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"Molecular Dissection of the genetic underpinnings of Congenital Sideroblastic Anemia, with a focus on SLC25A38 and mtATP6"

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“ Dissection of the genetic underpinnings of Congential Sideroblastic Anemia, with a focus on SLC25A38 and mtATP6”

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Inhaltsverzeichnis

Abkürzungsverzeichnis

Abkürzung	Bedeutung
CAS	Congenital Sideroblastic Anemia
ISC	Iron Sulfur Cluster
RCP	Respiratory Chain Protein
OP	Oxidative Phosphorylation
HBS	Heme Biosynthesis
NGS	Next Generation Sequencing
SLC25A38	Solute Carrier Family 25 Member 38
ALA	Aminolevulinate
MT-ATP6	Mitochondrially Encoded ATP Synthase Membrane Subunit 6
ATP6-SA	Mitochondrially Encoded ATP Synthase Membrane Subunit 6 – Sideroblastic Anemia
A-CSA	Acquired Congenital Sideroblastic Anemia
MDS	Myelodysplastic Syndrome
MPN	Myeloproliferative Neoplasms
MT-Translation	Mitochondrial Translation
PS	Pearson’s Syndrome
MLASA	Mitochondrial myopathy with lactic acidosis and sideroblastic anemia
YARS2	Tyrosyl-TRNA Synthetase 2 (YARS2).
SA	Sideroblastic Anemia
LA	Lactic Acidosis
ACSA	Autosomal Congenital Sideroblastic Anemia
XLSA	X linked Sideroblastic Anemia
HSPA9	Heat-shock protein A9
GLRX5	Glutaredoxin-related protein 5
PUS1	Pseudouridine Synthase 1
TRMA	tmRNA (uracil-C(5))-methyltransferase
SLC19A2	Solute Carrier Family 19 Member 2
ABCB7	ATP Binding Cassette Subfamily B Member 7
NDUFB11	NADH:Ubiquinone Oxidoreductase Subunit
HLASA	Hydrops, lactic acidosis, sideroblastic anemia
LARS2	Leucyl-TRNA Synthetase 2

SFID	Congenital sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay
TRNT1	TRNA Nucleotidyl Transferase 1
mtDNA	Mitochondrial DNA
PBS	Peripheral Blood Smear
BMA	Bone Mirrow Aspirate
PF	Predictive Factor

Publikationsliste

1. **Berhe, S**., Heeney, M. M., Campagna, D. R., Thompson, J. F., White, E. J., Ross, T., ... & Fleming, M. D. (2018). Recurrent heteroplasmy for the MT-ATP6 p. Ser148Asn (m. 8969G> A) mutation in patients with syndromic congenital sideroblastic anemia of variable clinical severity. *haematologica*, *103*(12), e561.

2. Heeney, M.*, **Berhe, S**.*, Campagna, D., Oved, J., Kurre, P., Shaw, P., ... & Fleming, M. (2021). SLC25A38 Congenital Sideroblastic Anemia: Phenotypes and genotypes of 31 individuals from 24 families, including 11 novel mutations, and a review of the literature. *Authorea Preprints*.

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Beitrag zu den Veröffentlichungen

Im Rahmen der beiden Veröffentlichungen „Recurrent heteroplasmy for the MT-ATP6 p.Ser148Asn (m.8969G>A) mutation in patients with syndromic congenital sideroblastic anemia of variable clinical severity" und " SLC25A38 congenital sideroblastic anemia: Phenotypes and genotypes of 31 individuals from 24 families, including 11 novel mutations, and a review of the literature" war ich von der Samplebeschaffung, -prozessierung und letztendlich Sequenzanalyse inklusive in-vitro und in-siliko Analyse (für detaillierte Beschreibung der Methoden, bitte siehe ‚Materials and Methods" in den jeweiligen Veröffentlichungen) bis hin zur Katalogisierung der Daten involviert.

Beide Manuskripte wurden schließlich von mir in Zusammenarbeit mit Herrn Prof. Dr. Fleming verfasst. Das Entwerfen des Manuskripts " SLC25A38 congenital sideroblastic anemia: Phenotypes and genotypes of 31 individuals from 24 families, including 11 novel mutations, and a review of the literature" ist in Kooperation mit Herrn Prof. Dr. Heeney erfolgt.

1. Einleitung

Congenital Sideroblastic Anemia (CSA) is a form of inherited anemia within the spectrum of mitochondrial dysfunction, which needs to be strictly differentiated from Acquired Congenital Sideroblastic Anemia (A-CSA) for diagnostic but also therapeutic purposes [1-3].

A-CSA can be for example related to exposure to certain drugs such as linezolid, nutritional deficiencies such as copper deficit or on the other hand bone marrow failure disorders such as myelodysplastic syndromes (MDS) or myeloproliferative neoplasms (MPN) [4-8].

CSA is secondary to mitochondrial defects and can thereby be systemized in to distinct mitochondrial pathways [1] :

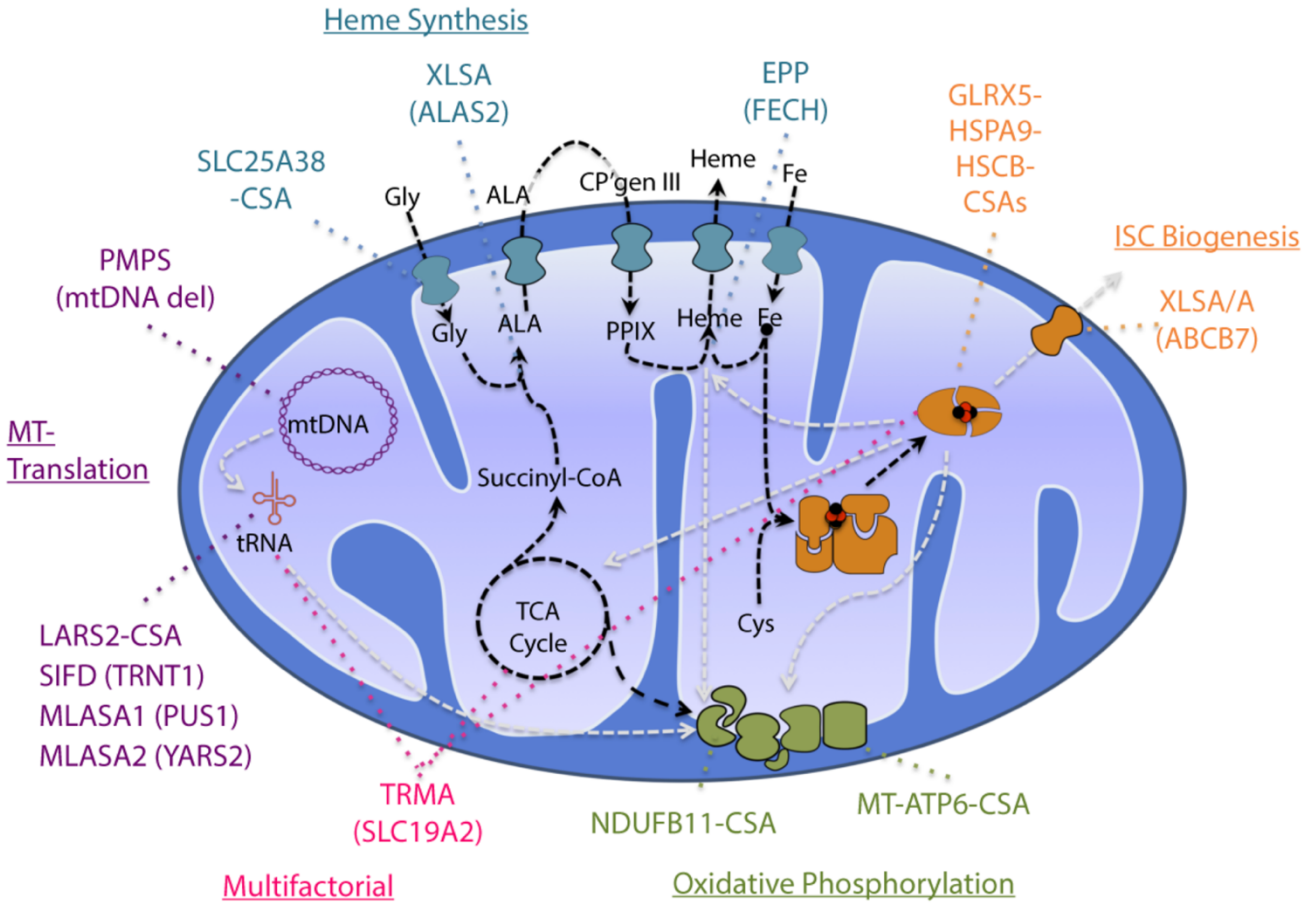

Figure 1: Defects in various mitochondrial pathways associated with CSA, courtesy Mark Fleming, MD, DPhil, Department of Pathology, Harvard Medical School

Iron Sulfur Cluster (ISC), Respiratory Chain Protein (RCP), Oxidative Phosphorylation (OP), Heme Biosynthesis (HBS) and Mitochondrial Biosynthesis, specifically MT-Translation (mitochondrial Translation) (Fig 1).

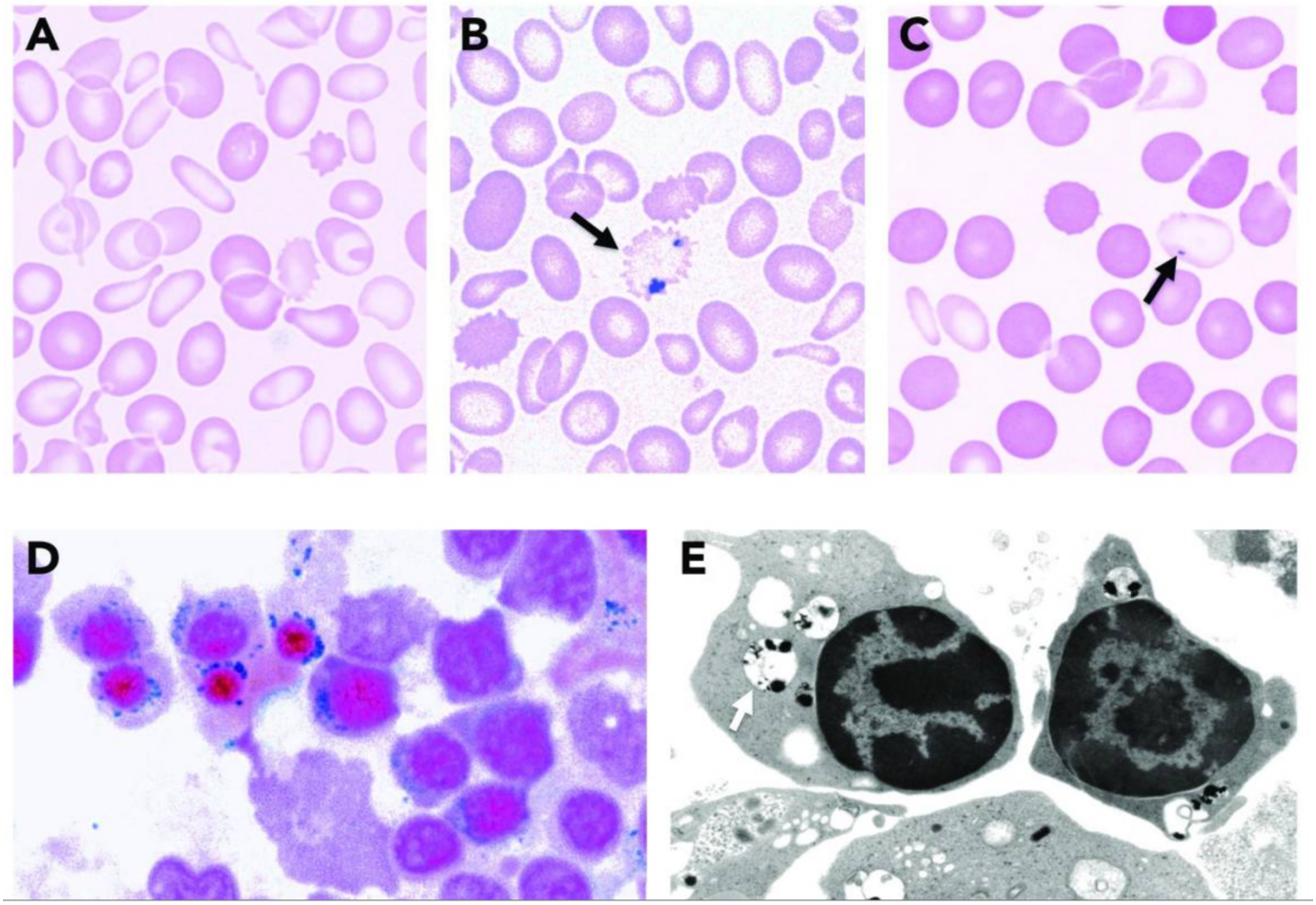

Fig.2: A) PBS w/ hypochromia, anisocytosis, and microcytosis

B) Prussian blue stained arrow indicationg siderocyte

(C) May Grunwald-Giemsa stained PBS w/ dimorphic RBCs and hypochromic microcytes containing Pappenheimer bodies

(D) Prussian blue BMA w/ ringed sideroblasts

(E) EMS w/ electron densities (black) within degenerating mitochondria (pale vacuoles, indicated by an arrow) ringing around erythroblast nuclei

Images are copies and courtesy of Marcel Seiler, Boston Veterans Affairs Medical Center [VAMC] and Mark Fleming, MD DPhil, Department of Pathology, Harvard Medical School, Boston, MA).

While anemia, with the distinct appearance of “ringed sideroblasts” on Prussian Blue (Fig. 2, thought to be secondary to intra-erythrocyte iron accumulation in erythrocyte precursors), can be a leading syndrome, CSA however can also be only one feature amongst multiple other phenotypes (Fig. 3) [1].

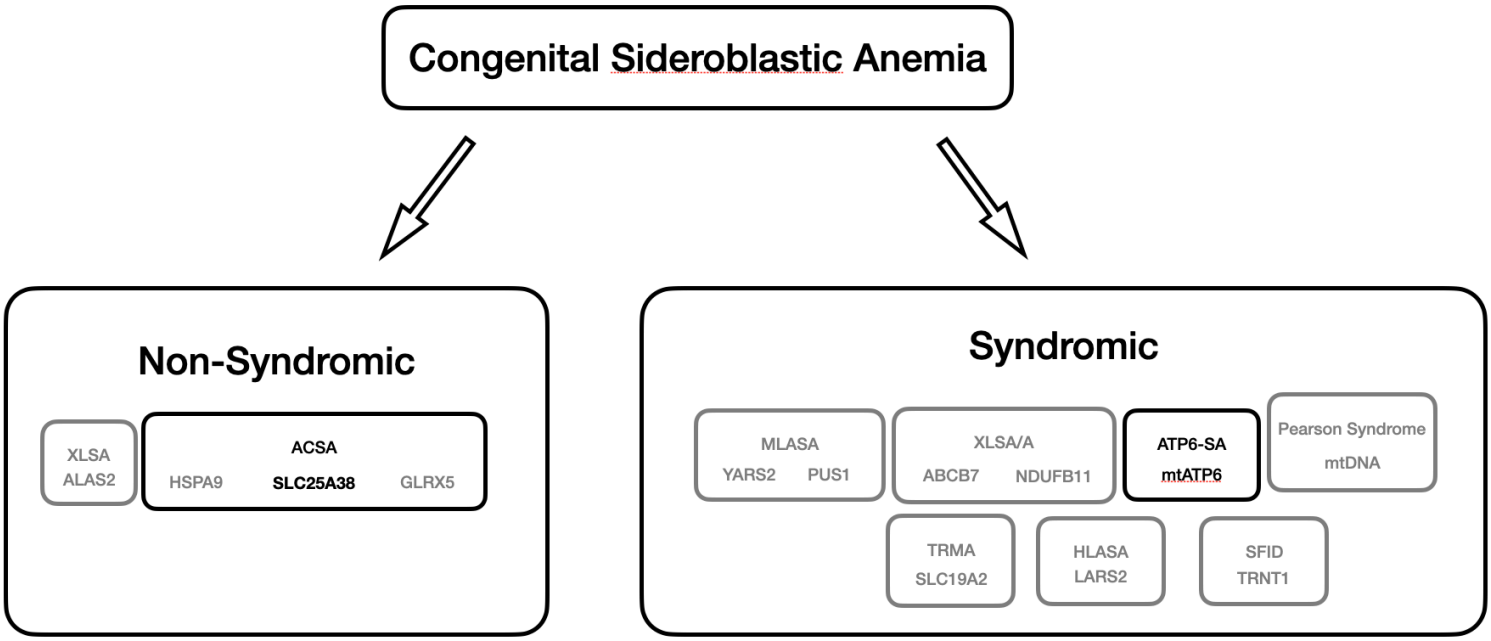

Fig. 3 --- Differentiation of non-syndromic versus syndromic forms of Congenital Sideroblastc Anemias, based on a figure by Mark D. Fleming, MD, DPhil, Department of Pathology, Harvard Medical School

While my work in the laboratory of Dr. Fleming included in-vitro and in-silico validation of various genetic variants supposedly associated with CSA, this paper focuses on two different entities exemplary for syndromic versus non-syndromic CSA (Fig3).

SLC25A38 - While variants affecting SLC25A38 (Solute Carrier Family 25 Member 38), a mitochondrial solute carrier transporting glycine (which is essential for the synthesis of 5 - Aminolevulinate (ALA), key step within the heme biosynthesis), can be regarded as mutations associated with non-syndromic CSA, related to Autosomal Congential Sideroblastic Anemia (ACSA) such as Gluta-

redoxin-related protein 5 (GLRX5), mt-ATP6 associated mutations can be found in multicomplex disorders [1,2,9-11] (Fig. 3).

MT-ATP6 – Within the spectrum of mitochondrial translation associated forms of CSA, two distinct entities have been described so far. The most common being Pearson's Syndrome (PS). A multicomplex disorder with symptoms including

ATP6-SA vs Pearson vs MLASA

	MLASA/PUS1	MLASA/ YARS2	ATP6-SA	**Pearson Marrow-Pancreas Syndrome**
Inheritance	autosomal recessive	autosomal recessive	maternal	maternal
Chromosome	14q24.33	12p11.21	mtDNA	mtDNA
Gene	PUS1	YARS2	mtATP6	variable
Gender distribution	M=F	M=F	M=F	M=F
Carrier Phenotype	-	-	NA	NA
Mean cell volume	Normal/⇑	Normal/⇑	Normal/⇓	⇑
Iron overload	–/+	–/+	–/+	–/+
Vitamin response	-	-	-	-
Transfusion	+/–	+/–	+/–	+
Associated phenotypes	Myopathy, lactic acidosis, craniofacial abnormalities, Sideroblastic anemia	Myopathy, lactic acidosis, sideroblastic anemia	„lactic acidosis", failure to thrive/grow, craniofacial abnormalities, mitochondrial encephalopathy (intellectual disability, myoclonic seizures), sideroblastic anemia	Mitochondrial encephalopathy, Exocrine, pancreas insufficiency, cytopenias, lactic acidosis, myopathy, pancytopenia, sideroblastic anemia

Table 1: clinical features Pearson's Syndrome, MLASA and ATP6-SA

exocrine pancreatic insufficiency, mitochondrial encephalopathy and lactic acidosis. (Tab. 1) associated with variable deletions (2 – 5 kB) in the mitochondrial DNA[2,12].

The second being Mitochondrial myopathy with lactic acidosis and sideroblastic anemia (MLASA) which has been described in patients carrying mutations in i.e. Pseudouridine Synthase 1 (PUS1) encoding Mitochondrial TRNA Pseudouridine Synthase A or Tyrosyl-TRNA Synthetase 2 (YARS2) (Tab.1) [2,12].

We describe a novel entity called ATP6-SA with a phenotypical pattern distinct from MLASA and Pearson's syndrome (Tab. 1) [13,14].

While Sideroblastic Anemia (SA) with Lactic Acidosis (LA) and myopathy seem to be the hallmark of all three entities ATP6-SA, MLASA and

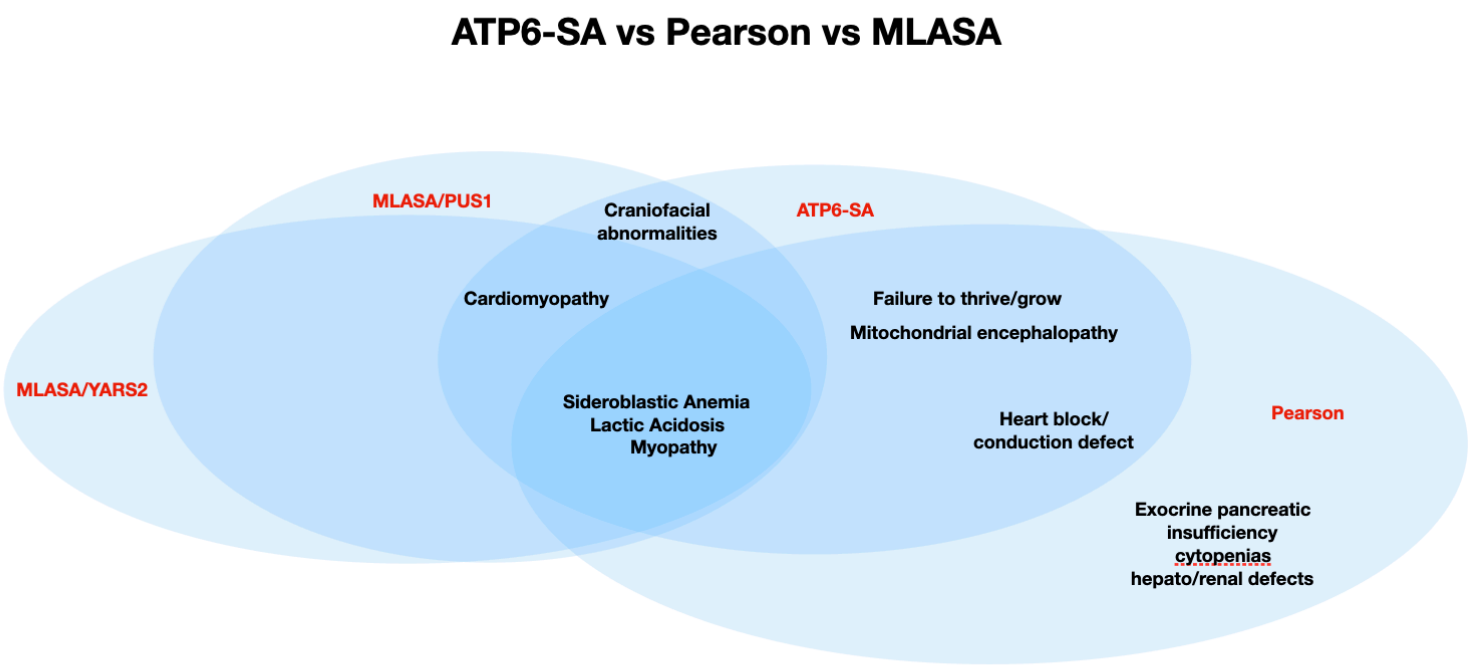

Figure 4 – multicomplex disorders of ATP6-SA versus Pearson versus MLASA

Pearson's Syndrome, there are still phenotypical features that are unique for each disease [9,12-18]. Our work illustrates the complexity of these pleiotropic congenital diseases and the importance for clear distinction of one from the other (Fig. 4).

2. Zusammenfassung

Im Rahmen meiner Promotion war ich involviert genetische Varianten via Whole Exome Sequencing, Next Generation Sequencing und Sanger/Candidate Gene Sequencing zu identifizieren, um diese dann im weiteren Schritt funktionell zu validieren.

Hierfür wurden verschiedenste Methoden benutzt. Mit z.B site directed mutagenesis von Plasmiden oder immoratlisierten Zelllinien via CRISPR/Cas 9 wurden „disease models“ konfiguriert, um schließlich die Auswirkung dieser genetischen Varianten zu analysieren. In Bezug auf die beigefügten Publikationen, waren die klinischen und bioinformatischen Daten aussagekräftig genug, um diese als „putative mutations“ zu identifizieren.

Die Arbeiten um das mitochondriale Transportprotein SLC25A38, welche die bis dato größte Studie in der Literatur ist und MT-ATP6, Komponente der ATPase 6 oder auch Komplex V (Teilschritt der Oxidativen Phosphorylierung), welche wiederum die bisher einzige Studie ist, die einen klaren Zusammenhang in mehreren Fällen zwischen Dysfunktionalitäten der ATPase 6 und kongenitaler Sideroblastischer Anämie aufzeigt, tragen zum besseren Verständnis der Pathophysiologie von CSA bei [1,2,10,11].

Herbei ist es uns auch gelungen eine neue Entität im Spektrum der kongenitalen Anämieformen assoziiert mit mitochondrialer genetischer Varianten zu beschreiben: In klarer Abgrenzung zu den bereits etablierten Krankheitsmodellen MLASA und Pearson Marrow Pancreas Syndrome, beschreiben wir eine neue, pleiotropische Kongenitale Anämieform, definiert als ATP6-SA.

Beide Arbeiten leisten dementsprechend einen essentiellen Beitrag zur molekularen Aufarbeitung kongenitaler Sideroblasticher Anämieformen bei [9,11,12,14,16-19].

Diese wiederum ermöglichen die Grundlage für zielgerichtete, personalisierte Therapieoptionen im komplexen Spektrum der Kongenitalen Sideroblastischen Anämie.

3. Abstract

In summary, CSA is a complex spectrum from the genetic background to the molecular pathophysiologic mechanisms and finally the diverse pattern of clinical presentations [20,21].

Illustrating the importance of in-depth molecular and equally important clinical analysis of patients with CSA facilitate the diagnostic work-up, individually shaped therapeutic approaches and guidance with regards to genetic counseling [20,22].

Our work, focusing on the roles of ATP6 and SLC25A38, provide further insight in to the mechanisms behind syndromic and non-syndromic types of CSA, clearly delineating those from the other entities within the family of CSA [21,23].

A detailed comprehension of ATP6-SA and SLC25A38 associated CSA will provide the ground stones for novel, curative therapeutic approaches such as gene therapy, making purely symptomatic management of anemia with all its harmful sequalae of iron overload such as cardiac complications or hepatic injury, hopefully a thing of the past [20,24,25].

4. Publikation I

Berhe, S., Heeney, M. M., Campagna, D. R., Thompson, J. F., White, E. J., Ross, T., ... & Fleming, M. D. (2018). Recurrent heteroplasmy for the MT-ATP6 p. Ser148Asn (m. 8969G> A) mutation in patients with syndromic congenital sideroblastic anemia of variable clinical severity. haematologica, 103(12), e561.

5. Publikation II

Heeney, M. M., Berhe, S., Campagna, D. R., Oved, J. H., Kurre, P., Shaw, P. J., ... & Fleming, M. D. (2021). SLC25A38 congenital sideroblastic anemia: Phenotypes and genotypes of 31 individuals from 24 families, including 11 novel mutations, and a review of the literature. *Human mutation*, *42*(11), 1367-1383.

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