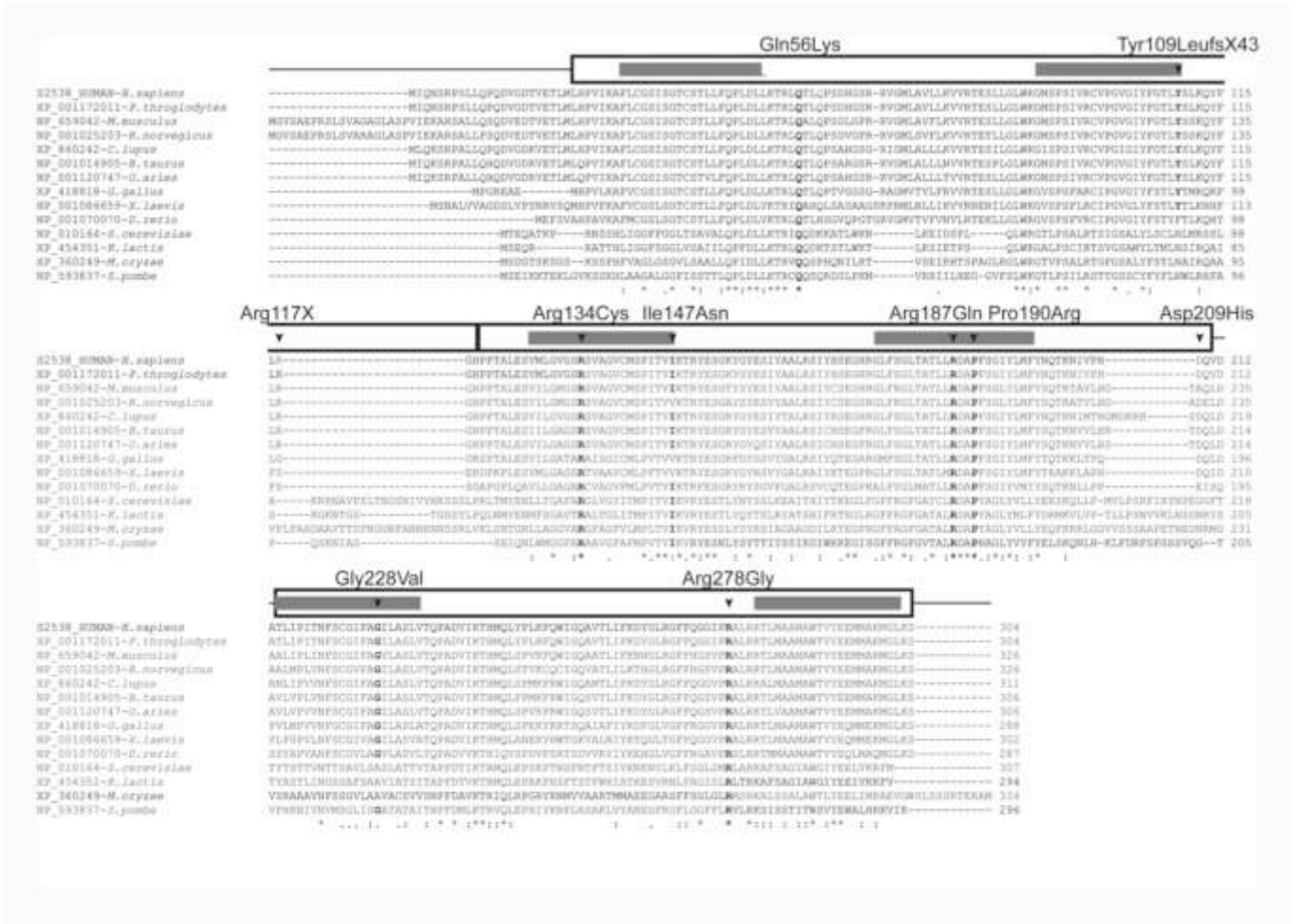

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Figure 2A

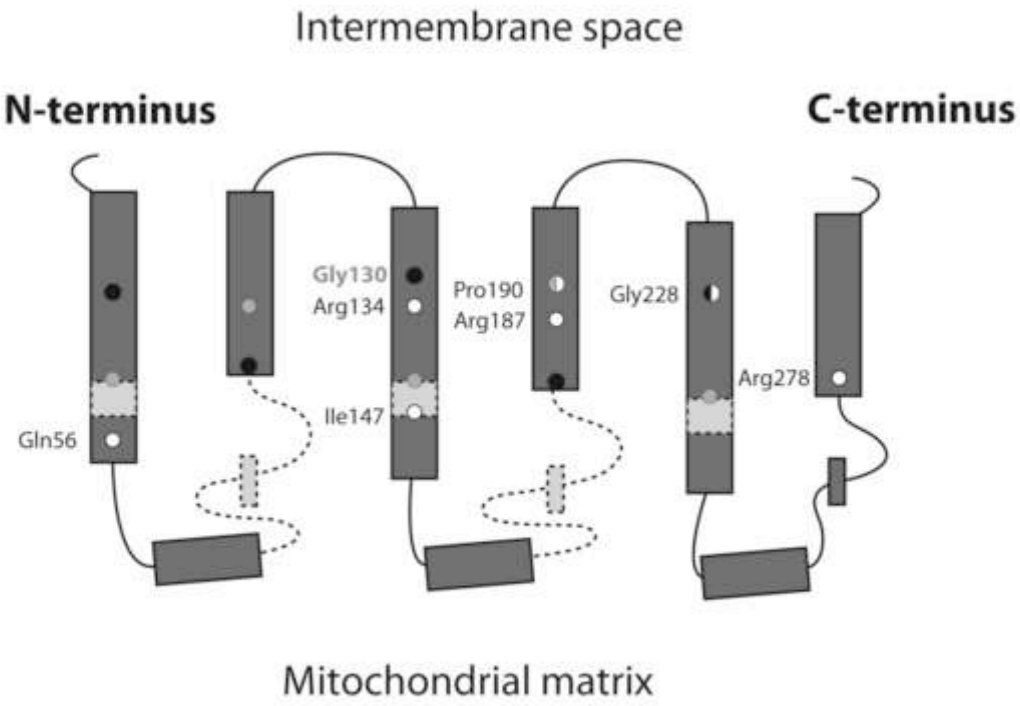

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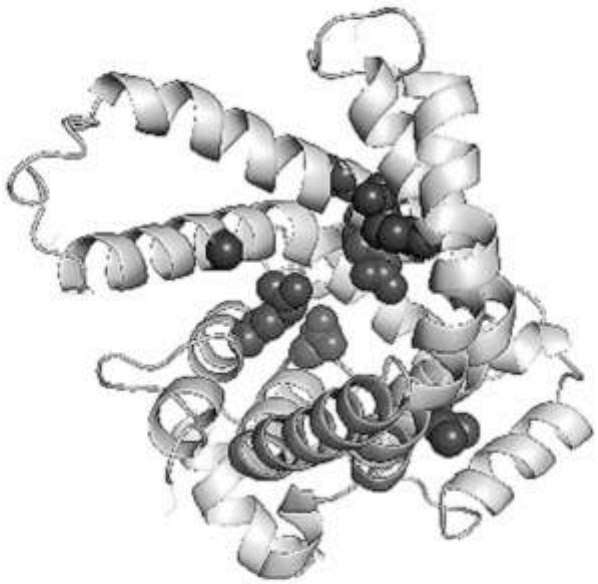
Figure 2B



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Supplementary Appendix

Design and methods supplement

Table S1

SLC25A38 primers used for PCR and sequence analysis

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 anaemia. These are visualized using Mutation Surveyor V3.25, SoftGenetics LLC, PA USA; distributed by BioGene Ltd, Cambridge, UK.

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 coloured in red, blue or green in the 2c3e structure or in lighter similar colours in the SLC25A38 model.

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Design and methods supplement

Isolation of DNA and mutation analysis

Cardiff (UHW)

Genomic DNA was extracted from peripheral blood white cells using salt extraction[1]. Primer pairs (Table I) were designed using the Primer3 programme (http://frodo.wi.mit.edu/primer3)[2] with subsequent specificity check using NCBI Blast, to amplify all 7 exons, exon-intron boundaries, 500bp upstream of the initiation codon and 700bp downstream of the chain termination codon of the gene (ENSG00000144659) in 9 PCR reactions using AmpliTaq Gold (Applied Biosystems, Foster City, California, USA). All primers were synthesised by Applied Biosystems, Cheshire,UK with one of two additional 17bp 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, Forster City, CA, USA). Terminated sequences were purified using DyeEx 2.0 spin kit (Qiagen, CA, USA) and analysed on the 3130xl sequence analyzer (Applied Biosystems, Foster City, California, 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 7 exons of SLC25A38 and the exon-intron junctions were amplified by PCR

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using previously published primer sequences[3] except for exon 7 (Table 1). 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 (PCR purification kit, Qiagen CA, USA), 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 analysed 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 9 PCR reactions with primers pairs (see Table 1) designed with the Primer3 programme (http://frodo.wi.mit.edu/primer3)[2] and synthesized by Invitrogen (Casrlsbad, 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 analysed on an Automatic Sequencer 23 ABI 3730XL (Applied Biosystems, Foster City, California, USA). Sequence traces were analyzed using the DNA variant analysis software Mutation Surveyor™ (Softgenetics**,** State College, PA, USA).

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[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.

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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

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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 anaemia. These are visualized using Mutation Surveyor V3.25, SoftGenetics LLC, PA USA; distributed by BioGene Ltd, Cambridge, UK.

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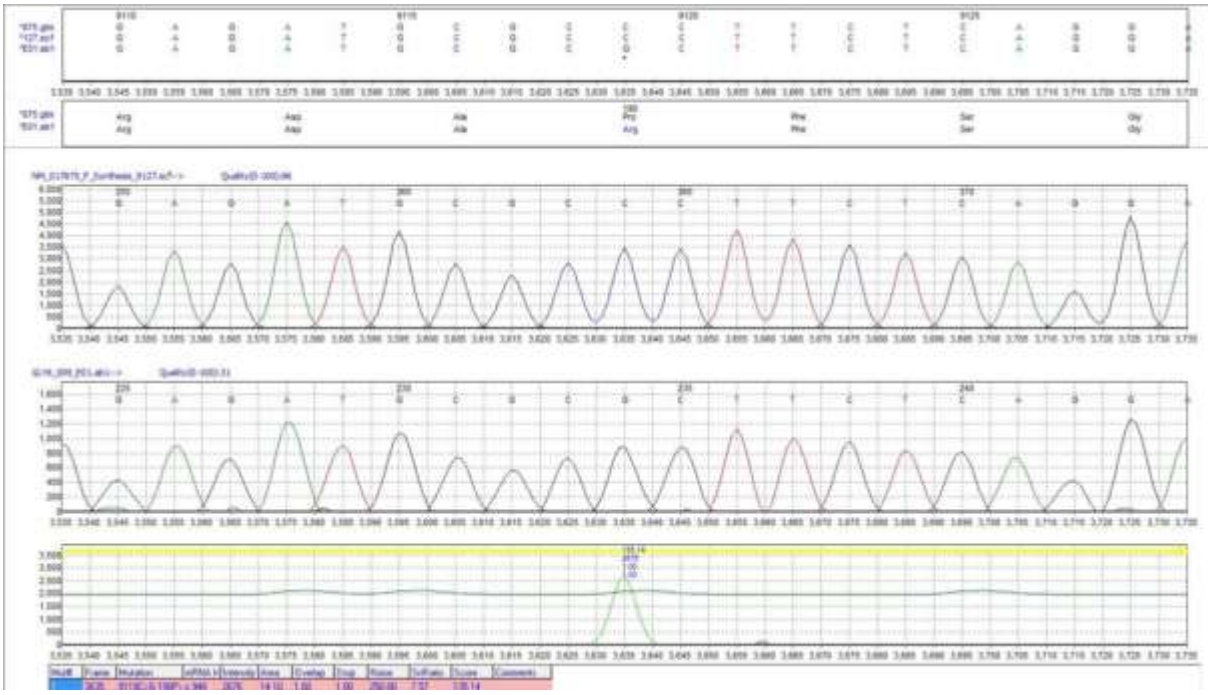
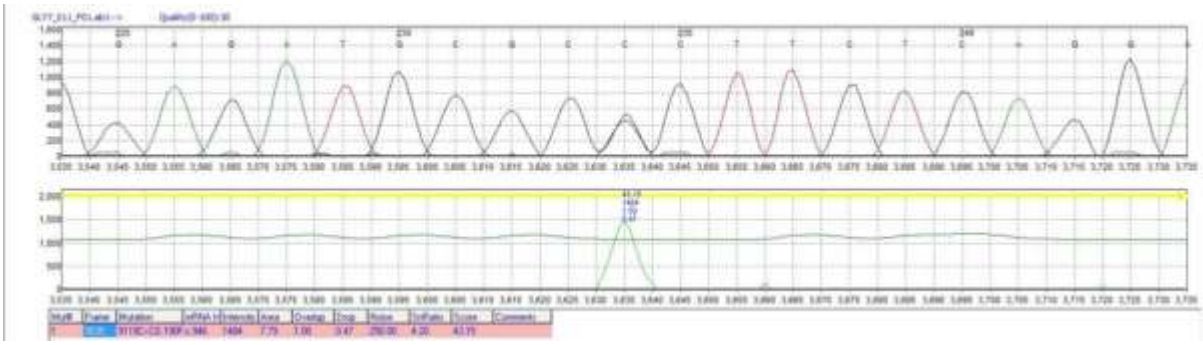
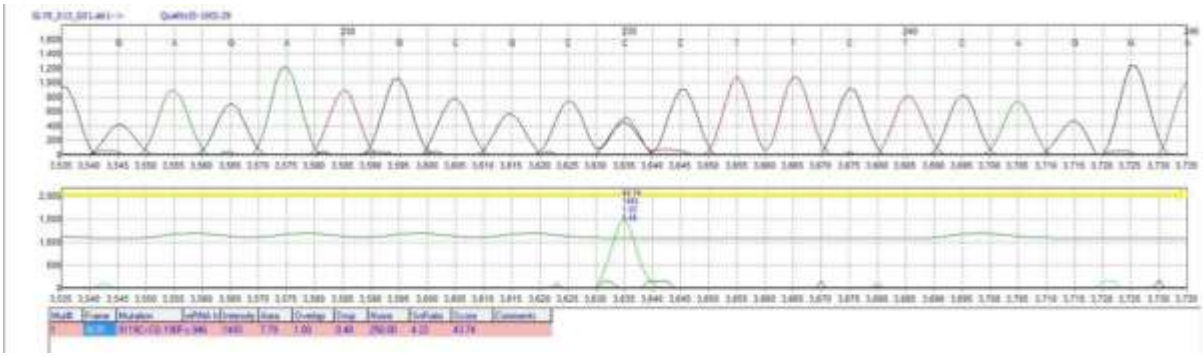
Case number 1
Exon 5 SLC25A38:c.569C>G;p.Pro190Arg
Proband:



Mother:



Father:



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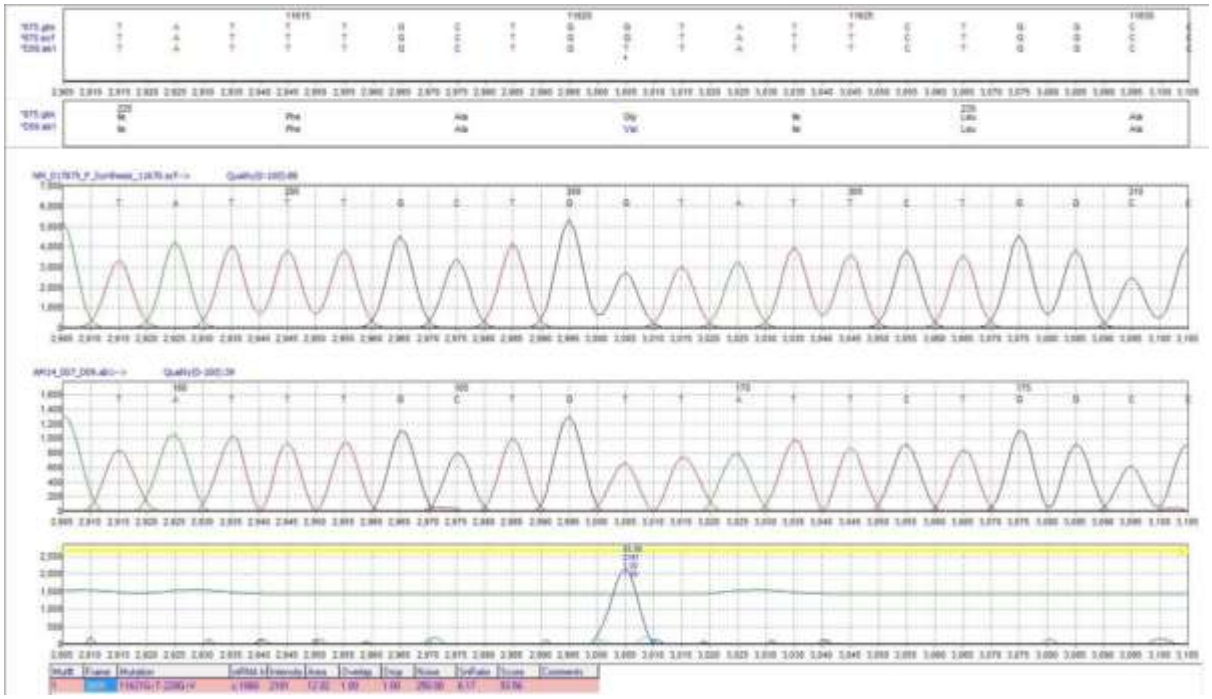
Case number 2
Exon 6 SLC25A38:c.683G>T,p.228Gly>Val
Proband:



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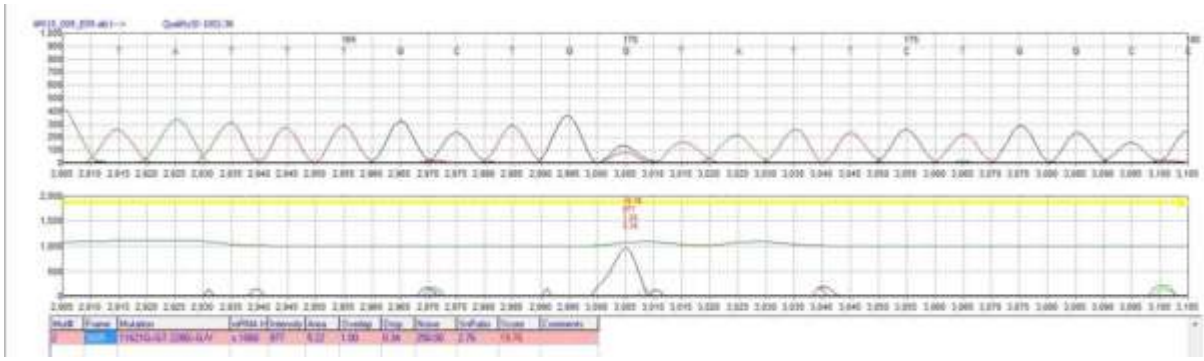

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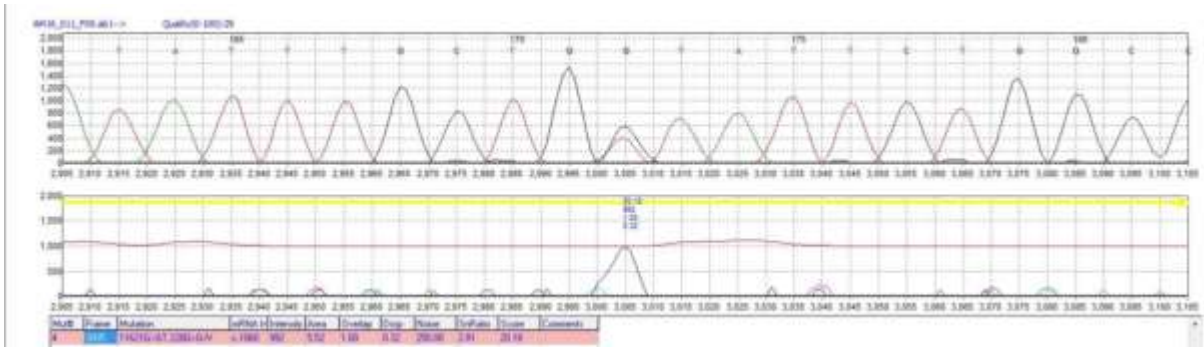

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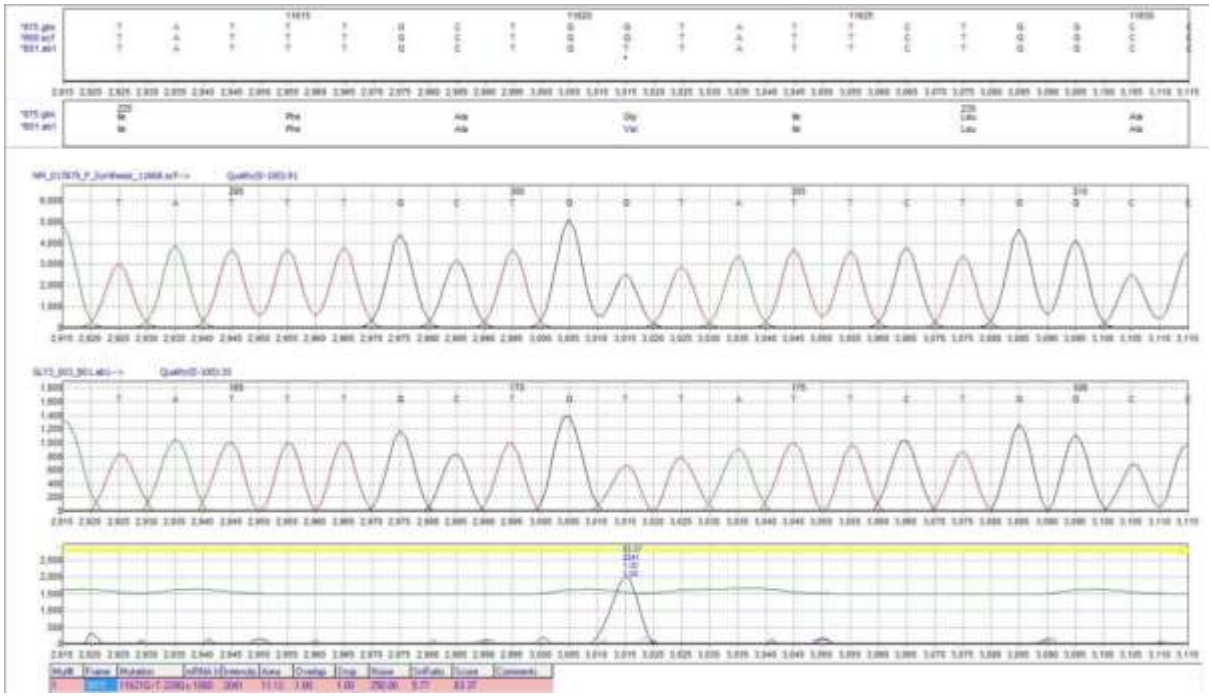
Case number 3
Exon 6 SLC25A38:c.683G>T;p.Gly228Val
Proband:



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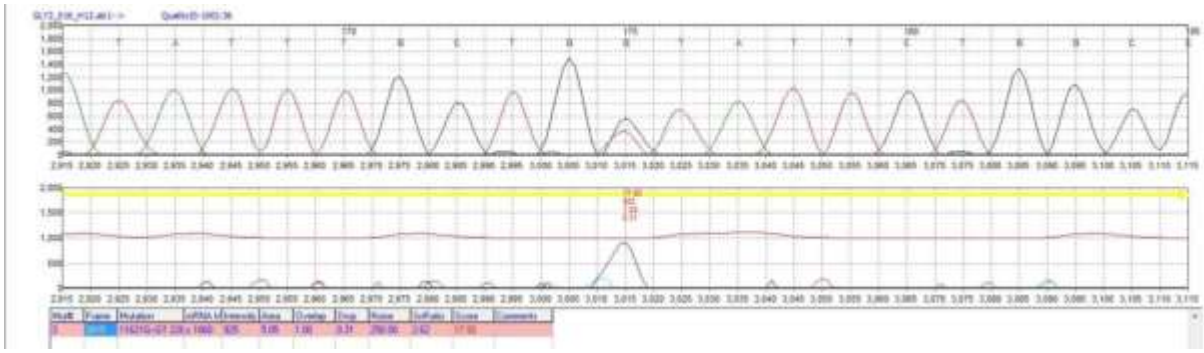

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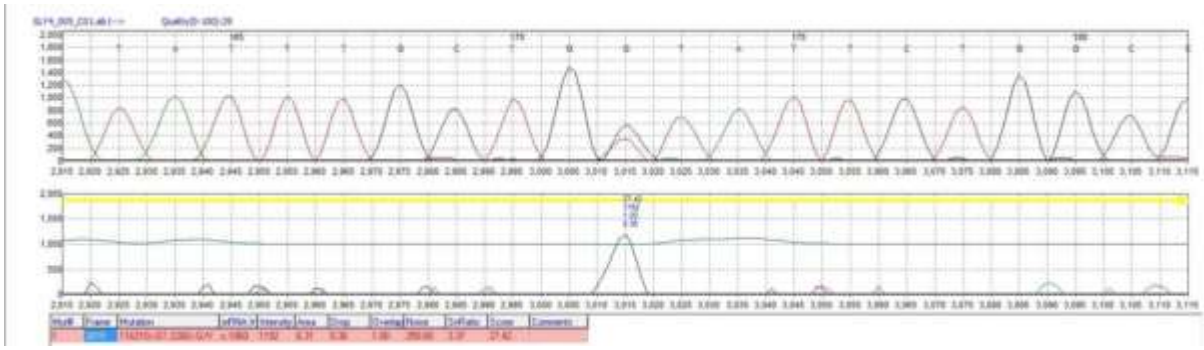

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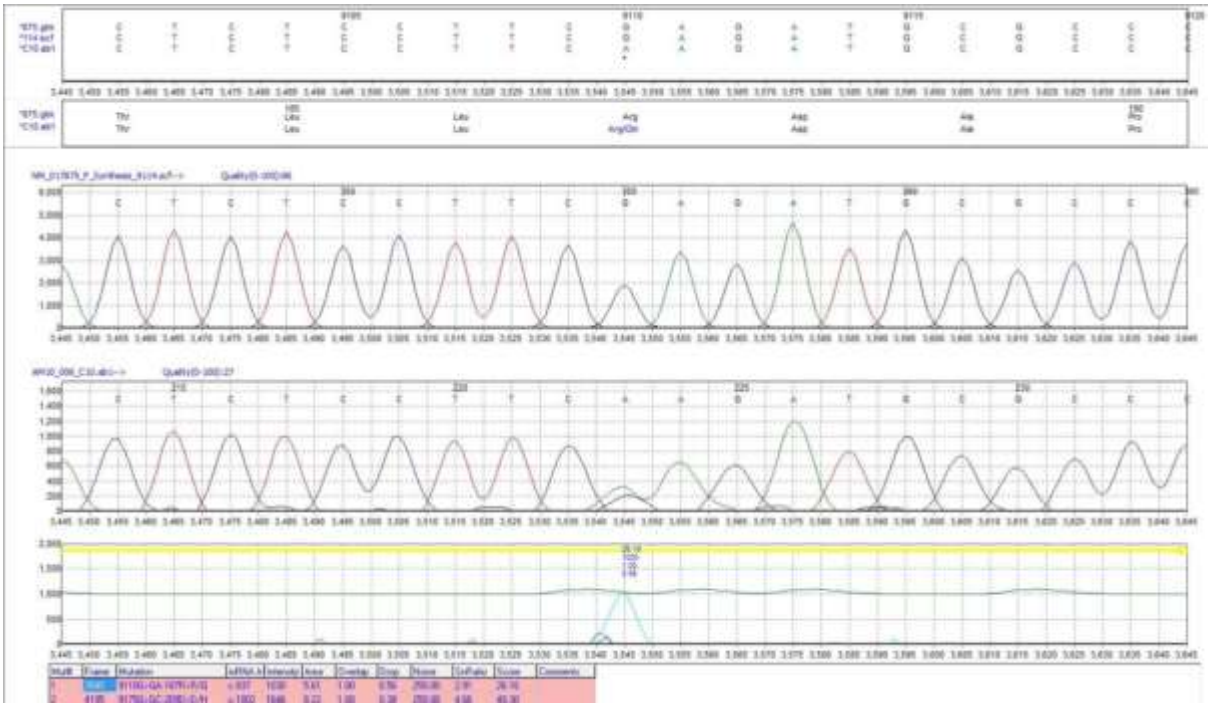
Case number 4 (compound heterozygote)
Exon 5 SLC24A38:c.[560G>A]+[625G>C];p.[Arg187Gln]+ [His209Asp]
Proband:



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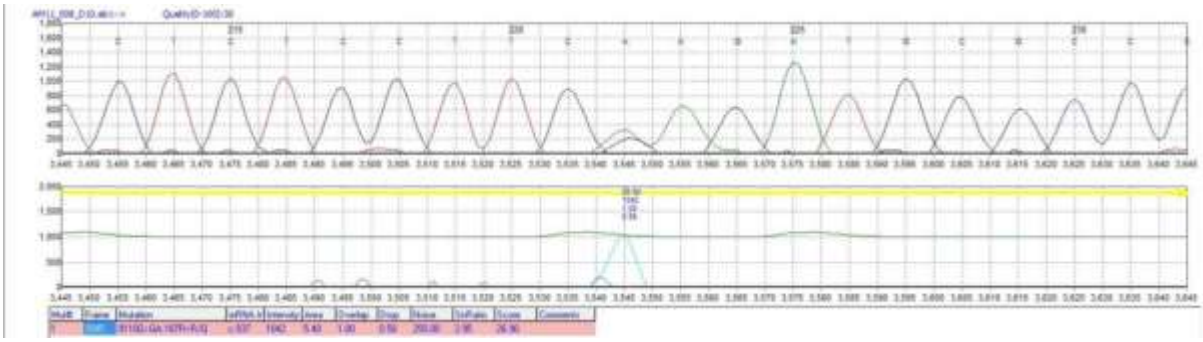
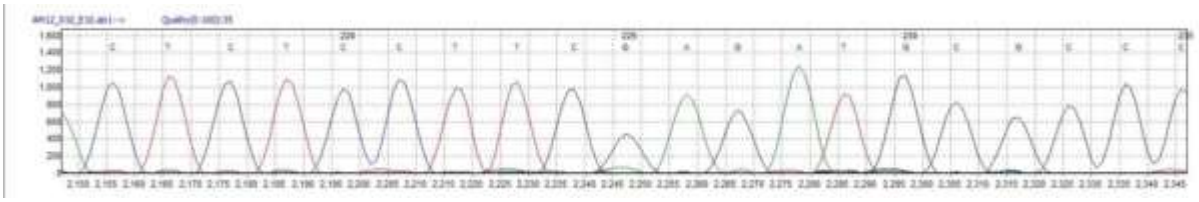


Father:



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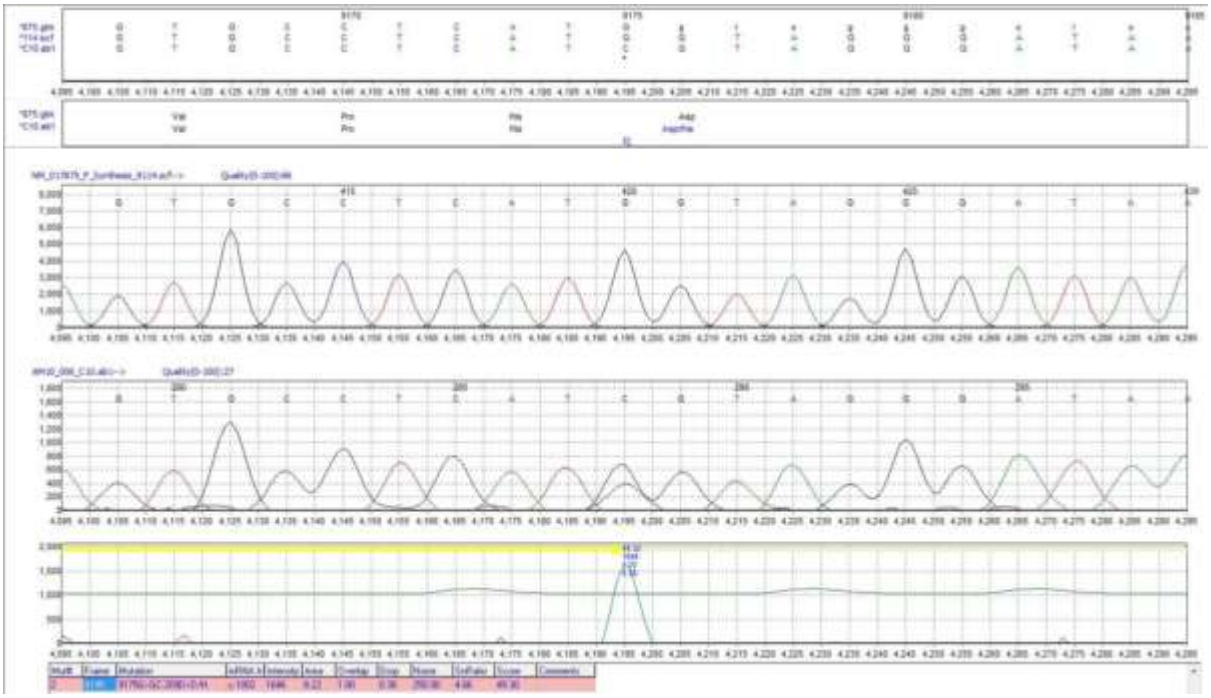
Exon 5 SLC25A38:c.[560G>A]+[625G>C];p.[Arg187Gln]+[His209Asp]

Proband:



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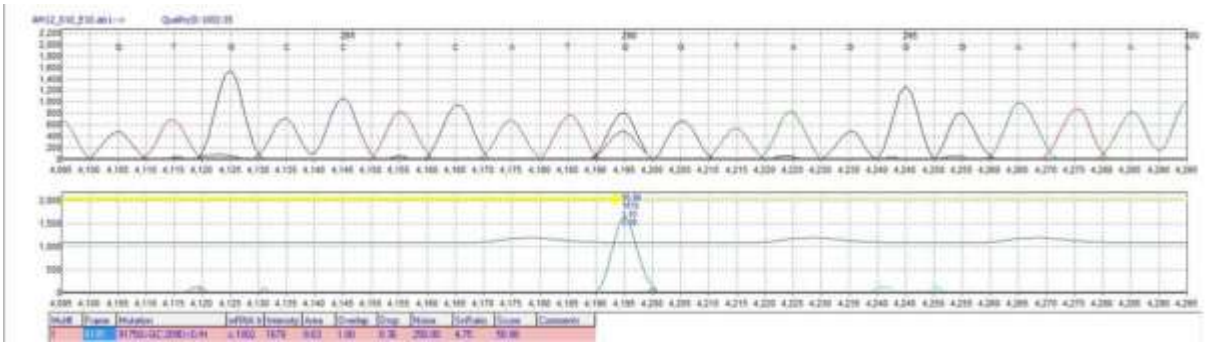

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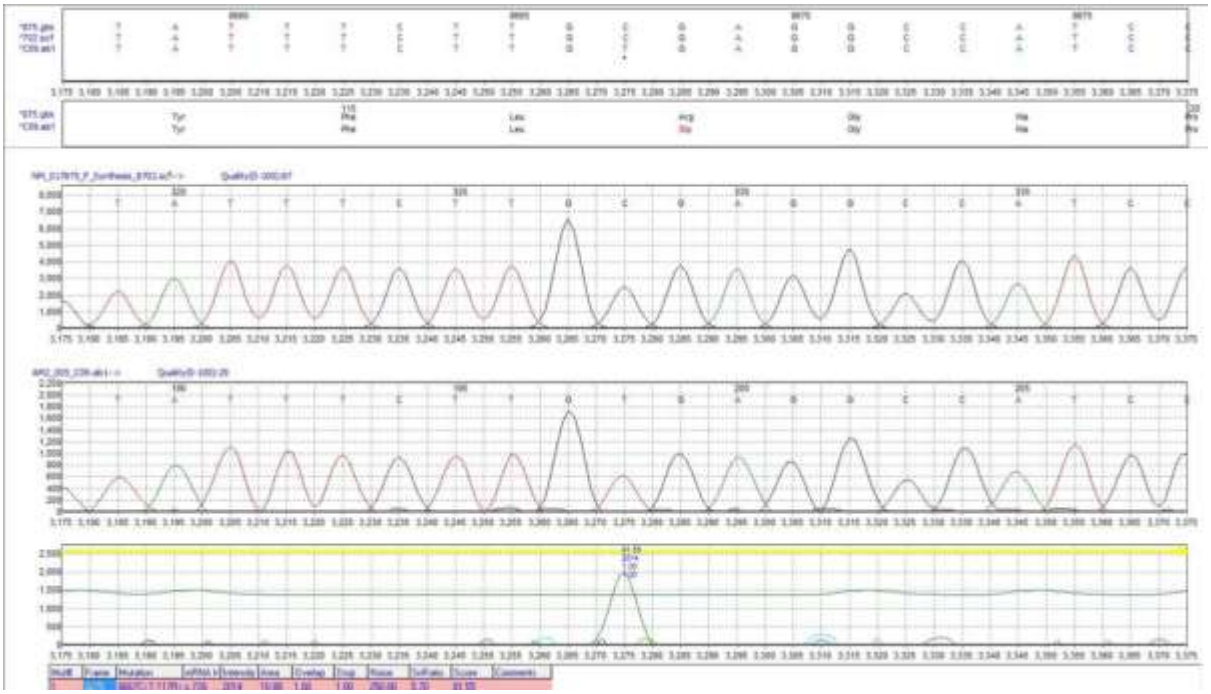
Case number 5
Exon 4 SLC25A38:c.349C>T;p.Arg117Stop
Proband:



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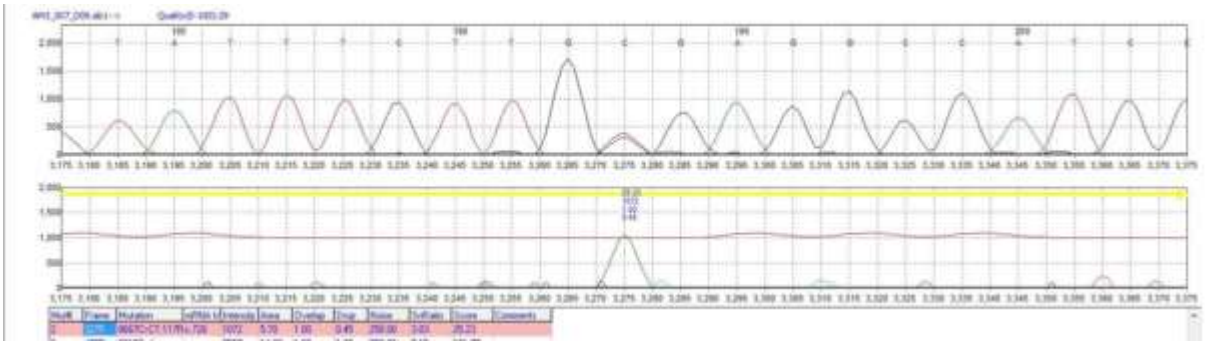

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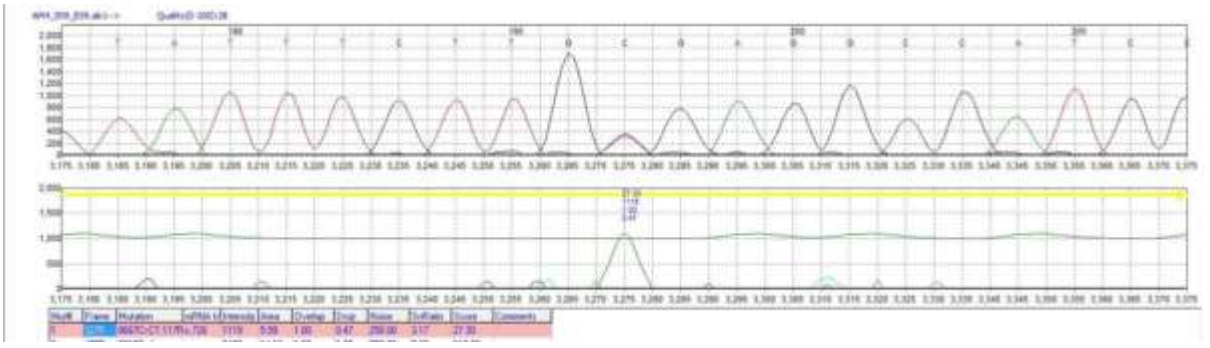

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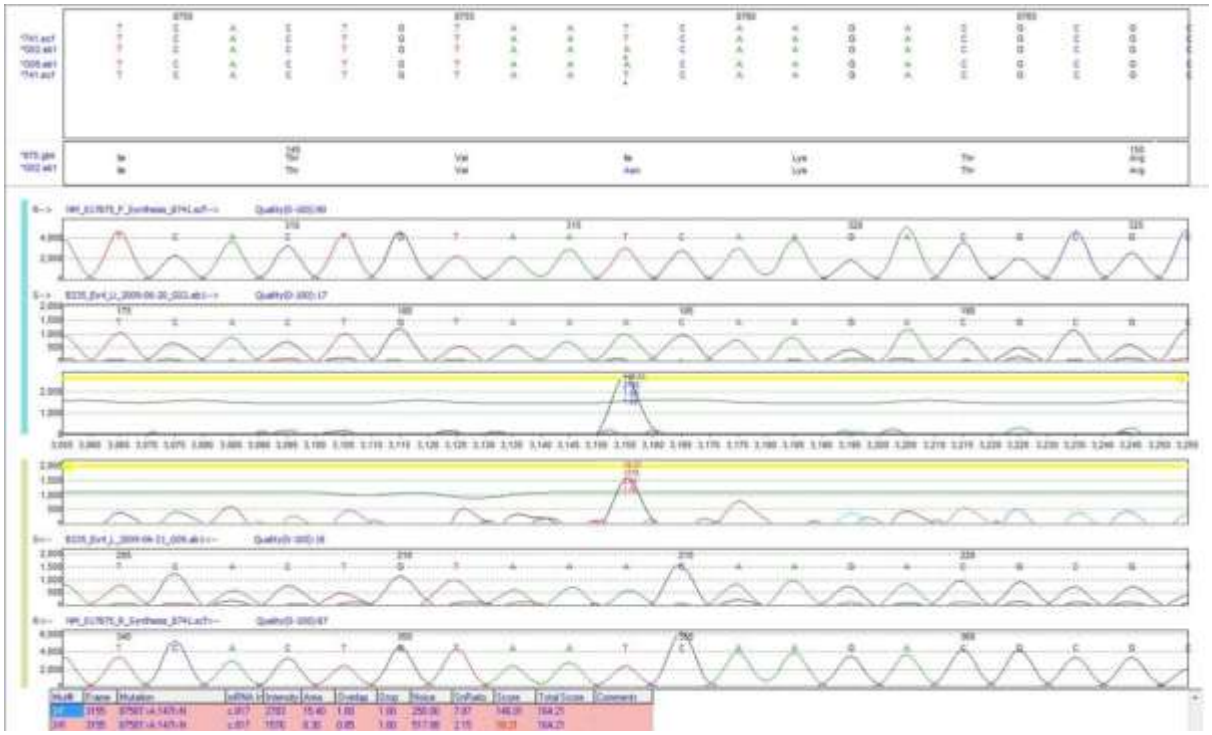
Case number 6:
Exon 4 SLC25A38:c.440T>A;p.Ile147Asn
Proband:



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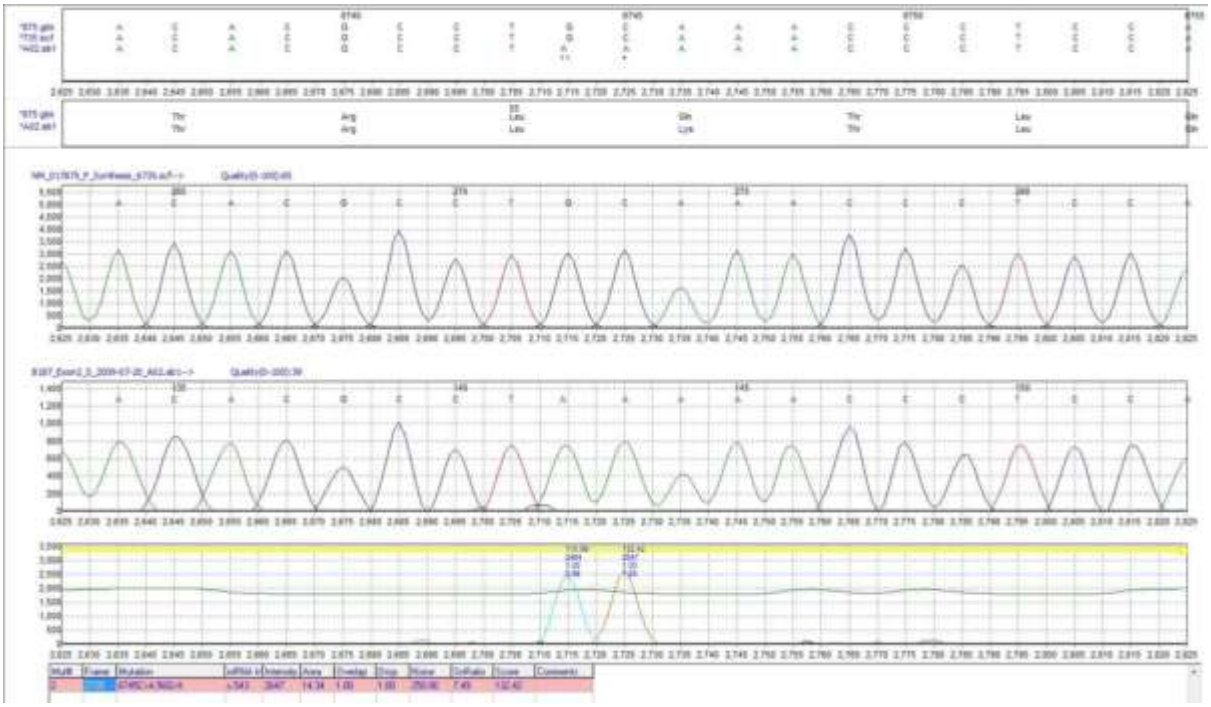
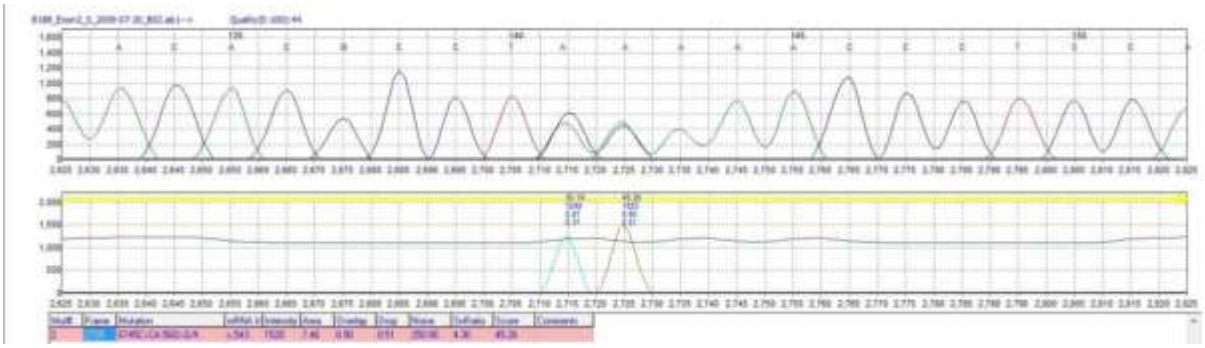
Case number 7:
Exon 2 SLC25A38:c.166C>A;p.Gln56Lys (please note c.165G>A is a common synonymous coding SNP)
Proband:



Parent 1:



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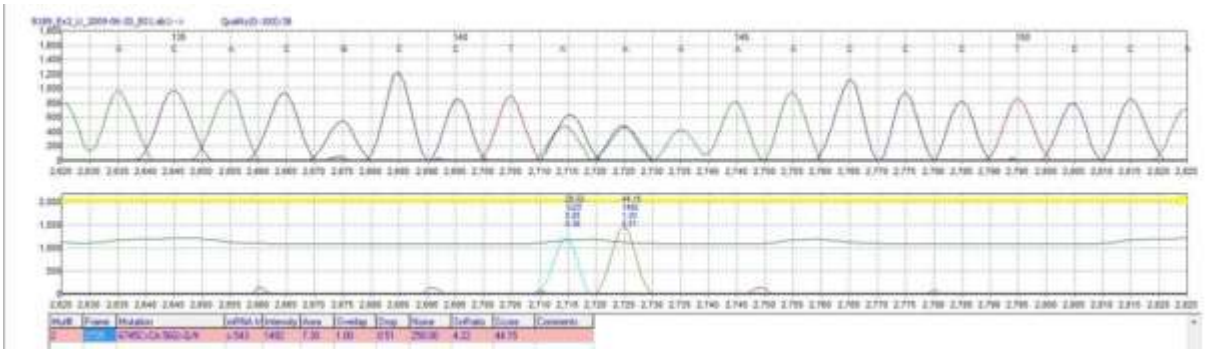

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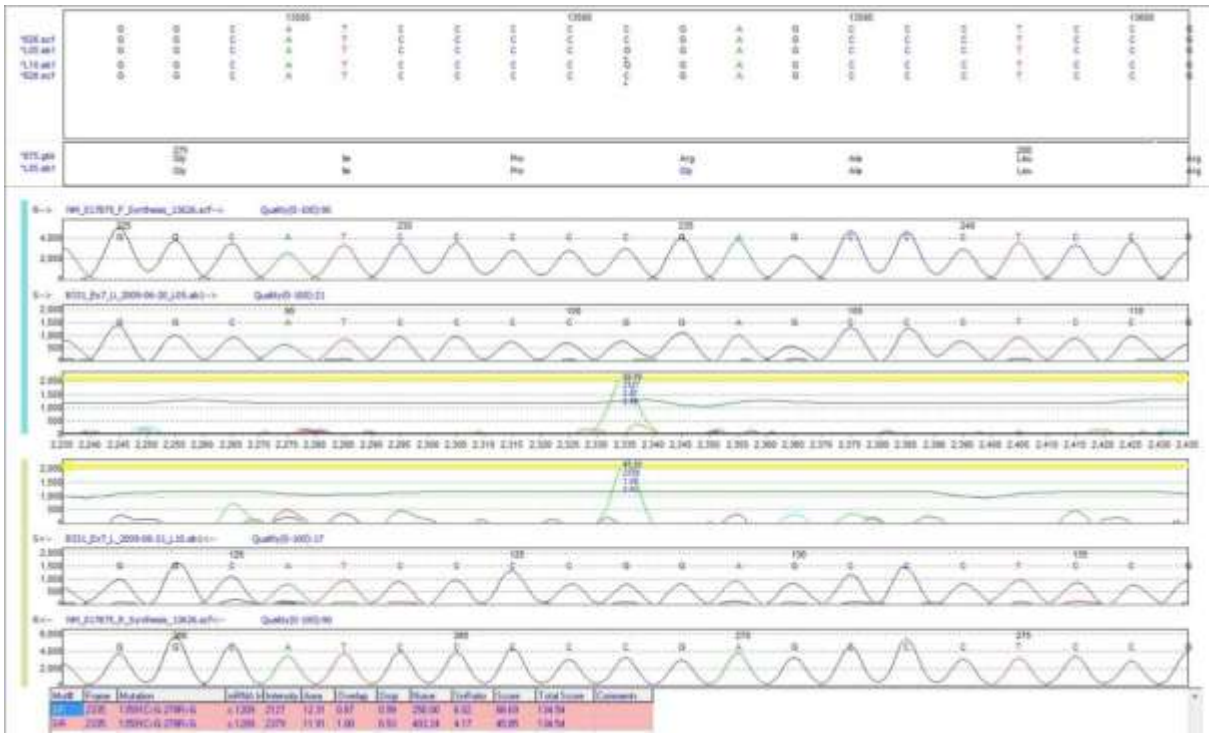
Case number 8:
Exon 7 SLC25A38:c.832C>G;p.Arg278Gly
Proband:



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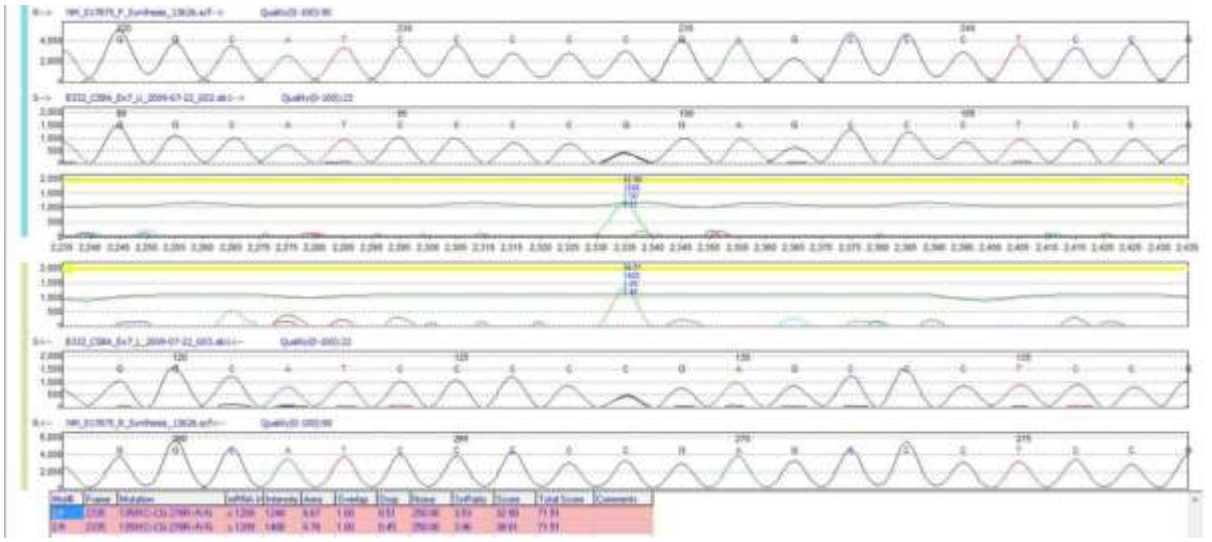

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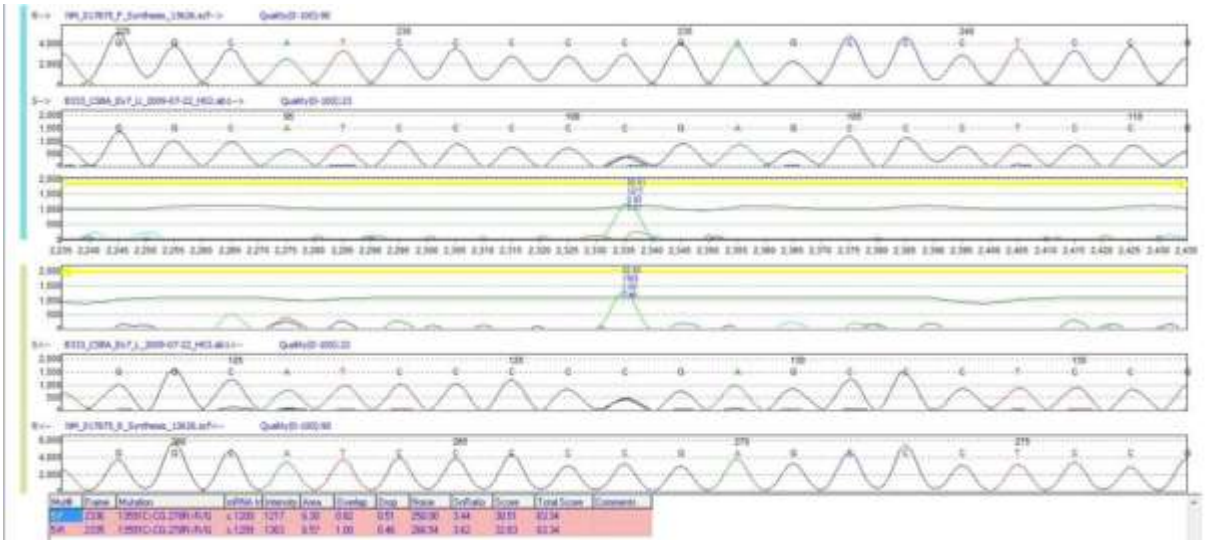

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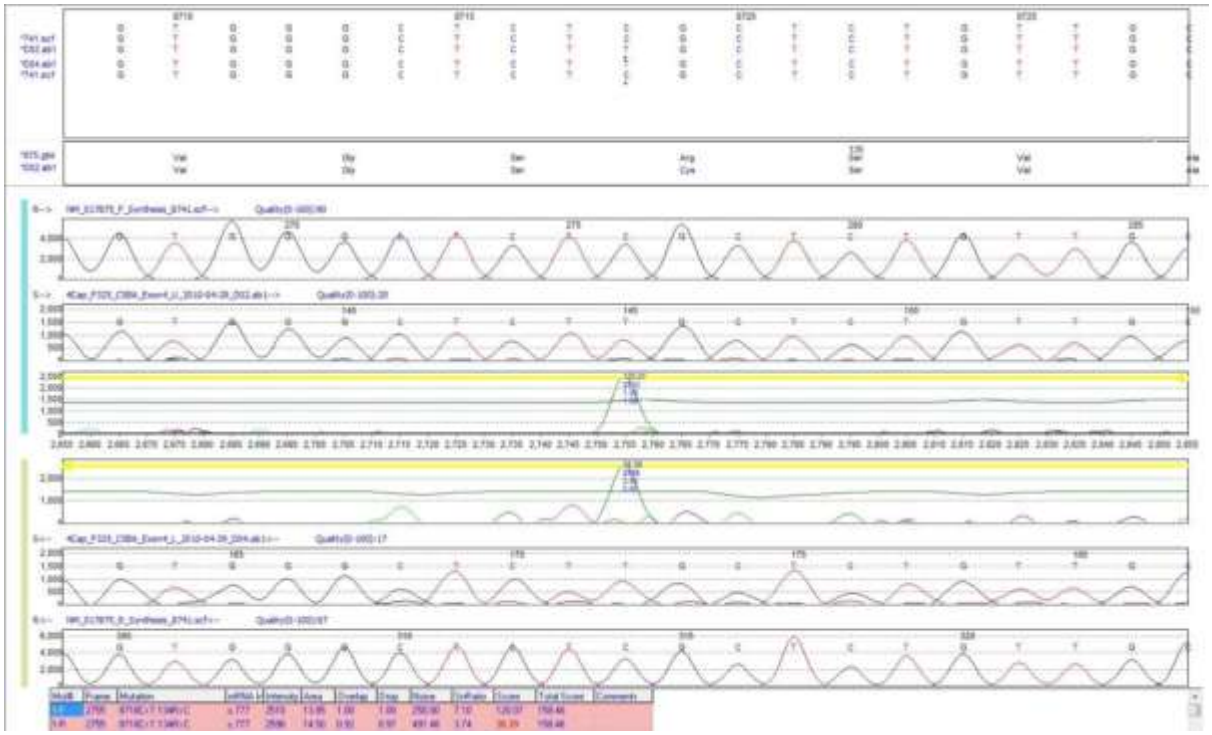
Case number 9:
Exon 4 SLC25A38:c.400C>T;p.Arg134Cys
Proband:



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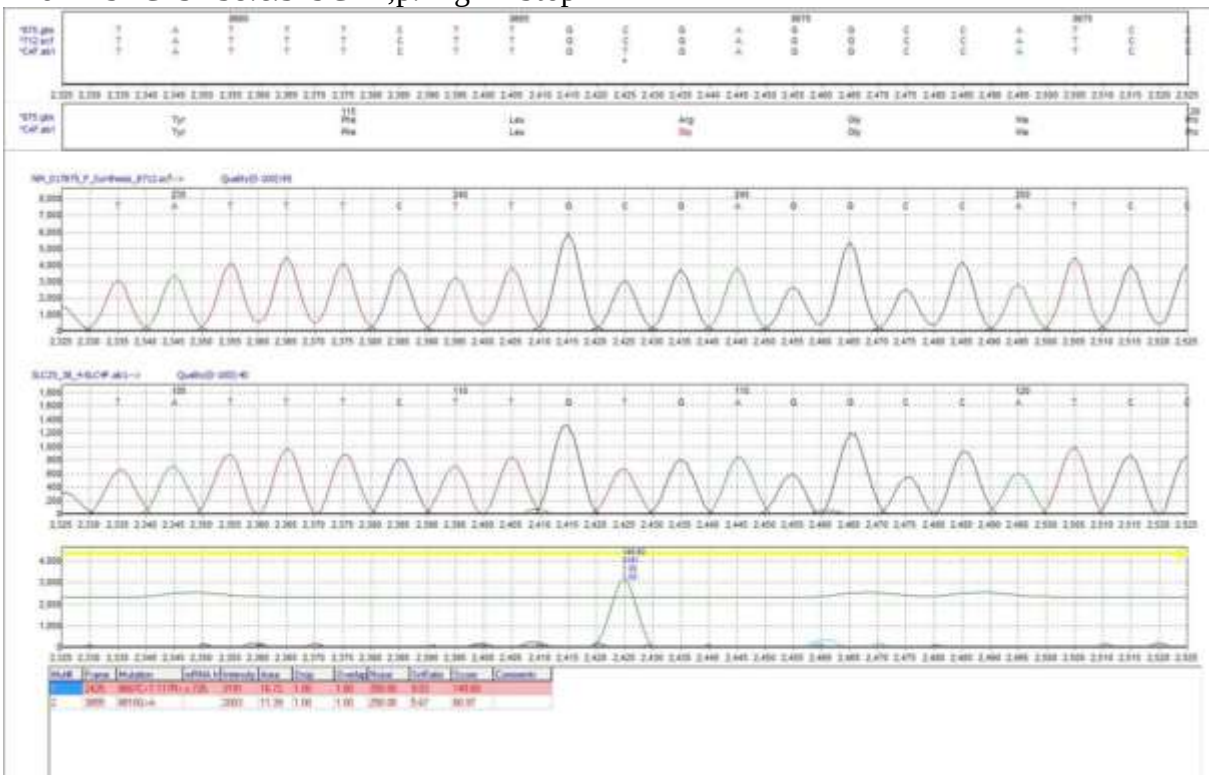
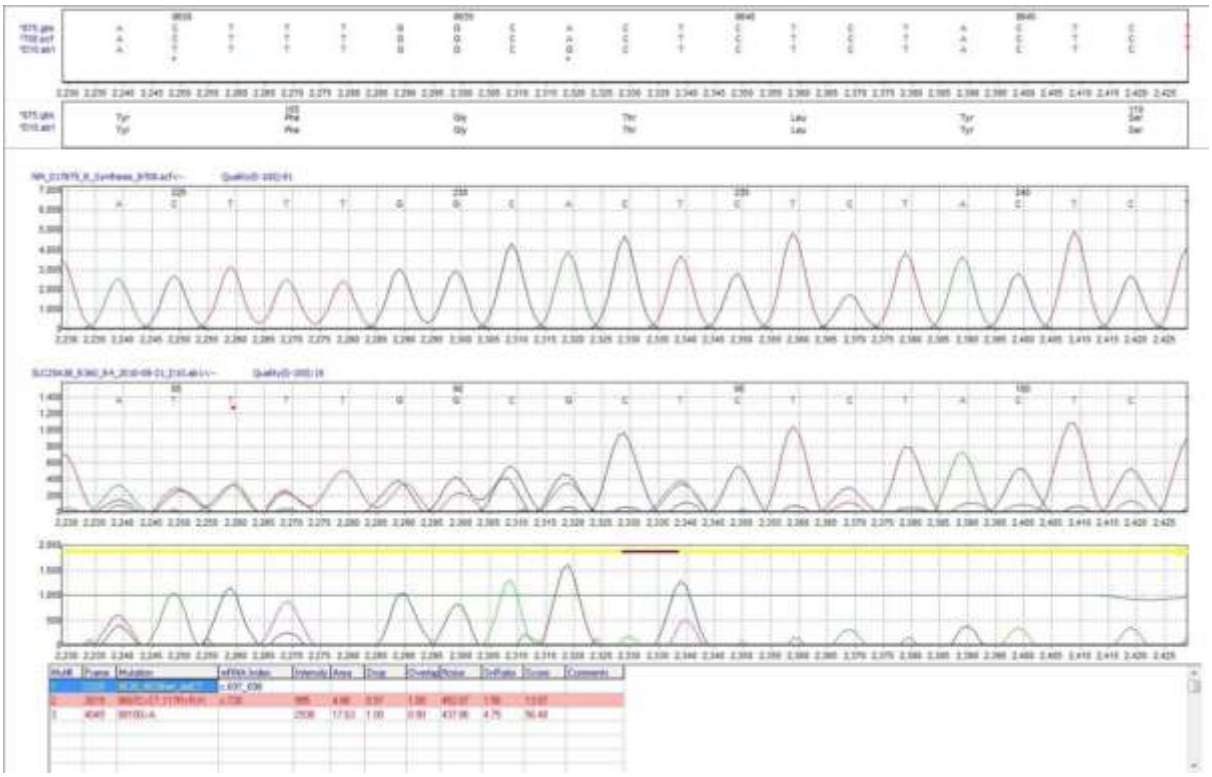
Case number 10
Exon 4 SLC25A38:c.349C>T;p.Arg117Stop



DOI: 10.3324/haematol.2010.039164

Case number 11: Exon 4
SLC25A38:[c.324_325delCT]+[349C>T];p.[Tyr109LeufsX43]+[Arg117Stop]
compound heterozygote
Proband:



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

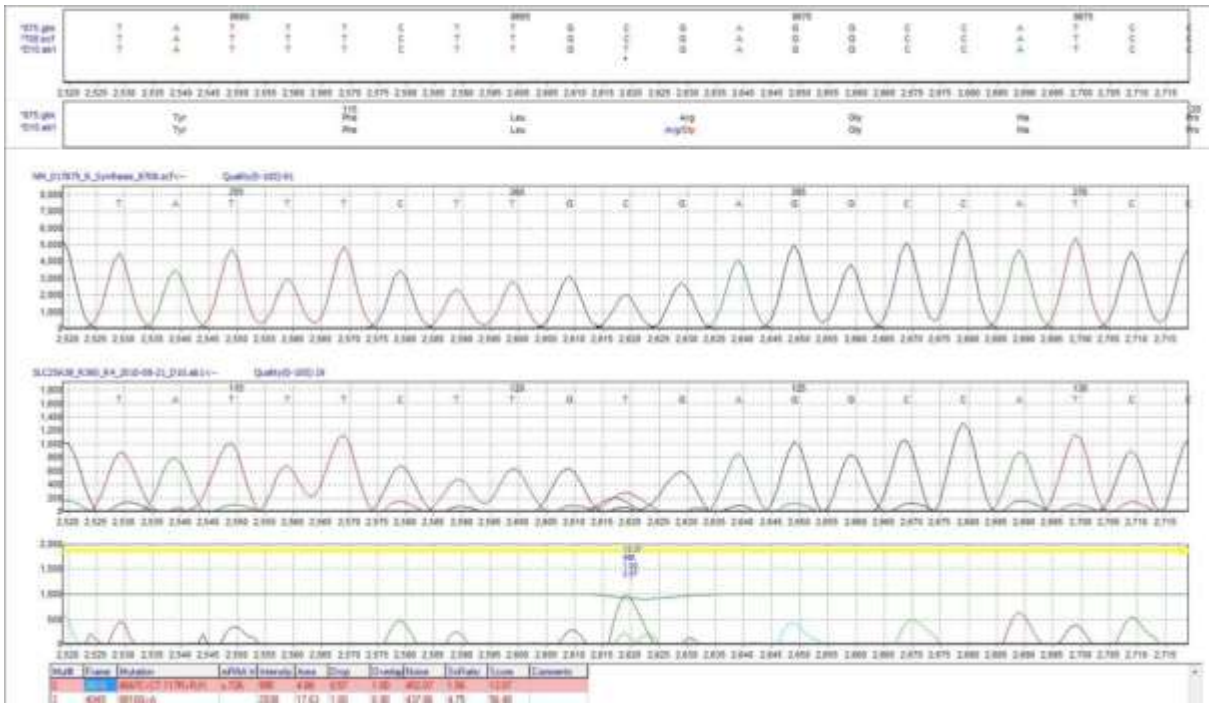


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Figure S2

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 coloured in red, blue or green in the 2c3e structure or in lighter similar colours in the SLC25A38 model.