The phenotypic spectrum of germline *YARS2* variants: from isolated sideroblastic anemia to mitochondrial myopathy, lactic acidosis and sideroblastic anemia 2

Lisa G. Riley,[1,2,*] Matthew M. Heeney,[3,4,*] Joëlle Rudinger-Thirion,[5] Magali Frugier,[5] Dean R. Campagna,[6] Ronghao Zhou,[3] Gregory A. Hale,[7] Lee M. Hilliard,[8] Joel A. Kaplan,[9] Janet L. Kwiatkowski,[10,11] Colin A. Sieff,[3,4] David P. Steensma,[12,13] Alexander J. Rennings,[14] Annet Simons,[15] Nicolaas Schaap,[16] Richard J. Roodenburg,[17] Tjitske Kleefstra,[15] Leonor Arenillas,[18] Josep Fita-Torró,[19] Rasha Ahmed,[20] Miguel Abboud,[20] Elie Bechara,[21] Roula Farah,[21] Rienk Y. J. Tamminga,[22] Sylvia S. Bottomley,[23] Mayka Sanchez,[19,24,25] Gerwin Huls,[26] Dorine W. Swinkels,[27] John Christodoulou[1,2,28,29,#] and Mark D. Fleming[3,6,13,#]

**LGR and MMH contributed equally to this work. #JC and MDF contributed equally to this work as co-senior authors.*

[1]Genetic Metabolic Disorders Research Unit, Kids Research Institute, Children's Hospital at Westmead, Sydney, Australia; [2]Discipline of Child & Adolescent Health, Sydney Medical School, University of Sydney, Australia; [3]Dana Farber-Boston Children's Center for Cancer and Blood Disorders, Boston, MA, USA; [4]Department of Pediatrics, Harvard Medical School, Boston, MA, USA; [5]Architecture et Réactivité de l'ARN, Université de Strasbourg, CNRS, IBMC, Strasbourg, France; [6]Department of Pathology, Boston Children's Hospital, Boston, MA, USA; [7]Johns Hopkins All Children's Hospital, St. Petersburg, FL, USA; [8]Division of Pediatric Hematology Oncology, University of Alabama at Birmingham, AL, USA; [9]Levine Children's Hospital, Charlotte, NC, USA; [10]The Children's Hospital of Philadelphia, Division of Hematology, Philadelphia, PA, USA; [11]University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [12]Adult Leukemia Program, Dana-Farber Cancer Institute, Boston, MA, USA; [13]Harvard Medical School, Boston, MA USA; [14]Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, the Netherlands; [15]Department of Human Genetics, Radboud University Medical Centre, Nijmegen, the Netherlands; [16]Department of Hematology, Radboud University Medical Centre, Nijmegen, the Netherlands; [17]Radboud Center for Mitochondrial Medicine, Translational Metabolic Laboratory, Department of Pediatrics, Radboud University Medical Centre, Nijmegen, the Netherlands; [18]Laboratorio Citología Hematológica, Servicio Patología, GRETNHE, IMIM Hospital del Mar Research Institute, Hospital del Mar, Barcelona, Spain; [19]Iron metabolism: regulation and disease group, Josep Carreras Leukaemia Research Institute (IJC), Campus ICO-Germans Trias i Pujol, Campus Can Ruti, Carretera de Can Ruti, Cami de les Escoles, Badalona, Spain; [20]Department of Pediatrics and Adolescents, American University of Beirut Medical Center, Beirut, Lebanon; [21]Department of Pediatrics, Saint George Hospital University Medical Center, Beirut, Lebanon; [22]Beatrix Children's Hospital, Department of Pediatric Hematology, University Medical Center Groningen, University of Groningen, the Netherlands; [23]Department of Medicine, University of Oklahoma College of Medicine, Oklahoma City, OK, USA; [24]Programme of Predictive and Personalized Medicine of Cancer, Germans Trias i Pujol Research Institute (PMPPC-IGTP), Badalona, Spain; [25]BloodGenetics, S.L., Esplugues de Llobregat, Barcelona, Spain; [26]Department of Hematology, University Medical Center Groningen, the Netherlands; [27]Department of Laboratory Medicine, Translational Metabolic Laboratory, Radboud University Medical Centre, Nijmegen, the Netherlands; [28]Neurodevelopmental Genomics Research Group, Murdoch Childrens Research Institute, Melbourne, Australia and [29]Department of Paediatrics, Melbourne Medical School, University of Melbourne, Australia

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Correspondence: john.christodoulou@mcri.edu.au

Supplemental Data

Clinical histories

Patient 1 (P1)

Patient 1 was the child of parents of Lebanese Christian ancestry. She was diagnosed with iron deficiency anemia with exercise intolerance at age 8 years, and was treated with a multivitamin containing iron. At age 13 years, she was found to have sinus tachycardia, and was treated with atenolol without response. She had a history of growth delay, and was diagnosed with ovarian failure at age 14 years. In the same year, a bone marrow aspirate and biopsy was performed to evaluate her chronic anemia and leukopenia/neutropenia. The bone marrow showed maturing trilineage hematopoiesis and ringed sideroblasts. Due to persistent elevated lactate, easy fatigability, and negative mitochondrial DNA analysis, she underwent muscle biopsy which was diagnostic of a mitochondrial myopathy. She was treated with dichloroacetate for her chronic lactic acidosis, but this was stopped as it may have contributed to her neutropenia, yet the neutropenia persisted even after cessation of dichloroacetate. Her symptoms of fatigue and exercise intolerance worsened, and a repeat bone marrow when she was age 16 years showed qualitative increased iron staining. Multiple experimental therapies for a presumed mitochondrial disorder were tried, but none were effective. Her condition remained stable for several years, until she developed progressive anemia and neutropenia at age 25 years and another bone marrow aspiration and biopsy showed ~10% ringed sideroblasts. She continued to be anemic with profound lactic acidosis, and became transfusion dependent age 27 years. She was admitted shortly before her 28th birthday for epigastric pain and shortness of breath from pericardial effusion. She underwent pericardial drainage. She subsequently developed severe

thrombocytopenia of unclear etiology. She was ultimately diagnosed with hemophagocytic lymphohistiocytosis treated with dexamethasone and etoposide. Her clinical condition progressively worsened including hypoxic respiratory failure and she died 2 months later at age 28 years due to multiorgan dysfunction (respiratory, circulatory, renal, and liver) and fungal sepsis.

Patient 2a (P2a)

Patient 2a is now a 9 year-old boy of consanguineous Lebanese Christian parentage with a history of an atrial septal defect status-post transcatheter repair who presented at the age of 6 with a new onset anemia, with a haemoglobin of 3.5 g/dL, an a normal WBC and platelet count. Direct antiglobulin and osmotic fragility tests were negative. Hemoglobin electrophoresis and G6PD were normal and parvovirus and hepatitis serologies were negative, as was a chromosomal breakage study for Fanconi Anemia. Since that time, he has required chronic transfusions. A bone marrow examination performed at age 8 showed a relative erythroid hyperplasia with dyserythropoiesis and numerous ringed sideroblasts. Exome sequencing of the patient, his sister (Patient 9b), and parents showed no other mutations associated with anemia or sideroblastic anemia.

Patient 2b (P2b)

Patient 2b is the younger sister of patient 2a, who, at age 4 years, at the time her brother was diagnosed with anemia, was screened and found to have a hemoglobin of 7 g/dL. A peripheral smear showed marked anisopoikilocytosis tending to slight microcytosis, slight hypochromia, some ovalocytes, rare teardrop RBCs, and stomatocytes, slight polychromasia and few

fragmented RBCs seen. Her hemoglobin has gradually increased in the subsequent 3 years, ranging from 9-12 g/dl without transfusion.

Patient 3 (P3)

Patient 3, now aged 10 years, is the second child of non-consanguineous Lebanese parents of Christian Maronite ancestry. He was born at term following an uncomplicated pregnancy by lower segment caesarean section. Birth weight was 1800 gm.

He was noted to have pallor and fatigue at the age of six years. A full blood count at that time revealed mild anemia, which was treated with intermittent oral iron for about three years. However, he continued to have pallor, fatigue and exercise intolerance. An echocardiogram was normal. Because of a tendency to diarrhea and growth retardation, an upper GI endoscopy was performed, which revealed no abnormalities. He was referred at that time to the hematology service with persistent anemia and pain in the lower extremities associated with exertion.

His most recent blood count revealed a mild (Hb 10 g/dL) normocytic anemia, with normal WBC and platelet counts, and with 2% reticulocytes. He has never received any blood transfusion. Blood lactate levels before and after exercise were 9.5 mmol/L and 13.9 mmol/L respectively (normal < 2.2). Urinalysis was normal. Repeat resting lactate levels ranged from 6 – 13.9 mmol/L. CPK was mildly elevated at 298 U/L (normal 24 – 190). Plasma total and free carnitine levels were normal. Viral serology was indicative of past CMV and EBV infections. A bone marrow aspirate and biopsy showed some hypercellularity, with full trilineage maturation and multiple ring sideroblasts (30% of total erythroblasts) and no evidence of blast excess. Mild eosinophilia with increased mast cells were also noted. A blood karyotype was also normal apart from a constitutional pericentric inversion of chromosome 9, and there was no excess

chromosomal breakage with mitomycin C. Abdominal ultrasound revealed mild hepatosplenomegaly.

When recently reviewed his weight was 27.5 kg (25^{th} percentile) and height was 132 cm (10^{th} – 25^{th} percentile). His older brother is healthy.

Patient 4 (P4)

Patient 4 was the first live birth of parents who were first cousins. He had a younger sibling with Filippi syndrome and an unaffected sibling. He presented to his general practitioner with headache and dyspnea at age 19 years, and was referred to hematology where he was found to have mild splenomegaly and a hypoproductive normocytic anemia (Hb 6.6 g/dL; MCV 81fL). Bone marrow aspirate and biopsy revealed ringed sideroblasts and features suggestive of myelodysplasia, with normal cytogenetics. Pyridoxine treatment did not improve the anemia. At age 20 years he was diagnosed with a myelodysplastic syndrome classified as Refractory Anemia with Ringed Sideroblasts (MDS-RARS) and required transfusions every two months for 6 months, thereafter his anemia recovered. However, by age 25 years, the anemia relapsed and a repeat trial of pyridoxine was initiated. Erythrocyte transfusions every 2 months were needed to keep his hemoglobin stable. By age 26 years he was diagnosed with MDS-Refractory Cytopenia with Multilineage Dysplasia (MDS-RCMD) with ringed sideroblasts and at age 27 years he became increasingly transfusion dependent. Additional treatment with Danazol and Cyclosporine A did not have any effect on the transfusion dependency. Variants in the *ALAS2* gene were excluded by Sanger sequencing and Fanconi Anemia was ruled out because of normal chromosomal breakage studies. At this age his transferrin saturation and his ferritin varied around 90% and 700 µ/L, respectively. An MRI of the liver and heart showed moderate to severe

iron overload in the liver but no myocardial iron overload and he received iron chelation. At age 28 years he underwent allogeneic hematopoietic stem cell transplantation from a matched unrelated donor to treat his anemia. He did not develop any graft-versus-host disease. After the transplant, his hemoglobin concentration increased from 9.7 g/dL to 12.6 g/dL (Supplementary table 1). At age 29 years he had no symptoms of myopathy, diabetes mellitus, vision or hearing loss, epilepsy or cognitive dysfunction but had moderate exercise induced lactic acidosis (pH 7.39 to 7.28 and whole blood lactate 1.5 mmol/L to 6.3 mmol/L), potentially indicative of mitochondrial disease, or related to cardiac dysfunction following transplantation. Biochemical analysis of patient fibroblasts showed normal respiratory chain enzymes (Supplementary table 2). However, fibroblast respiratory chain enzymes are normal in ~ 50% of cases where a deficiency has been detected in other tissues[1], including previously reported YARS2 cases[2]. 2.5 years following stem cell transplantation, he is in complete hematological remission, with a normal echocardiography and without graft-versus host disease. At last follow up, phlebotomy was begun for a ferritin of 906 μg/L.

Supplementary Table 1. Hemoglobin levels of P4 returned to normal following hematopoietic stem cell transplantation (HSCT).

Lab Parameters	**10 d pre-HSCT**	**16 d post-HSCT**	**4 m post-HSCT**	**6 m post-HSCT**
Hemoglobin (g/dL)	9.7	9.7	11.0	12.6
Leukocytes (× 10^9/L)	6.5	1.8	2.6	4,1
Thrombocytes (× 10^9/L)	261	116	122	139
Hematocrit (%)				36

Other lab parameters 6 m post-HSCT: pyruvate, 31 μmol/L; acetoacetic acid, 89 μmol/L; beta hydroxyboterzuur, 114 μmol/L; amino-acids analysis plasma, normal; no indications for glycosylation defects; elevated urine lactate; ECG, RSR 97/min, QRS 0.10 secs, no infarct repolarisation pattern or LVH.

Supplementary Table 2. Mitochondrial respiratory chain enzyme activities in fibroblasts of P4

	Enzyme Activity (mU/U CS)	
Enzyme	**Patient 4**	**Reference Values**
Complex I	318	163 - 599
Complex II	485	335 - 888
Complex III	902	570 - 1383
Complex IV	477	288 - 954
Complex V	629	193 - 819

Patient 5 (P5)

Patient 5, a girl, developed a profound normocytic nonregenerative anemia (Hb 3 g/dL) at age 2 years. Bone marrow aspiration and biopsy was hypercellular with erythroid hyperplasia, chromosomal analysis was normal, and mitochondrial DNA analysis was negative. She was treated with corticosteroids and folic acid for 2 months with no response, and received a total of six transfusions in a period of 4 months after diagnosis. Her anemia entered a spontaneous remission until the age of 6 years. Stool and urine had no evidence of blood, and a repeat bone marrow aspiration and biopsy revealed the presence of ringed sideroblasts. She was treated with pyridoxine for 4 months without hematologic response, and received monthly transfusions until her first unrelated donor HSCT under a reduced intensity regimen at age 9 years. She experienced secondary graft rejection, and subsequently had mild pancytopenia with episodic transfusion requirements for blood and platelets and filgrastim. She underwent a second

unrelated donor HSCT, after chelation. She died in 2013 at the age of 12 due to multi-organ failure dominated by liver dysfunction. No infectious etiology was detected. Lactate levels were always normal until just before her death.

Patient 6 (P6)

Patient 6 initially presented at 20 months of age, with a profound macrocytic anemia (Hb 2 g/dL, MCV 101fL) and severe iron overload. Bone marrow aspiration and biopsy revealed limited maturation of the erythroid lineage, dyserythropoiesis, and ringed sideroblasts (>15%). Cytogenetic analysis was normal, and lead level was normal. Mitochondrial DNA analysis was negative for deletions or duplications. She received a trial of pyridoxine, without hematological benefit. She is now age 19 years and has been maintained on chronic transfusion every three weeks, and iron chelation therapy. She has had mild lactic acidosis that worsens with intercurrent illnesses. She also has splenomegaly, thrombocytopenia, and intermittent neutropenia.

Patient 7 (P7)

Patient 7 presented at 3 months of age with normocytic hyporegenerative anemia (Hb 5.8g/dL). She received two transfusions and recovered a normal hemoglobin level. At age 16 months, she had high RBC at 5.31, normal hemoglobin and hematocrit, but microcytosis (MCV 66 fL) unresponsive to iron supplementation thought to represent alpha thalassemia trait. She returned at age 16 years with mild anemia that was unresponsive to iron treatment. She complained of overwhelming fatigue, and had intermittent diarrhea and abdominal pain. GI evaluation was negative for celiac disease and with normal stool elastase and normal upper and lower endoscopy. Bone marrow aspiration and biopsy showed 47% ringed sideroblasts. Electron

microscopy revealed normoblasts with cytoplasmic vacuolization and abnormal appearing mitochondria. She has no lactic acidosis. She describes easy fatigability, poor exercise tolerance and muscle pain and anecdotally is less fatigued when taking Coenzyme Q and B12 supplements. She is now 18 years old and was not anemic on her last two visits.

Patient 8a (P8a)

Patient 8a is a 53 year-old woman of mixed European background who developed extremity swelling at age 13 of unclear etiology. She was first diagnosed with anemia at age 18 years, but also reported a history of anemia during childhood based on pallor and was treated with supplemental iron in early adolescence. She joined the army at age 18 years, and reported rapid fatigability compared to her peers. She left the army after 3 years, related to fatigue and pain from bursitis. She completed two pregnancies but these were associated with extensive extremity swelling, leukopenia and thrombocytopenia requiring platelet transfusions for Caesarean sections. Bone marrow aspirate revealed a hypercellular marrow, moderately increased erythroid elements and proportionally decreased myeloid elements, mildly increased megakaryocytes, and ringed sideroblasts (40%). The peripheral blood smear showed macrocytic anemia and mild thrombocytopenia. Flow cytometric analysis and cytogenetic analysis were normal. Clinical targeted sequencing showed one unequivocal *YARS2* variant, but concern for MDS prompted further genetic screening that revealed a heterozygous mutation in *SF3B1* (p.Lys700Glu) that is commonly associated with acquired forms of sideroblastic anemia. She has normal lactate at baseline, but this has been demonstrated to significantly increase with minimal exertion.

Patient 8b (P8b)

Patient 8b is the asymptomatic sister of Patient 8a. She had no anemia or myopathy when investigated at age 48.

Patient 9a (P9a)

Patient 9a was found to have a profound macrocytic anemia (Hb 2.4 g/dL; MCV 113.8fL) at age 3 months. He was initially suspected to have Diamond-Blackfan Anemia, and was transfusion dependent for the first year of life to maintain a normal hemoglobin. His anemia spontaneously resolved at 1 year of age. Erythrocyte adenosine deaminase activity was slightly elevated, and mitochondrial DNA deletion analysis was negative. Transfusion dependent anemia recurred at 23 months of age for nearly 2 years, but he now continues in another period of remission, currently aged 6 years. Bone marrow aspiration and biopsy at the age of 3 revealed frequent presence of ringed sideroblasts (50%), and lymphocytosis with increased numbers of hematogones. The patient displayed some dysmorphic features with a triangular shaped head and wide set eyes.

Patient 9b (P9b)

Patient 9b is the asymptomatic fraternal twin of Patient 9a. He was not anemic at age 3 months when his brother presented, and again at age 4.

Patient 10 (P10)

Patient 10 is a young woman referred to the Hematology Department of Hospital del Mar-IMIM (Barcelona, Spain) at 23 years for the study of anemia. At that time, she had a normocytic anemia (Hb 9.6 g/dL; MCV 86 fL). She is an adopted child, and so family history is not

available. She reported a previous history of anemia during adolescence. Bone marrow aspirate revealed increased cellularity with a normal proportion of myeloid (erythroid, granulocytic and megakaryocytic) precursors. Approximately 20% of erythroblasts had an anomalous distribution of hemoglobin and basophilic stippling. On an iron-stained aspirate smear there were 32% ringed sideroblasts. Cytogenetic analysis was normal and no mutations in *SF3B1* were found in exons 14 and 15. She reported a moderate asthenia with no other symptoms.

Patient 11 (P11)

Patient 11 experienced progressive fatigue, tiredness and reduced exercise capacity at 13 years of age. Prior to then, he was considered healthy, though he did not have the endurance of his soccer teammates and his physical development was always slightly behind that of his older sister. He was assessed as having an average school performance.

Laboratory findings indicated anemia with reduced reticulocytes and a high transferrin saturation. No other abnormalities were found that could explain the normocytic anemia, including viral infections, vitamin deficiencies or intoxications. Bone marrow smear examination showed an increased cellularity with reduced dysplastic erythropoiesis, normal myelopoiesis and increased and dysplastic megakaryopoiesis; on the iron stain a large number of ringed sideroblasts as well as iron accumulation in the macrophages were present. EPO levels were greatly increased (967 U/l; reference range 4.5-19.6); hepcidin 17.1 nM (> upper level of reference range). Cytogenetics were normal and an MDS sequencing panel showed a heterozygous variant of uncertain significance in *DNMT3A*.

The patient was diagnosed with sideroblastic anemia, the cause of which was uncertain (*e.g.*, acquired MDS or congenital). Pyridoxine was prescribed without benefit. Since then, he has been maintained on erythrocyte transfusions every 3-5 weeks.

5 months after presentation, the serum ferritin was 583 ng/ml. MRI of heart (T2* 11.6 ms) and liver (T2* 22.0 ms) indicated iron loading in the heart (2.87 mg Fe/g) but not in the liver, and chelation was begun. Echocardiography showed a normal myocardial function with enhanced trabeculation especially in the left ventricle, no hypertrophy and an increased end-diastolic diameter of the left ventricle (48.6 mm), probably related to the anemia.

In addition, his motor performance was investigated (hand-held dynamometry and Bruininks-Oseretsky Test 2), showing reduced muscle strength (<1-2 SD) particularly after prolonged activity. His parents recognized this pattern of self-imposed physical restriction during daily life from early childhood. During this motor function test, the serum lactate increased from a baseline of 4.5 mmol/L (normal values 0.4-1.9) to 10.0 mmol/l. Moreover, plasma amino acid analyses showed an elevated alanine level (774 umol/l, reference range 174-450), consistent with an underlying mitochondrial defect.

He was eventually diagnosed with MLASA2 following a targeted gene sequencing panel for congenital sideroblastic anemia genes.

References

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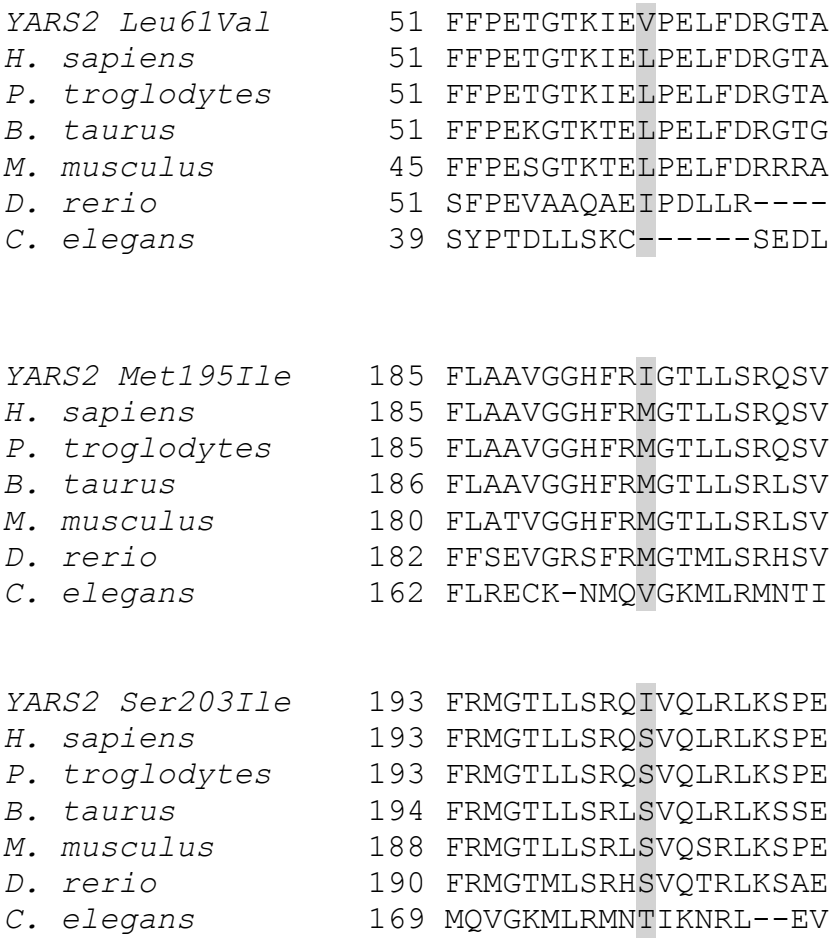
Supplementary Figure 1. YARS2 sequence alignments showing the location of p.Leu61Val, p.Met195Ile, p.Ser203Ile, p.Tyr236Cys, p.Gly244Ala (shaded) and their conservation among species.



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YARS2 Tyr236Cys    226 AYDFYYLFQRCGCRVQLGGS
H. sapiens         226 AYDFYYLFQRYGCRVQLGGS
P. troglodytes     226 AYDFYYLFQRYGCRVQLGGS
B. taurus          227 AYDFYYLFQHYGCRVQLGGS
M. musculus        221 AYDFYYLFQHYGCRVQLGGS
D. rerio           223 AFDFYQLHQLHGCRIQLGGS
C. elegans         200 AFDWYTLSEKYGCRFQLGGY


YARS2 Gly244Ala    234 QRYGCRVQLGASDQLGNIMS
H. sapiens         234 QRYGCRVQLGGSDQLGNIMS
P. troglodytes     234 QRYGCRVQLGGSDQLGNIMS
B. taurus          235 QHYGCRVQLGGSDQLGNIMS
M. musculus        229 QHYGCRVQLGGSDQLGNIMS
D. rerio           231 QLHGCRIQLGGTDQLGNIMS
C. elegans         208 EKYGCRFQLGGYDQLGHLDF
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