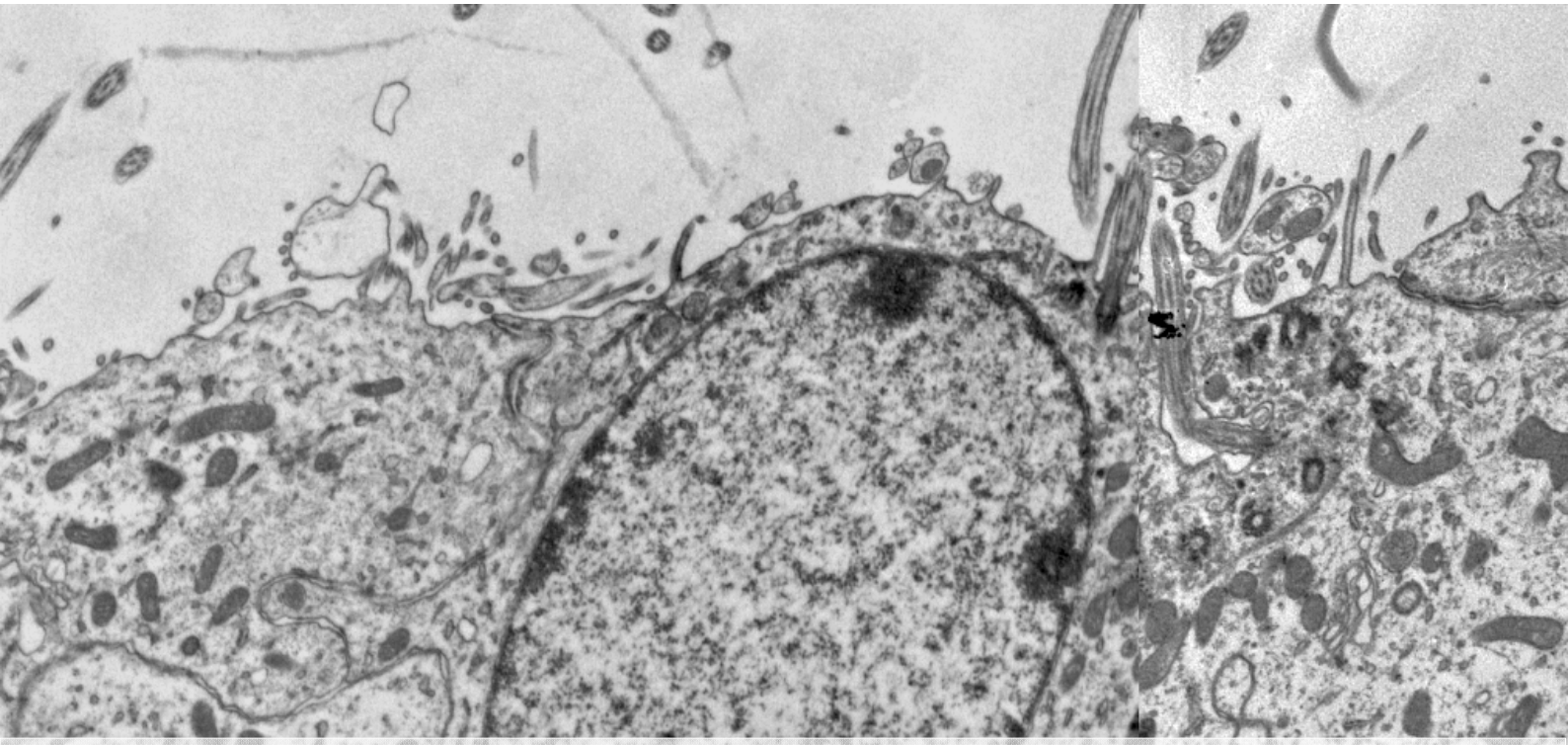
Universität Rostock Traditio et Innovatio

UNITED NEUROSCIENCE CAMPUS Lund - Rostock

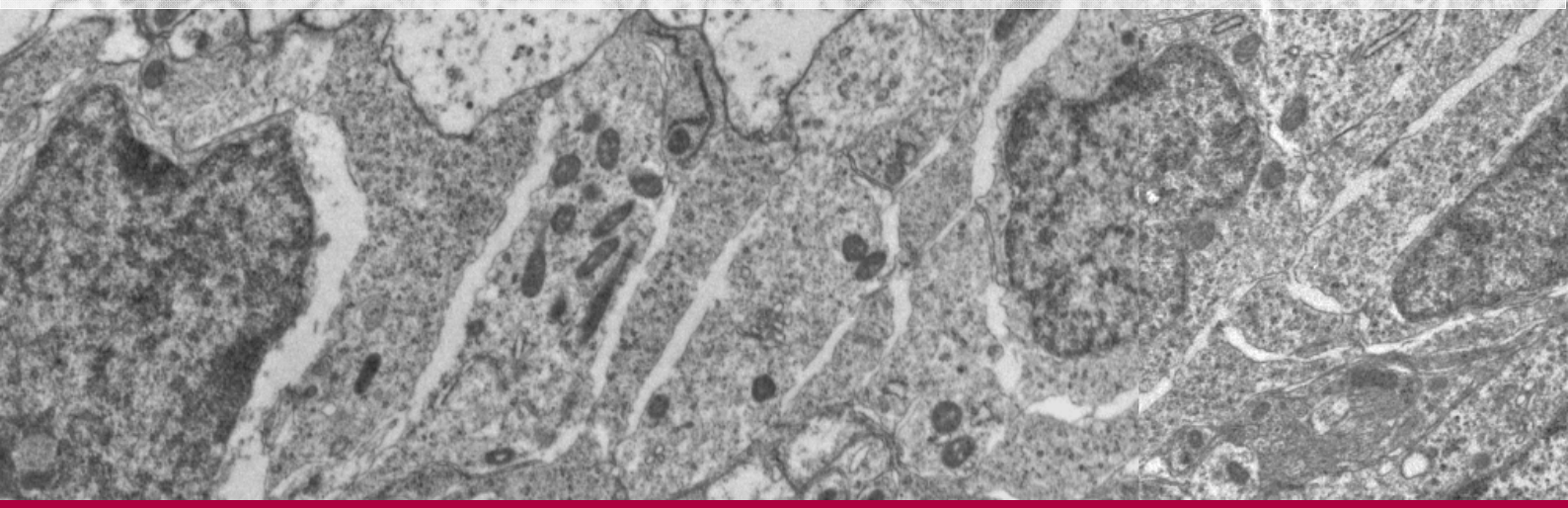
Universitätsmedizin Rostock



2. Rostock Summer School of Neurodegenerative Diseases

Program & Abstracts

17 & 18 September 2024



Universitätsmedizin Rostock

Dept Neurology

Translational Neurodegeneration Section „Albrecht Kossel“

Gehlsheimer Straße 20, 18147 Rostock

www.albrecht-kossel-institut.med.uni-rostock.de

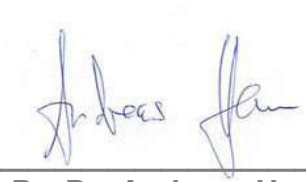
2. Rostock Summer School of Neurodegenerative Diseases

Dear Madam or Sir,

I cordially welcome you to the 2nd Rostock Summer School on Neurodegenerative Diseases, which consists of the Schilling Symposium and the Clinician Scientist day. The central topic is the connection from basic science to clinical translation in neurodegeneration with special focus on motor neuron diseases. I am honored to welcome so many well-known researchers in the field for this day, together with many young scientists which promises us a varied program with hopefully stimulating discussions. I also feel privileged that my colleagues from Rostock are once more willing to give insights in their clinical work on neurodegeneration, sharing with us state-of-the-art knowledge in the respective topic of neurodegenerative diseases.

I wish you all unique days in Rostock.

Yours sincerely,



Prof. Dr. Dr. Andreas Hermann
Head of Translational Neurodegeneration Section “Albrecht Kossel”

Universität Rostock Traditio et Innovatio

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Universitätsmedizin Rostock

Program

Universität Rostock Traditio et Innovatio

UNITED NEUROSCIENCE CAMPUS Lund - Rostock

Universitätsmedizin Rostock

17th September 2024
Schilling-Symposium

09:00 Welcome
Prof. Dr. Per Odin
Prof. Dr. Reisinger

Chair: Prof. Dr. Per Odin

09:30 Aging and neurodegeneration
Dr. Christiane Hartmann, Rostock

10:00 Hypothalamic and Metabolic Changes in Huntington's Disease Pathogenesis
Dr. Rana Soylu Kucharz, Lund

10:30 Neuronal migration and axonal reconnection in temporal lobe epilepsy
Prof. Dr. Dr. Thomas M. Freiman, Rostock

– 11:00-11:30 Coffee break –

Chair: Prof. Dr. Alexander Storch

11:30 Identification of molecular subgroups in ALS and implications for differential therapies
Prof. Dr. Paul Lingor, München

12:00 New multimodal multi-scale modalities to image amyloids in their native environment
Prof. Dr. Oxana Klementieva, Lund

12:30 Investigating the role of microglia in amyotrophic lateral sclerosis using human iPSC models
Dr. Dr. Björn F. Vahsen, Oxford

– 13.00-14.00 Lunch & Posters –

Chair: Prof. Dr. Dr. Andreas Hermann

14:00 Resolving the heterogeneity of the ALS spectrum using patients' hiPSCs
PD Dr. Alberto Catanese, Ulm

14:30 Capturing the glymphatic system in action - Different approaches to imaging brain clearance
Dr. Jari Jukkola, Lund

15:00 Modeling and Treating Neurological Disorders using Stem Cells, Reprogramming and Genome Engineering
Dr. Henrik Ahlenius, Lund

15:30 In Vitro Disease Modeling and Pathophysiological Characterization of Fatty Acid Hydroxylase-Associated Neurodegeneration
Fatima Efendic **(Data Blizz Young Investigators)**

15:45 Association of Brain Atrophy with Automated Digital Speech Features in Early Alzheimer's Disease
Qingyue Li **(Data Blizz Young Investigators)**

17th September 2024
Schilling-Symposium

Speakers

Dr. Henrik Ahlenius
Faculty of Medicine, Department of Experimental Medicine Science, Lund University

PD Dr. Alberto Catanese
Anatomy and Cell Biology, University Ulm

Fatima Efendic
Translational Neurodegeneration Section „Albrecht Kossel“, Rostock University Medical Center

Prof. Dr. Dr. Thomas M. Freiman
Department of Neurosurgery, Rostock University Medical Center

Dr. Christiane Hartmann
Translational Neurodegeneration Section „Albrecht Kossel“, Rostock University Medical Center

Dr. Jari Jukkola
Faculty of Medicine, Department of Experimental Medicine Science, Lund University

Prof. Dr. Oxana Klementieva
Faculty of Medicine, Department of Experimental Medicine Science, Lund University

Dr. Rana Soylu Kucharz
Faculty of Medicine, Department of Experimental Medicine Science, Lund University

Qingyue Li
Department of Psychosomatic Medicine, Rostock University Medical Center

Prof. Dr. Paul Lingor
Department of Neurology, Technical University of Munich, School of Medicine, Klinikum rechts der Isar & German Center for Neurodegenerative Diseases (DZNE)

Prof. Dr. Per Odin
Division of Neurology, Department of Clinical Sciences Lund, Lund University

Prof. Dr. Emil C. Reisinger, MBA
Dean and Scientifi c Director, Rostock University Medical Center

Prof. Dr. Alexander Storch
Department of Neurology, Rostock University Medical Center & German Center for Neurodegenerative Diseases (DZNE)

Dr. Dr. Björn F. Vahsen
Nuffield Department of Clinical Neurosciences, University of Oxford

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Universitätsmedizin Rostock

18th September 2024
Clinician Scientist Day

08:45 Welcome

09:00 Anatomie der Neurodegeneration
Dr. Sarah Joost

09:30 Neuropathology of neurodegenerative diseases
Prof. Dr. Dr. Andreas Hermann

10:00 Syndromatology I: Dementia – cognitive disorders in neurodegeneration
Prof. Dr. Stefan Teipel

- Coffee break –

11:00 Sleep in neurodegenerative diseases
Dr. Wiebke Hermann

11:30 Structural imaging of neurodegeneration
PD Dr. Ebba Beller

12:00 Molecular imaging of neurodegeneration
Prof. Dr. Bernd Krause

12:30 Ultrasound imaging neurodegeneration
Prof. Dr. Uwe Walter

- Lunch –

14:00 Syndromatology II: Movement disorders
Prof. Dr. Alexander Storch

14:30 Drug treatment for movement disorders
Dr. Matthias Löhle

15:00 Deep Brain Stimulation – current clinical practice and future directions
Dr. René Reese

15:30 Syndromatology III: Other neurodegenerative diseases
Prof. Dr. Dr. Andreas Hermann

Universität Rostock Traditio et Innovatio

UNITED NEUROSCIENCE CAMPUS Lund - Rostock

Universitätsmedizin Rostock

18th September 2024
Clinician Scientist Day

Speakers

PD Dr. Ebba Beller
Institute of Radiology, University Medical Center Rostock

Prof. Dr. Dr. Andreas Hermann
Department of Neurology, Translational Neurodegeneration Section „Albrecht Kossel“, University Medical Center Rostock
Center & German Center for Neurodegenerative Diseases (DZNE)

Dr. Wiebke Hermann
Department of Neurology, University Medical Center Rostock

Dr. Sarah Joost
Institute of Anatomy, University Medical Center Rostock

Prof. Dr. Bernd Krause
Department of Nuclear Medicine, University Medical Center Rostock

Dr. Matthias Löhle
Department of Neurology, University Medical Center Rostock

PD Dr. René Reese
Department of Neurology, University Medical Center Rostock

Prof. Dr. Alexander Storch
Department of Neurology, University Medical Center Rostock & German Center for Neurodegenerative Diseases (DZNE)

Prof. Dr. Sefan Teipel
University Medical Center Rostock Center & German Center for Neurodegenerative Diseases (DZNE)

Prof. Dr. Uwe Walter
Department of Neurology, University Medical Center Rostock

Universität Rostock Traditio et Innovatio

UNITED NEUROSCIENCE CAMPUS Lund - Rostock

Universitätsmedizin Rostock

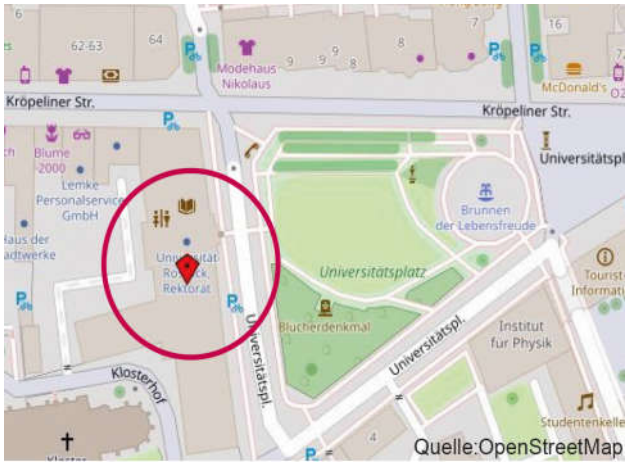
General informations

Locations

17 September 2024 – Universitätshauptgebäude

Universität Rostock, Rektorat
Aula, 1. OG

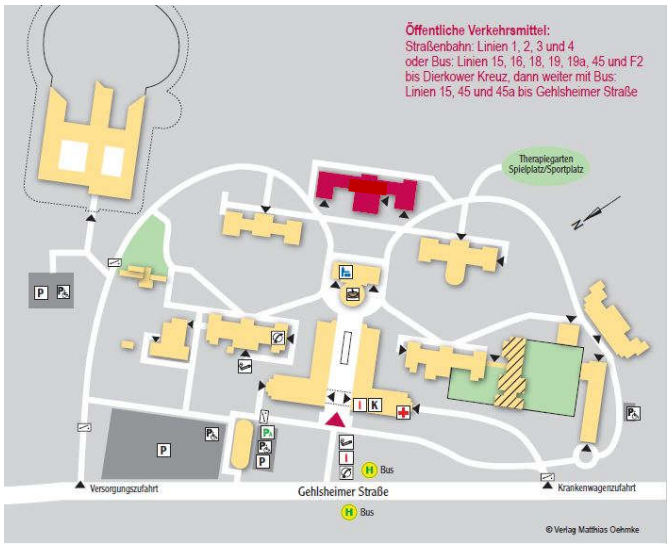
Universitätsplatz 1, 18055 Rostock



18 September 2024 - Campus Gehlsdorf

Universitätsmedizin Rostock
Klinik und Poliklinik für Neurologie
Sektion für Translationale Neurodegeneration „Albrecht Kossel“

Gehlsheimer Straße 20, 18147 Rostock



Contact & Organisation

Veranstalter *Organizer*
Prof. Dr. Dr. Andreas Hermann
Sektionsleiter
Sektion für Translationale Neurodegeneration „Albrecht Kossel“
Klinik und Poliklinik für Neurologie
Zentrum für Nervenheilkunde
Universitätsmedizin Rostock
Gehlsheimer Str. 20, 18147 Rostock

E-Mail: andreas.hermann@med.uni-rostock.de

www.albrecht-kossel-institut.med.uni-rostock.de

Anmeldung *Registration*
Martina Stoof
Sekretariat
Sektion für Translationale Neurodegeneration
Klinik und Poliklinik für Neurologie
Gehlsheimer Str. 20, 18147 Rostock
Phone: +49 (0) 381 494-9541

E-Mail: akos-veranstaltung@med.uni-rostock.de

www.albrecht-kossel-institut.med.uni-rostock.de

Universität Rostock Traditio et Innovatio

UNITED NEUROSCIENCE CAMPUS Lund - Rostock

Universitätsmedizin Rostock

Hermann und Lilly Schilling-Stiftung

Hermann und Lilly Schilling-Stiftung

The foundation was established in 1970 by Aloysia Schilling. She was the wife of Hermann Schilling, former State Finance Councillor of the Prussian State Bank and member of the Board of Directors of Vereinigte Elektrizitäts- und Bergwerks-Gesellschaft (VEBA), who died in 1961

Areas of Support

As part of its “Neuroscience in the Clinic” program (1997 to 2021), the foundation financed a total of seven departments/institutes for basic clinical research at neurological university clinics. These new establishments enable scientists working in the field of basic clinical research to take up questions from the clinic and transfer the results of their research into clinical application.
The following departments were established as part of the program:

- Institut für Klinische Neuroimmunologie, Klinikum Großhadern der Ludwig-Maximilians-Universität München
- Abteilung für Experimentelle Neurologie, Neurologische Klinik der Charité
- Abteilung Kognitive Neurologie, Eberhard-Karls-Universität Tübingen
- Abteilung Klinische Neurobiologie, Neurologische Universitätsklinik Heidelberg
- Institut für Klinische Neurobiologie, Neurologische Klinik und Poliklinik der Universität Würzburg
- Sektion für Klinische und Molekulare Neurogenetik, Klinik für Neurologie der Universität und des UK S-H, Campus Lübeck
- Abteilung für Kognitive Neurobiologie, Zentrum für Neurologische Medizin, Universitätsmedizin Göttingen

As part of the “Schilling Professorship and Translational Neuroscience Research Group” program launched in 2015, the following institutions are being funded:

- Schilling-Professur "Vaskuläre Neuroimmunologie" und Forschungsgruppe "Translationale Neurowissenschaften", Universitätsklinikum Hamburg-Eppendorf
- Schilling-Professur und Forschungsgruppe "Translationale Neurodegeneration", Universitätsmedizin Rostock
- Schilling-Professur "Neurologie und Translationale Neurowissenschaften" und "Translationale Schilling Forschungsgruppe für immunvermittelte Synaptopathien", Universitätsklinikum Jena.

In February 2021, a new “Schilling Professorship and Research Group Translational Neurosciences” was announced. These are currently being appointed.

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Universitätsmedizin Rostock

Albrecht Kossel

CHEMIE IN UNSERER ZEIT

DOI: 10.1002/ciuz.202010003

Ein Nobelpreisträger aus Mecklenburg

Albrecht Kossel und die Nukleinbasen

EDITH FRAMM | JOACHIM FRAMM

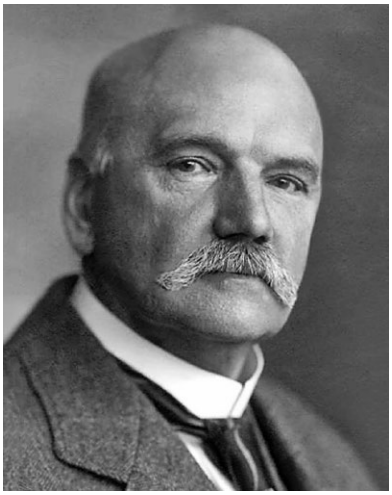

Abb. 1 ***Kossel um 1913.*** *(Archiv der Universität Heidelberg)*

Nobelpreisträger – und trotzdem kaum bekannt: Albrecht Kossel schuf mit seinen Arbeiten wesentliche Voraussetzungen für das Verständnis des Aufbaus von Nukleinsäuren und wurde 1910 mit dem Nobelpreis für Physiologie oder Medizin ausgezeichnet. Eine Spurensuche.

Am 16. September 2003 fand an der Universität Rostock ein akademischer Festakt zum 150. Geburtstag des Nobelpreisträgers Albrecht Kossel (1853–1927) statt. Im Anschluss wurde ein großer öffentlicher Platz in Rostock nach ihm benannt. Auch ein Institut der Universität trägt seinen Namen. Seit 2014 wird auch von der Gesellschaft Deutscher Chemiker alle zwei Jahre der „Albrecht-Kossel-Preis" für Biochemie verliehen. Doch weder in seiner Heimat Mecklenburg noch in den Fachkreisen ist Kossel ausreichend bekannt.

Sein Name ist mit der bedeutungsvollen Entdeckung der Nukleinbasen Adenin, Guanin, Thymin und Cytosin verbunden, deren Abfolge im Molekülfaden der DNA die Erbinformation bildet. Für die Arzneimitteltherapie haben die genetischen Grundlagen eine ganz besondere Bedeutung gewonnen [1].

Nachfolgend sollen Leben und Wirken Albrecht Kossels in Erinnerung gebracht werden.

Albrecht Kossels Lebensweg

Albrecht Kossel (Abbildung 1) wurde 1853 in Rostock geboren und wuchs dort auch auf. Der Vater war Kaufmann und Reeder, später preußischer Konsul und Bankdirektor. Erwähnenswert ist, dass der Großvater mütterlicherseits in Rostock einen Samenhandel betrieb und damit auch international großen Erfolg hatte. Albrechts Begabung und Begeisterung für die Botanik, mit der er schon in seiner Gymnasialzeit auffiel, mag er von diesem Großvater geerbt haben. Gern hätte Albrecht Botanik studiert, aber der Vater riet zu Medizin oder Pharmazie. Die Wahl fiel auf die Medizin.

Eine große Anziehungskraft ging von Straßburg aus. Dort wurde nach dem Deutsch-Französischen Krieg eine Elite-Universität gegründet. Die finanzielle Situation des Vaters war jedoch schwierig geworden, und auch die fünf jüngeren Brüder sollten eine Ausbildung bekommen. Nur ein in Rostock gestiftetes Stipendium ermöglichte das Studium in Straßburg. Die Auflagen dieses Stipendiums führten dazu, dass Kossel abwechselnd zwei Jahre auch in Rostock studierte, dort seine Examina ableistete und 1878 promoviert wurde.

Kossel begann am Pharmakologischen Institut der Universität Rostock bei Prof. Gaethgens (1839–1915) zu arbeiten, aber wenig später wurde eine Assistentenstelle bei Prof. Hoppe-Seyler (1825–1895) frei, dem Direktor des Straßburger Physiologisch-Chemischen Instituts. Kossel fühlte sich sehr zu dieser Persönlichkeit hingezogen, schon während des Studiums hatte er bei Hoppe-Seyler kleinere Untersuchungen durchgeführt. Niemand konnte ahnen, dass die Tätigkeit in Straßburg der Anfang bahnbrechender Forschungen bilden würde. Intensive, gewissenhafte Arbeiten führten 1881 zur Habilitation für die Fachgebiete Physiologische Chemie und Hygiene.

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Als dann Emil du Bois-Reymond, der Direktor des Physiologischen Instituts in Berlin, einen Leiter seiner chemischen Abteilung suchte, empfahl Hoppe-Seyler Albrecht Kossel. Dieser übernahm die Stelle im Oktober 1883. Dort gelangen ihm trotz der umfangreichen Lehraufgaben bedeutende Entdeckungen (Abbildung 2). Als er 1885 an einer Versammlung der deutschen Naturforscher und Ärzte in Straßburg teilnahm, lernte er Luise Holtzmann, seine spätere Frau, kennen. Drei Kinder wurden geboren, der Sohn Walther, der ein herausragender Physiker wurde, die Tochter Hedwig, die leider sehr früh verstarb und die Tochter Gertrud.

In Berlin wurde er 1887 zum außerordentlichen Professor berufen. Aber ein eigener Lehrstuhl blieb ihm zunächst versagt. 1895 fügte es sich, dass er in Marburg Ordinarius für Physiologie wurde. Kossel konnte dort seine Forschungen unvermindert fortsetzen. Doch noch einmal sollte er sich verändern: Er nahm den Ruf auf den Lehrstuhl für Physiologie in Heidelberg an. Die Forschung mit zahlreichen Schülern und Mitarbeitern ging weiter. Auszeichnungen, vor allem sechs Ehrendoktorate (in den Jahren 1904–1923) sowie Mitgliedschaften in wissenschaftlichen Akademien des In- und Auslands, häuften sich.

Albrecht Kossel wurde die Leitung des 7. Internationalen Physiologenkongresses übertragen, der 1907 in Heidelberg stattfand. 1908 wurde er Prorektor in Heidelberg. 1910 erhielt er den Nobelpreis. 1913 ereilte Kossel ein schwerer Schicksalsschlag, denn seine Frau verstarb mit 49 Jahren an einer Bauchspeicheldrüsenentzündung. Dann folgte die Katastrophe des Ersten Weltkrieges. Kossel pflegte eine weltweite Zusammenarbeit mit bedeutenden Forschern. Sie brach nun zu seinem großen Bedauern ab. Nach dem Krieg waren die Deutschen nicht erwünscht, nur langsam normalisierte sich der wissenschaftliche Austausch. 1924 wurde Kossel emeritiert. Albrecht Kossel konnte weiterhin eigene wissenschaftliche Untersuchungen durchführen. Bis 1927 forschend tätig, wurde Kossel wie einst Hoppe-Seyler erst durch den Tod das Handwerkzeug aus der Hand genommen.

Die Entdeckung der Nukleinbasen

1878 begann Albrecht Kossel in Straßburg, die Arbeiten von Friedrich Miescher (1844–1895) fortzusetzen. Miescher hatte 1869 im Laboratorium von Felix Hoppe-Seyler in Tübingen aus den isolierten Zellkernen der Leukozyten des Eiters eine bisher unbekannte, phosphorhaltige Substanz gewonnen, die er Nuklein nannte [3]. Kossel konnte zunächst 1883 nachweisen, dass zellkernreiche Gewebe und Organe auch mehr Nuklein-Phosphorsäure enthalten. Gezielte Hungerversuche an Hühnern und Tauben zeigten, dass das Nuklein kein Reservestoff ist: Die Menge an Nuklein veränderte sich nur wenig, unabhängig davon, ob ein Organismus hungerte oder nicht. Daraus folgerte Kossel, dass die Funktion des Nukleins eher bei der Neubildung des Gewebes zu suchen sei. Außerdem konnte er beweisen, dass Guanin ein Spaltprodukt des aus Gänseblut gewonnenen Nukleins ist [4]. Guanin war seit 1844 als eine stickstoffreiche Base bekannt, die sich in den Exkrementen von Säugetieren und Vögeln anreichert. Erste Erkenntnisse zum Vorkommen des Guanins im Nuklein gab es schon seit 1874. Sie gingen auf den Schweizer Chemiker Jules Piccard (1840–1933) zurück, der Mieschers „Nuclein des Lachsspermas“ (Nukleinsäure) untersucht hatte [5].

Abb. 2 ***Das ehemalige Physiologische Institut in Berlin, Dorotheenstraße 96 (Teilansicht). Hier gelang es Albrecht Kossel und seinen Mitarbeitern in den Jahren 1890–1894, die Nukleinbasen Adenin, Guanin, Thymin und Cytosin als Spaltprodukte der Nukleinsäure nachzuweisen.*** *(Foto Framm, 2018)*

Am 12. Januar 1885 berichtete Kossel vor der Berliner Chemischen Gesellschaft über eine bedeutende Entdeckung. Er konnte aus einer größeren Menge Rinder-Bauchspeicheldrüse, die in der Berliner Firma C. A. F. Kahlbaum von dem Chemiker Adolph Bannow (1844–1919) aufbereitet worden war, eine stickstoffreiche Base mit der Summenformel $C_5H_5N_5$ isolieren, für die er, abgeleitet von dem griechischen Wort „aden“ für Drüse, den Namen Adenin vorschlug. Kossel wies sie wenig später auch als Spaltprodukt des Hefenukleins nach [6, 7].

Richard Altmann (1852–1900) in Leipzig war es 1889 gelungen, aus dem Nuklein der Hefe den Eiweißanteil abzutrennen und eine phosphorhaltige organische Säure zu isolieren. Er gab ihr den Namen Nukleinsäure [8]. Kossel konnte nach Altmanns Verfahren diese Nukleinsäure herstellen und dann Adenin und Guanin als Spaltprodukte nachweisen. Es stellte sich dabei heraus, dass auch ein Kohlenhydrat Bestandteil der Nukleinsäure sein musste. Kossel wählte für die basischen Substanzen Adenin, Guanin und seine Derivate den zusammenfassenden Namen Nukleinbasen [9].

Im November 1893 berichtete Kossel von weiteren Entdeckungen. Aus den Thymusdrüsen des Kalbes hatte er mit dem Assistenten Albert Neumann Nukleinsäure gewonnen und mit Schwefelsäure behandelt. Es bildete sich ein gut kristallisiertes Spaltprodukt, für das der Name Thymin vorgeschlagen wurde. 1894 konnten sie aus den Thymusdrüsen noch eine weitere Substanz isolieren. Sie gaben ihr den Namen Cytosin [10].

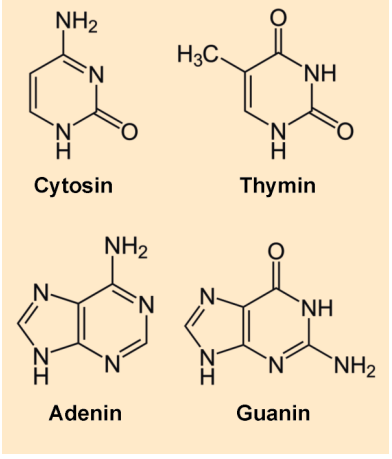
Nachdem am Ende des 19. Jahrhunderts – im Wesentlichen durch die Synthesen Emil Fischers (1852–1919) – die Strukturformeln des Guanins und Adenins als Purinkörper

und die des Thymins als Pyrimidinkörper endgültig aufgeklärt worden waren, konnte Kossel mit seinem Mitarbeiter Hermann Steudel (1871–1969) auch die Strukturformel der Nukleinbase Cytosin als Pyrimidinkörper zweifelsfrei feststellen [11].

Es hatte sich inzwischen erwiesen, dass Guanin, Adenin sowie Thymin und Cytosin (Abbildung 3) in allen entwicklungsfähigen Zellen zu finden sind. Die Erkenntnisse über diese vier Nukleinbasen sollten für die weiteren Forschungen einen unerschütterlichen Grundstein legen. Albrecht Kossel konnte sie eindeutig als Bausteine der Nukleinsäure charakterisieren. In seinem Nobelvortrag am 12. Dezember 1910 hob er hervor: *„Es gelang mir, eine Reihe von Bruchstücken zu erhalten … welche durch eine ganz eigentümliche Ansammlung von Stickstoffatomen gekennzeichnet sind. Es sind hier nebeneinander … das Cytosin, das Thymin, das Adenin und das Guanin.“* [12]

Mit diesen Erkenntnissen schuf Kossel wesentliche Voraussetzungen für das berühmte Doppel-Helix-Modell der Desoxyribonukleinsäure, das 1953 von James D. Watson und Francis Crick entwickelt wurde. Die Entdeckung der fünften primären Nukleinbase im Jahr 1900 in Marburg geht auf Alberto Ascoli (1877–1957) zurück. Kossel war jedoch offenbar (s. u.) maßgebend daran beteiligt [13].

ABB. 3 | DIE VIER NUKLEINBASEN



Weitere Arbeiten

Ende Oktober 1888 teilte Albrecht Kossel in Berlin eine andere Entdeckung mit. In einem Tee-Extrakt, der ihm vom Rostocker Apotheker und Fabrikant Friedrich Witte (1829–1893) zugeleitet worden war, wurde außer dem Adenin eine weitere, bisher unbekannte Substanz gefunden. Es erwies sich, dass diese mit Theobromin und Coffein verwandt war. Beide Stoffe hatte Emil Fischer hinreichend charakterisiert. Aufbauend auf Fischers Erkenntnissen stellte Kossel nicht nur die Summenformel, sondern auch die Strukturformel auf. Er schlug für die neue Substanz den Namen Theophyllin vor [14]. Sieben Jahre später gelang Emil Fischer die synthetische Darstellung. Nachdem es ab 1970 möglich geworden war, Theophyllin in retardierter Arzneiform herzustellen, wurde es für einige Jahrzehnte das weltweit bevorzugte Mittel für die Dauertherapie bei Asthma.

Schwerpunkt der Arbeiten Kossels blieb jedoch die Erforschung der Chemie des Zellkerns. Schon 1884 gelang ihm der Nachweis eines eiweißartigen Körpers im Nuklein des Gänseblutes. Damit war die bereits in seiner Habilitationsschrift geäußerte Vermutung, dass die Nukleine aus einem Eiweißkörper und der phosphorhaltigen Substanz bestehen, bestätigt worden. Für diesen Eiweißkörper schlug Kossel den Namen Histon vor [15].

In den Samenzellen des Lachses hatte Friedrich Mieschers eine basische Substanz gefunden, die mit dem Nuklein salzartig verbunden war, und sie Protamin genannt. Kossel wies deren Eiweißnatur nach. Im Protamin der Samenzellen des Störs, das den Namen Sturin erhielt, entdeckte er zeitgleich mit Sven Gustav Hedin (1859–1933) eine neue basische Substanz, das Histidin [16]. Außerdem wies er die bereits bekannten basischen Aminosäuren Arginin und Lysin nach. Auch die Protamine des Lachses (Salmin) und des Herings (Clupein) dienten Kossel als Ausgangsstoffe.

Protamine und Histone waren Eiweiße, die vergleichsweise weniger Aminosäuren enthielten. Sie bildeten den Gegenstand vielfältiger weiterer Untersuchungen. Gemeinsam mit seinem Mitarbeiter Friedrich Kutscher entwickelte Kossel eine neue Analysenmethode für Eiweiße [17]. Dieses Silberbarytverfahren blieb viele Jahre die beste Analysenmethode für Histidin, Arginin und Lysin: Die Eiweiße wurden hydrolysiert und die drei basischen Aminosäuren abgetrennt. Wieder in Lösung gebracht wurde das Gemisch mit Silbersulfat oder Silbernitrat bei bestimmter Alkalität (Bariumhydroxidlösung) gefällt. Bei ganz schwacher alkalischer Reaktion fällt Histidinsilber, bei stark alkalischer fällt Argininsilber aus. Im Filtrat dieser Fällungen lässt sich dann das Lysin nach Entfernung des Silbers als Pikrat isolieren. Mit dieser Methode konnte der Anteil der drei basischen Aminosäuren in verschiedensten Eiweißen quantitativ bestimmt werden, und sie war der Ausgangspunkt für Kossels intensive Suche nach einem Ordnungsprinzip für die Eiweiße. Später verwendete er für die Analysen Flaviansäure, die mit Arginin ein fast unlösliches Salz bildet [18]. Es zeigte sich, dass der Guanidinteil des Arginins, der Imidazolteil des Histidins und die endständige Aminogruppe im Lysin nicht an der Peptidbindung der Eiweiße beteiligt sind. Kossel vermutete, dass diese ungebundenen stickstoffhaltigen Teilstrukturen eine bestimmte biologische Bedeutung haben [19]:

„Ich stelle mir das Eiweißmolekül so vor, dass es jederzeit auf einen chemischen Angriff mit irgendeiner seiner charakteristischen Gruppen antworten kann. So, wie an einem Weinstock die Trauben hängen, so verfügt das Eiweißmolekül über eine große Anzahl charakteristischer Gruppen … Werden irgendwelche speziellen Kombinationen benötigt, so liegen sie in angreifbarer Form schon da.“

Kossel vermutete außerdem, dass die Funktionen der Eiweiße von ihrer chemischen Struktur abgeleitet werden müssen. Von besonderer Bedeutung für die Entwicklung und das Grundverständnis der Biochemie sollte sich die

Baustein-Hypothese Kossels erweisen, die er bei Amtsantritt als Prorektor in der Aula der Universität Heidelberg vortrug [20] und nach der Kohlenhydrate und Eiweiße oft aus lauter gleichartigen, kleineren Teilstücken bestehen. Als Beispiel führte er Stärke und Glykogen an, die aus der einfachen Substanz Traubenzucker gebildet werden. Auch die Eiweiße seien aus Teilstücken zusammengesetzt, den Aminosäuren: *„Einzelne dieser Stücke oder Segmente, zum Beispiel das Leucin, können sich zwar vielfach wiederholen, aber dann sind andere dazwischen gefügt. … Die Art der Zusammenfügung … ist eine gesetzmäßige.“ „Die Proteinstoffe, welche dem Hühnchen als Nahrung dienen, müssen gewissermaßen umrangiert werden, um später in den Horngebilden der Haut oder im Blut oder im Knorpel als neue Eiweißart zu erscheinen.“* Aus den pflanzlichen Proteinmolekülen, die dem tierischen Organismus zugeführt werden, entstünden durch Verdauungsvorgänge die Aminosäuren. Aus diesen Bausteinen würden dann im Organismus die körpereigenen Proteine aufgebaut.

Diese Bausteinhypothese bezog Kossel nicht nur auf die Eiweiße, sondern auch auf Fette, Kohlenhydrate und Nukleinsäuren. Um zu beweisen, dass die Bausteine in allen Lebewesen identisch sind, untersuchte er mit seinen Mitarbeitern viele Organismen. Kossel fand sie in den Schuppen der Ostseefische, in den Heidelberger Glühwürmchen, in der Bäckerhefe, in mecklenburgischen Gänsen und Rindern, in Schmetterlingen und Löwenmäulchen und in indischen Teeblättern.

KOSSEL SCHUF WESENTLICHE VORAUSSETZUNGEN FÜR DAS DOPPEL-HELIX-MODELL DER DNA VON WATSON UND CRICK

Gemeinsam mit Henry Drysdale Dakin (1880–1952) entdeckte Kossel das Enzym Arginase, das Arginin in Ornithin und Harnstoff spaltet [21].

Überlegungen zu den Erbvorgängen finden sich in seinem Nobelvortrag am 12.12.1910 [12]. Dort betonte er, dass die an die Nukleinsäure locker gebundenen Proteine des Zellkerns einen ungewöhnlich hohen Anteil an stickstoffreichen Aminosäuren haben. Der hohe Stickstoffanteil gelte auch für die Nukleinsäuren selbst und grenzt beide Gruppen scharf von den übrigen Bestandteilen der Zelle ab. *„Diese stickstoffreichen und phosphorhaltigen Atomgruppen sind es, deren Ablagerungsstätten … bei der Zellteilung zuerst in Bewegung gebracht werden und deren Übertragung auf andere Zellen einen wesentlichen Teil des Befruchtungsvorgangs ausmacht.“*

In einer Rede bei der Jahresfeier der Heidelberger Akademie formulierte er 1921 [22]: *„… Erbfaktoren werden bei der Befruchtung übertragen und müssen also in dem befruchteten Ei in kleinster Dimension niedergelegt sein. Wir können uns heute kaum eine andere Vorstellung von der Festlegung so vieler Form und Stoff bestimmender Anlagen auf engstem Raum machen, als dadurch, dass wir sie auf die Lagerung der Moleküle und Atome beziehen.“ … „Denkt man sich an Stelle eines jeden Eiweißbausteins einen Buchstaben, so kann durch geeignete Zusammenstellung derselben schon eine genaue Aufzählung der Eigenschaften eines Organismus geliefert werden. … Neben ihnen finden wir andere Stoffe, welche die Möglichkeiten der Kombination erhöhen können!“*

Noch in seinen letzten Lebensjahren konnte Kossel wichtige Befunde, auch zur Natur der Eiweiße im Allgemeinen, gewinnen. Kurz vor seinem Tod vollendete er eine größere Monographie mit dem Titel „Protamine und Histone“ [23].

Herausgeber der Zeitschrift für physiologische Chemie

1877 hatte Felix Hoppe-Seyler für sein neues Fachgebiet die „Zeitschrift für physiologische Chemie“ gegründet. Kossel wurde 1895 Mitglied des Redaktionskollegiums. Als Hoppe-Seyler im gleichen Jahr verstarb, übernahm Kossel gemeinsam mit Eugen Baumann (1846–1896) die Herausgabe der Zeitschrift, die zu Ehren ihres Lehrers nun „Hoppe-Seylers Zeitschrift für physiologische Chemie“ genannt wurde. Kossel blieb auch nach Baumanns Tod 1896 der Herausgeber.

Diese Zeitschrift hatte für die Entwicklung der Physiologischen Chemie eine besondere Bedeutung, denn sie war viele Jahre die einzige Zeitschrift, die ausschließlich der Biochemie gewidmet war. Namhafte Wissenschaftler aus dem In- und Ausland waren Mitglieder der Redaktion. 1914 erreichte der Jahrgang einen Umfang von 3000 Seiten. In den Kriegsjahren ging der Umfang stark zurück, weil nur noch Biochemiker aus dem Deutschen Reich und Österreich-Ungarn sowie dem neutralen Ausland Beiträge einreichten. Die russischen Gelehrten Wladimir Gulewitsch (1867–1933) und Ivan Pavlov (1849–1936), die Mitglieder der Redaktion waren, schieden jedoch während des Ersten Weltkriegs nicht aus. Es blieb eine Verbindung erhalten, die diese Repräsentanten der internationalen Wissenschaft geschaffen hatten. Bereits 1920 traf bei Kossel wieder ein Beitrag aus St. Petersburg (Petrograd) für die Zeitschrift ein. Als 1923 der 130. Band aus Anlass seines 70. Geburtstages Kossel gewidmet wurde, gab es auch Beiträge eines englischen und eines US-amerikanischen Schülers. Seit 1996 erscheint die Zeitschrift unter dem Namen „Biological Chemistry“.

Werk und Persönlichkeit

Albrecht Kossel entwickelte sein Lebenswerk in der Epoche der aufblühenden deutschen Naturwissenschaften, aus der viele Nobelpreisträger hervorgingen. 1954 schrieb der Biologe Fritz Kaudewitz (1921–2001) über Kossel [24]: *„Mit ihm ging nicht nur ein großer Wissenschaftler dahin. Ein Mensch, dessen Güte und innere Ausgeglichenheit, dessen Wahrhaftigkeit und Pflichtgefühl schlechthin vollkommen waren, hatte sein Leben vollendet. Denen, die nach*

ihm kamen und sein Werk weiterführten, mag er als ein leuchtendes Beispiel dafür erschienen sein, wie sehr echte wissenschaftliche Leistung von der seelischen Kraft einer in sich ausgeglichenen großen Persönlichkeit getragen wird."

Schon früh entdeckte Kossel seine Neigung für die Botanik. Doch verankert hat er sich im neuen Fachgebiet der Physiologischen Chemie. Instinktsicher drang er in unerforschte Bereiche vor, arbeitete mit Zielstrebigkeit und Genauigkeit. Nicht plötzliche geniale Eingebungen waren es, sondern gewissenhafte Glied an Glied anfügende Einzelforschungen, die ihn auszeichneten. Angriffen anderer Forscher trat er entschieden und selbstbewusst mit Beweisführung entgegen. Kossel wurde sich immer sicherer über die Bedeutung der Biochemie und sprach 1908 [20]: *„Die Biochemie ist leer ausgegangen, als man an den deutschen Hochschulen die Welt des Geistes in Professuren verteilte, und doch suchen wir die Lösung der wichtigsten und tiefsten Probleme des Lebens in ihrer Werkstätte."*

AUCH AN DER DER ENTDECKUNG DER FÜNFTEN PRIMÄREN NUKLEINBASE URACIL WAR KOSSEL BETEILIGT

Albrecht Kossel hatte großen Anteil daran, dass sich die Biochemie noch zu seinen Lebzeiten als selbständiges Fachgebiet an den deutschen Universitäten durchsetzen konnte.

Kossel neigte nicht dazu, Hypothesen aufzustellen; nur was bewiesen werden konnte, hatte Bedeutung. Aber man darf davon ausgehen, dass er etwas ahnte von dem sich entwickelnden Gewaltigen, das in diesen Anfängen lag. 50 Jahre hat er ununterbrochen geforscht, eine tiefe Freude an der experimentellen Arbeit blieb ihm ein Leben lang erhalten. Sie fand in 120 Veröffentlichungen ihren Niederschlag. Kossel lehnte es ab, dass sein Name bei Publikationen erschien, die zwar ihren Ursprung in seinem Institut hatten sowie von ihm gefördert und begleitet wurden, wenn er nicht selbst bei den Experimenten mit tätig geworden war. So kam es dazu, dass Alberto Ascoli diese bedeutsame Veröffentlichung über die Entdeckung des Uracils lediglich mit einem „innigsten Dank" an seinen Lehrer abschließen konnte. Dieser habe ein nie versiegendes Interesse an den Forschungen gehabt, so schrieb er, und eine unerschöpfliche Bereitwilligkeit, jederzeit mit Rat und Tat zu helfen. Ascoli fügte noch an: *„ ... wie oft wäre ich sonst entmutigt den sich vor mir auftürmenden Schwierigkeiten machtlos gegenübergestanden!"* [13]

Kossel pflegte weitverzweigte internationale Kontakte, viele Reisen führten ihn ins Ausland. Scheinbar unpolitisch zeigte er im Ersten Weltkrieg beachtenswerte Haltungen. Er verweigerte die Unterschrift unter das propagandische Manifest des Deutschen Reiches „An die Kulturwelt". Viele berühmte Persönlichkeiten, unter ihnen die meisten deutschen Nobelpreisträger, unterzeichneten sie. Als er im Hungerwinter als Ernährungsexperte bedrängt wurde, ließ er wissen [25]: *„Auf Ersuchen der Reichsregierung soll ich der Bevölkerung klarmachen, dass die Lebensmittelrationen ausreichend sind? Diese Anstiftung zur Lüge weise ich mit Empörung und Entrüstung weit von mir."*

Biographen erwähnen Kossels Bescheidenheit und verbinden sie mit seinem norddeutschen Charakter. Siegfried Edlbacher schrieb über sein Wesen [19]: *„Nachdenklich und ernst, ja manchmal melancholisch, doch stets getragen von einem leisen Humor."*

Besonders eindrucksvoll würdigte ihn Ulf Lagerkvist [29]: *„ ... his elucidation of the chemical nature of some building blocks that make up nucleic acids and chromatine has secured immortality for this exceedingly modest and almost shy man."*

Viele Jahre glaubte man, dass die Nukleinsäure bei allen Lebewesen uniform sei und deshalb nicht Träger der Erbinformation sein konnte. Außerdem verlagerte sich die biochemische Forschung nach den beiden Weltkriegen ins Ausland. So geschah es, dass Werk und Persönlichkeit Albrecht Kossels zum großen Teil in Vergessenheit geraten sind. Thomas Müller-Bohn schrieb jetzt [26]: Es *„... drängt sich der Gedanke auf, dass Albrecht Kossel als Mensch und Forscher nachträglich mehr Würdigung verdient. Da die DNA-Bestandteile Adenin, Cytosin, Guanin und Thymin von ihm beschrieben wurden, läge es nahe, sie als Kosselsche Nukleinbasen zu bezeichnen."*

Zusammenfassung

Das 21. Jahrhundert wird bereits als das Zeitalter der Genetik bezeichnet. Tiefgreifende gesellschaftliche Veränderungen sind zu erwarten. Mit dieser Entwicklung sind viele berühmte Namen verbunden. Doch Albrecht Kossel, der die bedeutungsvollen Bausteine der Nukleinsäure entdeckt und erstmalig charakterisiert hat, ist wenig bekannt. Er erhielt 1910 den Nobelpreis, geriet später in Vergessenheit. Mehr als vier Jahrzehnte vergingen, bis man verstand, dass es die Kosselschen Nukleinbasen Adenin, Guanin, Thymin und Cytosin sind, die in der DNA die Erbinformation kodieren. Es ist das Anliegen, Leben und Werk Albrecht Kossels zu beschreiben und die Erinnerung an diesen herausragenden Biochemiker zu fördern. Seine große Persönlichkeit ragt vorbildhaft in die Gegenwart hinein.

Summary

The 21st century has already been described as the age of genetics. Far-reaching social changes are to be expected. Many famous names are associated with this development. However, little is known about Albrecht Kossel, who discovered the important building blocks of nucleic acids and characterised them for the first time. He was awarded the Nobel Prize in 1910 and later fell into oblivion. More than four decades passed before it was understood that it is the Kossel's

WAS MAN WISSEN MUSS

Albrecht Kossel (1853–1927) entdeckte die Basen Adenin, Guanin, Thymin und Cytosin als Bausteine der Nukleinsäure.

Kossel wurde zum Pionier der Genetik, denn diese Kosselschen Nukleinbasen codieren in der DNA die Erbinformation.

1910 wurde Albrecht Kossel für seine herausragenden Leistungen auf dem Gebiet der Biochemie mit dem Nobelpreis geehrt.

Albrecht Kossel (1853–1927) discovered the bases adenine, guanine, thymine and cytosine as building blocks of nucleic acid.

Kossel became a pioneer in genetics, because these Kossel's nucleic bases encode the genetic information in the DNA.

In 1910 Albrecht Kossel was honoured with the Nobel Prize for his outstanding achievements in the field of biochemistry.

nucleic bases adenine, guanine, thymine and cytosine that encode the genetic information in DNA. It is our aim to describe the life and work of Albrecht Kossel and to promote the memory of this outstanding biochemist. His great personality projects exemplarily into the present.

Schlagwörter

Albrecht Kossel, Nobelpreis, Proteinforschung, Nukleinbasen, DNA.

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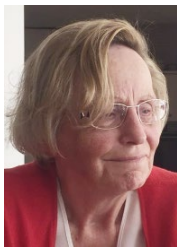
Die Autoren



Dr. Edith Framm, geboren 1944 in Wismar. Medizinstudium in Berlin, Rostock und Halle. 1969 erfolgte die Promotion und danach die Ausbildung zur Fachärztin für Allgemeinmedizin. Sie war zunächst in einer Poliklinik in Wismar tätig, bis sie sich in eigener Praxis als Landärztin in Blowatz bei Wismar niederließ. Seit 2003 wurden von ihr mehrere Romanbiografien verfasst, darunter „Albrecht Kossel und die DNA – ein Nobelpreisträger aus Mecklenburg", Koch und Raum, Wismar 2019.

Dr. Joachim Framm, geb. 1944 in Wismar. Pharmaziestudium in Rostock und Jena, Promotion in Halle. Seit 1972 Apotheker in Wismar, von 1978 bis 2009 Leiter der Hirsch-Apotheke. Er widmete sich in Publikationen und Vorträgen der Beratungstätigkeit in Apotheken, hält seit 1994 Vorlesungen und Seminare für Studierende der Pharmazie. Er ist ein Autor der vom Deutschen Apotheker Verlag herausgegebenen „Arzneimittelprofile" (6. Auflage 2018, Stuttgart).

Korrespondenzadresse:
Dr. Edith Framm
Dr. Joachim Framm
Am Markt 29
D-23966 Wismar
E-Mail: joachim.framm@t-online.de

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Speakers

Modeling and Treating Neurological Disorders using Stem Cells, Reprogramming and Genome Engineering

Henrik Ahlenius

Faculty of Medicine, Department of Experimental Medicine Science, Lund University

Human pluripotent stem cells (PSCs) have shown high potential for cell replacement therapy and modeling of neurodegenerative disorders. However, traditional differentiation of PSCs, using small molecules, can be time consuming, technically difficult and yield heterogenous populations of cells.

In addition, patient to patient and line to line variability can mask phenotypes in disease modeling efforts. Furthermore, iPSC derived cells are rejuvenated and might not be ideal for modeling age related diseases.

To overcome these hurdles, we have developed efficient forward programming and direct conversion methods to generate neural cells from PSCs and directly from fibroblasts.

We now combine these technologies with CRISPR engineering to model and develop therapeutics for neurodegenerative disorders such as frontotemporal dementia and brain tumors with a focus on astrocytes.

Resolving the heterogeneity of the ALS spectrum using patients‘ hiPSCs

Alberto Catanese

Anatomy and Cell Biology, University Ulm

Amyotrophic Lateral Sclerosis (ALS) is a genetically-heterogenous, fatal neurodegenerative disease mainly affecting upper and lower motoneurons. This extremely complex pathogenic landscape represents one of the major obstacles still limiting our understandings of the disease. In fact, the genetic heterogeneity characterizing ALS has limited the identification of an effective therapy, and this bleak prognosis will only improve with a greater understanding of convergent disease mechanisms. The use of hiSPCs derived from patients has provided the opportunity to investigate the pathological mechanisms underlying the different ALS genes and also provided evidence for a common pathological portrait shared across the disease spectrum. Additionally, this tool can be effectively used to clarify the pathogenity of specific mutations with uncertain clinical relevance, which represents a great advantage to address rare clinincal cases besides the ones affected by major pathogenic variants. In our work, we applied multi-omics analysis to an heterogenous cohort of hiPSC lines differentiated into motor neurons to i) identify a common synaptic phenotype unifying *TBK1, SOD1, FUS, C9orf72* and *TARDBP* cases, ii) clarify the pathogenic impact of missense variants observed in NEK1 patients.

In Vitro Disease Modeling and Pathophysiological Characterization of Fatty Acid Hydroxylase-Associated Neurodegeneration

Fatima Efendic1 ,*, Andreas Hermann1, 2, 3 and Moritz J. Frech1, 3

1. Sektion für Translationale Neurodegeneration „Albrecht Kossel“, Neurology, Universitätsmedizin Rostock
2. Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald
3. Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, 18147 Rostock, Germany
*Presenting author

Demyelination and degeneration are the most common pathophysiological hallmarks of neurodegenerative disorders. Fatty acid hydroxylase-associated neurodegeneration (FAHN) is a rare neurodegenerative disorder caused by mutations in the FA2H gene. This mutation results in a reduction in enzymatic activity, which in turn leads to instability of the myelin sheath, demyelination, and axonal degradation. In this study, we generated FAHN patient iPSCs-derived neurons and oligodendrocytes and employed the combination of these two cell types to investigate studies on myelination and myelin sheath in vitro. Immunocytochemistry and Western blot demonstrated a significant impairment in the colocalisation and a markedly reduced expression of myelin proteins in single OPCs and in coculture. The examination of impaired myelination in FA2H-deficient cell line was accompanied by the observation of reduced colocalisation, with a notable decrease in the amount of MBP and PLP protein. The balance of myelin proteins in myelin membrane synthesis is essential for the formation of a compact myelin sheath. The results were corroborated by a significant variation in the myelinated axon structure, including axon length, internode length, and the number of Ranvier nodes. Another common finding in neurodegenerative disorders is a defect in the recycling process. Consequently, we sought to ascertain whether this defect in autophagy was applicable to FA2H deficient cell line. The results demonstrated a reduction in p62 expression and progression, indicated by elevated LC3BII levels and a compromised fusion of autophagosomes and lysosomes. The generation of a human iPSC-derived myelin model for FAHN disorder has not only highlighted the pathophysiological phenotype of the FAHN cell model but also provided important directions for further investigation. Consequently, it is postulated that a mutation in FA2H affects the formation of a compact and stable myelin sheath and impairs the functionality of the autophagy recycling machinery in neuronal cells.

Neuronal migration and axonal reconnection in temporal lobe epilepsy

Thomas M. Freiman

Full-Professor of Neurosurgery and Chairman of the Department of Neurosurgery

Abstract:

Temporal lobe epilepsy is the most frequent reason for of pharmacoresistant seizures and often histologically a sclerosis of the hippocampus is seen. It consists of a selective neuronal loss of the subregions cornu ammonis (CA) 1 and CA4. As well as a reconnection of the mossy fibres, which are axons of the surviving granule cells. Granule cells not only survive but also show migration, which leads to a dispersion of the normally thin granule cell layer. It will be shown, that the excitatory mossy fibres will make new connections to other excitatory granule cells as well as the inhibitory basket cells, which are also spared from cell death. The potential inhibition of a single basket cells is multiple time more effective than the excitation of a granule cell thus resulting in an increased inhibition. We hypothesize that an unbalanced hyperinhibited network leads to a synchronised random excitation.

Microglia in Aging and Neurodegeneration

Christiane Hartmann

Translational Neurodegeneration Section „Albrecht Kossel“, Rostock University Medical Center

Aging, a process that affects nearly all multicellular organisms, is the primary risk factor for neurodegenerative diseases such as amyotrophic lateral sclerosis. Microglia, the brain's immune protectors, play a crucial role in maintaining brain homeostasis. During aging or neurodegenerative diseases, microglia undergo changes in morphology, phenotype, and function, which can significantly contribute to the onset or worsening of neurodegenerative processes.

To explore the role of microglia in brain homeostasis, we developed a human microglia model that can be prematurely aged through Progerin expression. Our previously developed AgeScore, a user-friendly biomarker-based panel of classical aging markers designed to estimate the biological age of in vitro cell culture systems, showed an increase in the biological age of this model following Progerin induction. Additionally, in aged microglia we observed a decline in microglial function as well as in nucleo-cytoplasmic transport.

The findings from this study are critical for understanding the role of microglia in aging and neurodegenerative processes and may contribute to the development of new therapies for age-related and neurodegenerative diseases in the future.

Capturing the glymphatic system in action - Diffrerent approaches to imaging brain clearance

Jari Jukkola

Faculty of Medicine, Department of ExperimentalMedicine Science, Lund University

The glymphatic system, which is a mechanism for brain clearance of metabolites from the brain parenchyma and the cranium, has been widely studied in the past 12 years. Initially, with her in vivo multiphoton microscopy investigation, the renowned professor Maiken Nedergaard proposed the pulsation of cerebral arteries to be the source of power for brain clearance. Since then, the glymphatic field has expanded both in scope and methodology to better understand how exactly the brain rids itself of harmful metabolites. In my talk, we will discuss the pros and cons of past and current methodologies and what they reveal about the glymphatic system. We will end with defining an ideal approach to capture the still-elusive phenomena of macromolecular parenchymal clearance, which acts as the core tenet for the glymphatic system.

New multimodal multi-scale modalities to image amyloids in their native environment

Oxana Klementieva

Faculty of Medicine, Lund University, Sweden

Spatiotemporal alterations in the chemical and structural makeup of biomolecules play an essential role in the onset and progression of various diseases, including Alzheimer's Disease. Early structural changes at the submicron level often occur well before disease symptoms can be recognized and before morphological changes can be detected using conventional tissue-level methodologies such as spatial proteomics, histology, or immunohistochemical staining. Consequently, there is a critical need for structure-sensitive techniques. Here, I present an approach capable of spatiotemporal chemical imaging of amyloid structures at submicron resolution within their native environment.

Using a recently established technique, the Medical Microspectroscopy Group from Lund University conducted groundbreaking experiments that enabled the monitoring of amyloids in the process of formation, proliferation, and cellular damage directly within living tissues.

To assess structural changes with sub-micron precision, we employed optical photothermal infrared (O-PTIR) microspectroscopy, a technique sensitive to amyloid structures. By applying O-PTIR to freshly extracted brain tissue from APP/PS1 mice, we documented structural changes in functioning brain tissue, observing the appearance of newly formed amyloids spatially and temporally colocalized with lipid damage. Achieving time-resolved submicron in situ imaging of amyloid structures marks a significant technological advancement that opens new avenues for in-depth molecular analysis of amyloid formation within their natural environment, thus facilitating an understanding of why amyloids begin to form, accumulate, and damage the tissue.

Hypothalamic and Metabolic Changes in Huntington's Disease Pathogenesis

Rana Soylu Kucharz

Faculty of Medicine, Department of Experimental Medicine Science, Lund University

Huntington's disease (HD) is a neurodegenerative disorder characterized by motor, cognitive, and metabolic symptoms. Studies have revealed that hypothalamic dysfunction is critical in HD-related metabolic disturbances. Expression of mutant huntingtin (mHTT) in the hypothalamus disrupts neurocircuits that regulate metabolism, leading to insulin and leptin resistance and dysfunction in brown adipose tissue thermogenesis. These changes contribute to obesity and metabolic imbalance in HD models. Additionally, mHTT in the hypothalamus can potentially contribute to the pathology of other brain regions through non-cell-autonomous mechanisms, suggesting a broader role of hypothalamic alterations in HD pathogenesis. We will discuss hypothalamic and metabolic changes in HD and the emerging evidence on how hypothalamic dysfunction connects metabolic disturbances to neurodegenerative changes in HD, providing insights into the disease's multifaceted nature.

Association of Brain Atrophy with Automated Digital Speech Features in Early Alzheimer's Disease

Qingyue Li[1], Alexandra König[3,4], Nicklas Linz[4], Fedor Levin[2], Martin Dyrba[2], Annika Spottke[5,6], Björn Falkenburger[7,8], Christoph Laske[9,10,11], Emrah Düzel[12,13], Frank Jessen[5,14,15], Inga Zerr[16,17], Jens Wiltfang[16,18,19], Josef Priller[20,21,22,23], Michael T. Heneka[5,24,25], Michael Wagner[5,24], Stefan Teipel[1,2], Stefanie Köhler[2]

[1] Department of Psychosomatic Medicine, Rostock University Medical Center, Rostock
[2] German Center for Neurodegenerative Diseases (DZNE), Rostock, Germany
[3] Cognition-Behavior-Technogoly group (Cobtek), Université Côte d'Azur, Nice, France
[4] ki:elements GmbH, Saarbrücken, Germany
[5] German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany
[6] Department of Neurology, University of Bonn, Germany
[7] German Center for Neurodegenerative Diseases (DZNE), Dresden, Germany
[8] Department of Neurology, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[9] German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany
[10] Section for Dementia Research, Hertie Institute for Clinical Brain Research and Department of Psychiatry and Psychotherapy, University of Tübingen, Tübingen, Germany
[11] Department of Psychiatry and Psychotherapy, University of Tübingen, Tübingen, Germany
[12] German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany
[13] Institute of Cognitive Neurology and Dementia Research (IKND), Otto-von-Guericke University, Magdeburg, Germany
[14] Department of Psychiatry, University of Cologne, Medical Faculty, Cologne, Germany
[15] Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany
[16] German Center for Neurodegenerative Diseases (DZNE), Goettingen, Germany
[17] Department of Neurology, University Medical Center, Georg August University, Goettingen, Germany
[18] Department of Psychiatry and Psychotherapy, University Medical Center Goettingen, University of Goettingen, Goettingen, Germany
[19] Neurosciences and Signaling Group, Institute of Biomedicine (iBiMED), Department of Medical Sciences, University of Aveiro, Aveiro, Portugal
[20] German Center for Neurodegenerative Diseases (DZNE), Berlin, Germany
[21] Department of Psychiatry and Psychotherapy, Charité, Berlin, Germany
[22] School of Medicine, Technical University of Munich; Department of Psychiatry and Psychotherapy, Munich, Germany
[23] University of Edinburgh and UK DRI, Edinburgh, UK
[24] University of Bonn Medical Center, Dept. of Neurodegenerative Disease and Geriatric Psychiatry/Psychiatry, Bonn, Germany
[25] Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Belvaux, Luxembourg

Background

Subtle changes in speech are increasingly recognized as indicators of cognitive deficits in Alzheimer's disease (AD), including its preclinical and prodromal stages, and can now be assessed using automated methods that capture digital speech features. Here, we investigated whether MRI measures of brain atrophy, particularly in the basal forebrain and cortical speech areas, are associated with digital speech features in older adults within the AD spectrum.

Methods

The ongoing Prospect-AD study aims to validate automatically identified speech markers in reference to established gold standard biomarkers, it is conducted as an add-on study of the ongoing German national cohort studies DELCODE and DESCRIBE. Within Prospect-AD, speech recordings were collected via automated phone calls from both healthy controls and individuals with preclinical or prodromal AD. Speech features were extracted from the semantic verbal fluency (SVF) task during these calls, using artificial intelligence-based analysis of the audio recordings. These features were then integrated with clinical and imaging data from DELCODE and DESCRIBE, including socio-demographic information, baseline diagnoses, neuropsychological assessments, and MRI scans. Linear regression models were used to estimate the association between characteristics of

automatically obtained speech features and baseline brain volumes, and linear mixed effect models for the rate of regional brain atrophy.

Results

To date, 226 participants have been enrolled in the Prospect-AD study, with 102 included in this report. A subset of 84 participants was selected for longitudinal analyses based on their qualified follow-up scans. Performance on the SVF task correlated with brain volumes in corresponding functional areas. Additionally, SVF performance was associated with rates of atrophy in regions critically implicated in the progression of AD (see Figure 1). Various text-based and temporal features exhibited differing degrees of correlation with brain volume changes preceding the speech assessment. Detailed results will be presented at the summer school.

Conclusions

Our results support the use of automatically extracted features from remote cognitive assessments for screening populations suspected of early-stage AD. Automated speech-based cognitive testing may facilitate the identification of older adults at increased risk for clinical progression to AD and could guide further clinical and MRI evaluations, aiding in the effective allocation of clinical resources.

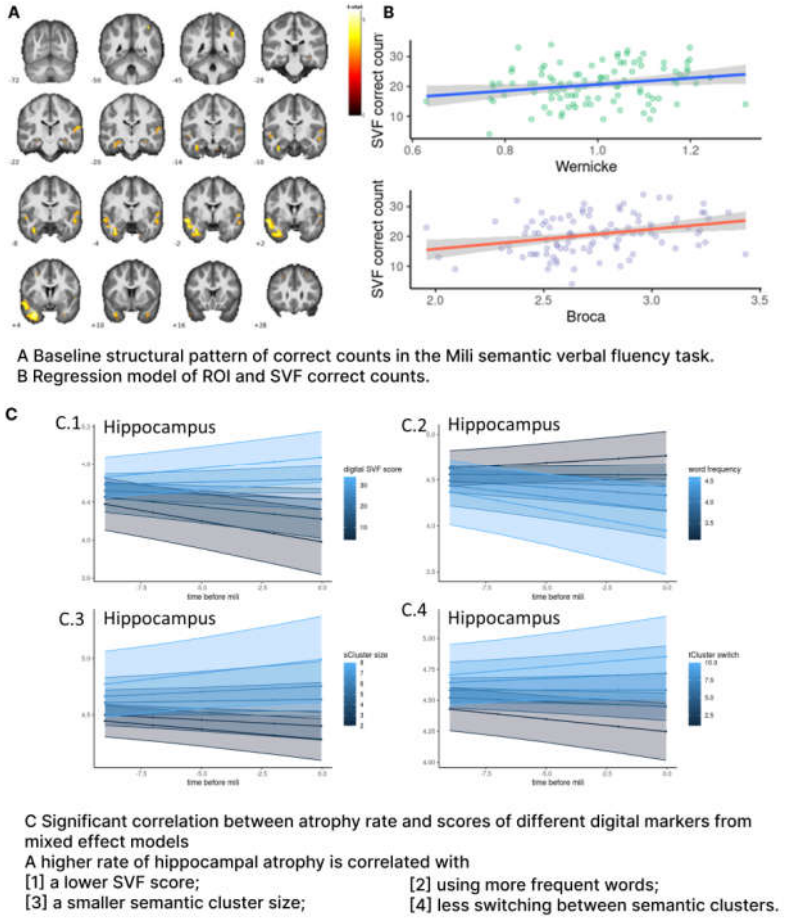

Figure1 Baseline and longitudinal results

Identification of Molecular Subgroups in ALS and Implications for Differential Therapies

Paul Lingor

Department of Neurology, Technical University of Munich, School of Medicine, Klinikum rechts der Isar & German Center for Neurodegenerative Diseases (DZNE)

Amyotrophic lateral sclerosis (ALS) is a complex and heterogeneous neurodegenerative disorder characterized by diverse clinical phenotypes, varying rates of progression, and a wide range of genetic backgrounds. The limited success of current therapeutic approaches is partly due to the failure to adequately account for this heterogeneity in treatment strategies.

In this presentation, I will discuss recent findings from the MAXOMOD consortium, where we utilized multi-omic analyses to identify distinct molecular subgroups within ALS. Our research reveals significant sex-specific molecular heterogeneity and identifies molecular subgroups based on analyses of the prefrontal cortex in human brains. These subgroups highlight key disease mechanisms, including immune response, mitochondrial dysfunction, and RNA metabolism. Notably, our findings suggest that the MAPK pathway represents a promising therapeutic target for ALS treatment.

Furthermore, we demonstrate that proteomic analysis of cerebrospinal fluid (CSF) can identify subclusters that correspond to the molecular mechanisms observed in brain tissue. These identified subgroups in both ALS patient tissue and CSF offer a potential framework for categorizing patients according to their molecular profiles, thereby enabling more tailored and individualized therapeutic approaches.

Investigating the role of microglia in amyotrophic lateral sclerosis using human iPSC models

Björn F. Vahsen

Nufield Department of Clinical Neurosciences

University of OxfordAbstract: Amyotrophic lateral sclerosis (ALS) is the third commonest neurodegenerative disease, primarily leading to the degeneration of motor neurons. In addition, neuroinflammation and activation of microglia is frequently observed in ALS patients, particularly in cases with the commonest ALS-associated mutation found in the *C9orf72* gene. Here, we generated induced pluripotent stem cell (iPSC)-derived microglia from C9orf72-ALS patients to study the effect of the C9orf72 mutation on microglial biology. We showed that C9orf72 mutant microglia have a pro-inflammatory phenotype with increased activation of matrix metalloprotease 9 (MMP-9). Using microglia-motor neuron co-cultures, we further demonstrated neurotoxic effects of C9orf72 mutant microglia on motor neurons, which was ameliorated by MMP9 inhibition. These findings suggest that microglia play an important role in C9orf72-ALS pathophysiology and identify microglial MMP9 expression as a potential therapeutic target.

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Posters

Investigating General Practitioner´s Perspectives on Blood Based Alzheimer´s Biomarkers

Gamze Altas, Julia Perry, Felix G. Rebitschek, Johanna Knöferle, Anja Schneider, Silke Schicktanz, Stefan Teipel, Marina Boccardi

Background: Alzheimer´s positive biomarkers begin decades before clinical symptoms emerge. Minimally-invasive blood-based biomarkers (BBBM) may soon be available. However, in unimpaired individuals they can only denote increased risk to develop clinical symptoms of Alzheimer´s disease (AD). This raises important considerations regarding their clinical benefit. General practitioners (GPs) represent the primary contact for individuals seeking information, guidance, and support on pathological aging, however, their perspectives regarding the use of BBBM in primary care have not yet been explored. Our PREPARE-study aims to explore GPs´ attitudes, experiences, expectations and needs regarding the adoption of BBBM in general practice as well as AD risk assessment and communication. We aim to examine practical as well as social and ethical implications of the clinical use of BBBM.

Methods: Our study will use a mixed-methods approach to process anonymous answers to an online survey to be circulated among GPs in Germany to assess their perspectives. We are defining the survey in 2 phases: 1) identification of main and specific research questions by a transdisciplinary working group (6 biomarker and dementia experts, 2 bioethicists and a risk assessment expert); 2) revision by general medicine experts. With subsequent semi-structured interviews with GPs, we aim to gain deeper understanding and clarify ambiguities from the survey data. To maximize GP participation, we are reaching all GP associations identifiable online or suggested by collaborators.

Results: So far, we completed phase 1) and defined 30 specific questions investigating 6 major research questions, i.e., GPs´ a) perspectives on ageing b) knowledge about risk factors of AD as well as the diagnostic and therapeutic options for patients with mild cognitive impairment or mild AD dementia, c) available clinical examination in primary care regarding dementia diagnostics, d) perceived informative value of Alzheimer´s biomarkers for asymptomatic individuals, e) capacity and interest in providing counseling for BBBM f) if and how would the adoption of BBBMs change GPs decision making. Two general medicine experts are processing Phase 2. The questionnaire can be completed in 20 minutes, and investigates both practical and ethical considerations relevant to adopting BBBM in general practice.

Discussion: While the utility of BBBM for screening, case-finding, or pre-diagnostic triage is still debated, our study aims to collect the perspective of primary care practitioners. By initiating a bilateral dialogue with them, we will be able to assist and provide targeted key information, communication, and decision-making tools and strategies, enabling sensible implementation of new biomarkers at the light of local resources, constraints, societal preferences. Moreover, the findings collected from GPs will help to dynamically adjust such strategies based on the rapidly evolving preventive and therapeutic options.

Investigation of the effect of disease- associated mutations in *RHOT1* and *VPS13A* on calcium homeostasis and lipid transfer

Anna Bartalis[1], My Uyen Dang Thi[1], Kevin Peikert[1,2,3], Julia Fitzgerald[4], Hannes Glaß[1], Andreas Hermann[1,2,3,5], Dajana Großmann[1]

1 Translational Neurodegeneration Section "Albrecht Kossel", Department of Neurology, Rostock University Medical Center, University of Rostock, 18147 Rostock, Germany.
2 Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, Rostock, Germany
3 United Neuroscience Campus Lund-Rostock (UNC), Rostock site.
4 Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany
5 Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, Rostock, Germany.

Background: Neurodegenerative diseases (NDDs) result from complex interactions and dysfunctions across multiple organelles, including mitochondria, lysosomes, the endoplasmic reticulum (ER), and peroxisomes. These diseases disrupt crucial cellular processes such as calcium, protein, and lipid homeostasis. Recent research emphasizes the importance of organelle contact sites (CS), particularly those between mitochondria and the ER (MERCS). At these sites, VPS13 proteins facilitate bulk lipid transport, and mutations in these genes are linked to NDDs like Chorea-Acanthocytosis (ChAc). VPS13D, another member of the VPS13 protein family, interacts with Miro1, a protein linked to Parkinson's disease (PD). Miro1 is situated on the outer mitochondrial membrane and is involved in regulating mitochondrial transport, dynamics, MERCS, and calcium homeostasis. Our recent research identified mutations in the *RHOT1* gene, which encodes Miro1, in four patients with Parkinson's disease. Although lipids are known to be crucial in neurodegenerative diseases, the details of their biosynthesis and transport are still not well understood.

Methods: To investigate lipid trafficking in neurodegenerative diseases, fibroblasts from ChAc patients and iPSC-derived neurons with CRISPR/Cas9-induced *RHOT1* mutations were used. To examine *RHOT1* we are using an isogenic quartet with one mutation site found in a PD patient and two engineered mutations to study the interaction with PINK1 and Parkin, two key proteins in PD pathology. We are using high-resolution live cell imaging to study the effects of these mutations on mitochondria, lipid biosynthesis and neuronal function.

Results: Our findings demonstrate that mutations in Miro1 lead to significant alterations in calcium handling. Mutant neurons showed elevated mitochondrial calcium levels compared to controls, suggesting potential impairment of neuronal function. Additionally, we observed differences in lipid droplet formation and lipid trafficking at mitochondrial-ER contact sites (MERCS) between control and mutant neurons. In VPS13A fibroblasts, there was an increase in contact sites and a decrease in mitochondrial lipid content under both normal and starvation conditions.

Conclusion: This study provides insight into how mutations in Miro1 and VPS13A contribute to disrupted organelle interactions, leading to calcium and lipid homeostasis impairments associated with PD and ChAc pathology.

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Calcium dysregulation in iPSC-derived neurons from patients with VPS13A disease

Emily Fischer[1], Kevin Peikert[1,2,3], Hannes Glaß[1], Andreas Hermann[1,2,3,4], Dajana Großmann[1]

[1]Translational Neurodegeneration Section “Albrecht Kossel”, Department of Neurology, Rostock University Medical Center, University of Rostock, Germany.
[2]Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, Rostock, Germany
[3] United Neuroscience Campus Lund-Rostock (UNC), Rostock site
[4]Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, Rostock, Germany.

Background:

VPS13A disease, also called chorea-acanthocytosis, is a rare neurodegenerative disorder of the young adulthood, which is caused by loss-of-function-mutations in the gene coding for VPS13A. VPS13A is a bridge-like lipid transfer protein (BTLP) residing at membrane contact sites (MCS). In addition to bulk lipid transfer, MCSs also play a crucial role in the regulation of the cellular calcium homeostasis, especially between the endoplasmic reticulum (ER) and the plasma membrane or mitochondria.

VPS13A has been shown to be involved in calcium homeostasis via ORAI1-mediated store operated calcium entry (SOCE). Previous studies showed a reduction of the calcium channels ORAI1 and STIM1 with an impairment of SOCE in VPS13A deficient neurons. However, the exact mechanisms behind as well as the consequences on cellular calcium homeostasis remain elusive.

Hypothesis:

The mechanism of SOCE is important for maintaining cellular calcium homeostasis and a disruption can potentially result in an alteration of calcium distribution. As VPS13A is crucially involved in mitochondria-ER contact sites (MERCS), we hypothese that VPS13A deficiency leads to disruption of MERCS, thereby contributing to calcium dyshomeostasis.

Methods:

We used induced pluripotent stem cell (iPSC)-derived neurons from healthy donors and from patients with VPS13A disease. For visualization of the MERCS, neurons were transfected with a specific split-GFP marker. The cells were treated with BTP2, an inhibitor of the calcium release-activated channel (CRAC) ORAI1, 24 hours post transfection. Neurons were then stained with the mitochondria-specific dye MitoTracker deep red and the calcium dye Rhod-2, AM. Super resolution live-cell imaging was performed on a LSM900 confocal microscope with Airyscan 2 module (Zeiss) over two minutes before and after treatments with Thapsigargin, an inhibitor of the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), Ru360, an inhibitor of the mitochondrial calcium uniporter (MCU), or the calcium ionophore Ionomycin, respectively. For measuring of SOCE neurons were stained with the calcium dye Fluo-4, AM. Then, intracellular calcium stores were depleted by exposing neurons in calcium-free medium and Thapsigargin. The addition of extracellular calcium, as well as the continued presence of

Thapsigargin, results in a rapid increase in intracellular calcium, which reflects SOCE. TECAN Spark was used to performe the measurement of SOCE.

Results:

VPS13A deficient neurons showed a dysregulated calcium homeostasis with a disruption of SOCE, increased cytosolic calcium level and a mitochondrial calcium overload. Under Thapsigargin-induced calcium stress, control neurons increased the amount of MERCS in order to facilitate mitochondrial calcium uptake, while VPS13A deficient neurons were not able to adapt the amount of MERCS under these conditions. Furthermore, the combined treatment with Ru360 and Thapsigargin revealed an MCU-independent mitochondrial calcium uptake in VPS13A deficient neurons. The mitochondrial calcium phenotype was partially reversed by inhibition of SOCE with BTP2.

Conclusion:

Our results support the hypothesis that VPS13A plays a role in neuronal calcium homeostasis, involving SOCE and mitochondrial calcium handling. It further shows that mitochondrial-ER interaction is disturbed, leading to an MCU-independent calcium overload in mitochondria. However, the relationships between bulk lipid transfer via BLTPs and calcium homeostasis remains enigmatic and require further investigation.

Funding:

Nachwuchsforderung 2020 der Deutschen Gesellschaft für Parkinson und Bewegungsstörungen e.V.; Veränderungen der ER-Mitochondrien Kontaktstellen und der inneren Mitochondrienmembran in iPSC-basierten Neuronen von Parkinsonpatienten mit Mutation in PINK1 oder Parkin; Nachwuchsforderung 2021 der Deutschen Gesellschaft für Parkinson und Bewegungsstörungen e.V.; LipiSYN: Untersuchung des Zusammenhangs zwischen Störungen der Membranlipiddynamik und der α-Synuclein-Proteostase. DFG (GR 6326/2-1): The role of ER-mitochondria signalling in PRKN-associated Parkinson´s disease. DFG Großgeräteantrag für LSM 800 Airyscan. FKZ: INST 264/175-1 FUGG.K.P. is supported by the Rostock Academy of Science (RAS). A. H. is supported by the Hermann und Lilly Schilling-Stiftung für medizinische Forschung im Stifterverband.

Analysis of lipid peroxidation and ferroptosis in iPSC-derived neuronal models with mutations in *RHOT1*

Jack Genschmar[1], Anna E. Bartalis[1], Julia Fitzgerald[2], Dajana Großmann[1], Andreas Hermann[1,3,4]

1) Translational Neurodegeneration Section "Albrecht Kossel", Department of Neurology, University Medical Center Rostock, University of Rostock, Rostock, Germany
2) Department of Neurodegeneration, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany.
3) Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, University of Rostock, 18147 Rostock, Germany
4) Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, 18147 Rostock, Germany

Background: Ferroptosis is a form of programmed cell death triggered by the accumulation of iron ions or reactive oxygen species (ROS), leading to irreversible cell membrane damage due to lipid peroxidation. The process is exacerbated by the loss of antioxidant defenses, e.g. GPX4 inhibition or glutathione exhaustion and plays a role in the demise of neurons in various neurodegenerative diseases. Miro1 is a mitochondrial protein that functions as a cytosolic calcium sensor and plays a crucial role in regulating mitochondria-ER contact sites (MERCS). Mutations in *RHOT1*, the gene encoding Miro1, have been identified in Parkinson's disease (PD) patients. Previous studies showed that mutant Miro1 disrupted calcium homeostasis, potentially increasing ROS formation. Thus we hypothesize that dysfunction of Miro1 predisposes cells to ferroptosis.

Methods: To explore Miro1's role in ferroptosis, we used iPSC-derived neurons with CRISPR/Cas9-generated point mutations in the *RHOT1* gene. We assessed cell viability using the AquaBluer assay. Results were normalized per nucleus, using Hoechst staining. Lipid peroxidation was measured using the Bodipy665/676 dye to determine oxidative damage of membranes. Ferroptosis was induced using different concentrations RSL3 to inhibit GPX-4, an enzyme which is crucial for reduction of lipid peroxides. Liproxstatin-1 served as a protective agent to prevent lipid peroxidation.

Results: Lipid peroxidation was induced by RSL3 in the isogenic control neurons. Treatment with inhibitors of different mitochondrial calcium channels (Ru265, Ru360, Dantrolene, CGP3725) or with the iron ion chelator Desferoxamine (DFO) did not mitigate this effect, potentially suggesting that neither calcium dyshomeostasis, nor accumulation of iron ions are the underlying causes for increased vulnerability aginst ferroptosis in Miro1-mutant neurons. Application of increasing concentrations of RSL3 revealed an increased vulnerability of Miro1-mutant neurons to ferroptosis, compared to the isogenic control neurons. Liproxstatin-1 rescued cell viability of all neuron lines, suggesting that cell death was indeed caused by ferroptosis.. However, results have to be further validated.

This study underscores the potential link between Miro1 dysfunction and increased susceptibility to ferroptosis, suggesting avenues for targeted neuroprotective strategies in PD.

Funding: Nachwuchsförderung 2020 der Deutschen Gesellschaft für Parkinson und Bewegungsstörungen e.V.; Veränderungen der ER-Mitochondrien Kontaktstellen und der inneren Mitochondrienmembran in iPSC-basierten Neuronen von Parkinsonpatienten mit Mutation in PINK1 oder Parkin; Nachwuchsförderung 2021 der Deutschen

Gesellschaft für Parkinson und Bewegungsstörungen e.V.; LipiSYN: Untersuchung des Zusammenhangs zwischen Störungen der Membranlipiddynamik und der α-Synuclein-Proteostase. DFG (GR 6326/2-1): The role of ER-mitochondria signalling in PRKN-associated Parkinson´s disease. DFG Großgeräteantrag für LSM 800 Airyscan. FKZ: INST 264/175-1 FUGG. Hermann and Lilly Schilling-Stiftung für medizinische Forschung im Stifterverband.

Mitochondria as aggregation hub in neurons

Hannes Glaß[1], Julia Pfeiffer[1], Jette Abel[1], Hans Jurchow[1], Andreas Hermann[1, 2, 3]

[1] Translational Neurodegeneration Section “Albrecht Kossel”, Department of Neurology, University Medical Center Rostock, University of Rostock, Germany.
[2] Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, Rostock, Germany
[3] Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, Rostock, Germany.

Insoluble aggregates are a hallmark of many neurodegenerative diseases. Mutations in fused in sarcoma (FUS) cause amyotrophic lateral sclerosis, where FUS aggregates have been observed. The exact mechanism of FUS aggregation remains elusive. Cytoplasmic mislocalization of nuclear FUS and subsequent sequestration into stress granules (SG), which can serve as aggregation seeds, are currently in the focus in understanding the origin of FUS aggregates.

In order to gain a better understanding of FUS aggregate dynamics we treated cells with different pH value adjusted media and induced SG with sodium arsenite. We found that a basic pH value increased the half-live time of fluorescence recovery after photo bleaching (FRAP) of wt-FUS_eGFP containing SG in HeLa cells to a level comparable to that of p.P525L-FUS_eGFP carrying cells. Furthermore, we observed spontaneous SG formation upon treatment with media exceeding pH of 8.75. Proteins are most prone to aggregation at their isoelectric point, since they have the least charge and thus little electrostatic repelling force. The calculated isoelectric point of FUS is at pH 9.4.

The mitochondria matrix is a compartment with high pH value (~7.8), because protons being constantly pumped into the cristae to drive ATP production from oxidative phosphorylation. Since FUS has been shown to be present in mitochondria, we were wondering whether mitochondrial FUS is prone to aggregation. Filter trap assay revealed elevated FUS aggregation in mitochondrial fractions of p.P525L-FUS_eGFP motor neurons (MN) compared to isogenic wt-FUS_eGFP MN but not nuclear fractions. We conclude that basic compartments like the mitochondrial matrix can provide a pro-aggregating environment for proteins like FUS that can be the source of FUS aggregation in FUS-ALS patients.

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Npc1 deficiency impairs microglia function via Trem2-mTOR signaling in Niemann-Pick disease type C

Xiaojing Han[1], Yunxiao Li[1], Andreas Hermann[1,2,3], Xin Yan[1], and jiankai Luo[1]

[1] Translational Neurodegeneration Section "Albrecht Kossel", University Medical Center Rostock, University of Rostock, Rostock, Germany,
[2] Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, University of Rostock, 18147 Rostock, Germany,
[3] Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, 18147 Rostock, Germany

Niemann-Pick disease Type C (NPC) is a neurodegenerative disease mainly caused by the mutation in *NPC1* gene, leading to massive accumulation of unesterified cholesterol in the late endosome/lysosome of cells. Impaired phenotype of microglia is a hallmark in *Npc1* mutant mice (*Npc1*$^{-/-}$ mice). However, the mechanism of Npc1 in regulating microglial function is still unclear. Here, we showed that the reactive microglia in the neonatal *Npc1*$^{-/-}$ mice indicated by the increased lysosome protein Cd68 and phagocytic activity were associated with disrupted Trem2-mTOR signaling in microglia. Furthermore, in *Npc1*-deficient BV2 cells, genetic deletion of *Trem2* partially restored microglial function, probably via restored mTOR signaling. Taken together, our findings indicated that loss of *Npc1* in microglia caused changes of their morphologies and the impairment of lysosomal function, which were linked to the Trem2-mTOR signaling pathway.

Role of Ferroptosis in FUS-ALS: Mitochondrial Dysfunction and Therapeutic Targets

Muhammad Ismail 1, Jette Abel1, Dajana Großmann1 and Andreas Hermann 1, 2,3 *

[1] Translational Neurodegeneration Section „Albrecht Kossel“, Department of Neurology, University Medical Center Rostock, University of Rostock, Rostock, Germany
[2] German Center for Neurodegenerative Diseases (DZNE) Rostock/Greifswald, 18147 Rostock, Germany
3 Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, University of Rostock, 18147 Rostock, Germany

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the selective degeneration of motor neurons in the brain and spinal cord. About 10% of cases are familial with mutations in fused in sarcoma (FUS) being amongst the four most common genes affected in European countries. Ferroptosis is a regulated form of cell death that relies on iron and is characterized by phospholipid peroxidation and metabolic constraints in cell membranes, leading to the destruction of membrane integrity and consequently cell death. Pathophysiological hallmarks of (FUS-) ALS involves mitochondrial dysfunction and oxidative damage, implicating ferroptosis as a putative cell-death pathway in motor neuron demise. However, a mechanistic understanding of ferroptosis contributing to ALS, particularly FUS-ALS, remains limited. To address this gap, Hela cells, induced pluripotent stem cell-derived neural progenitor cells (NPCs), and NPCs generating motor neurons expressing FUS-eGFP-WT and FUS-eGFP-P525L were employed to investigate ferroptosis in the context of FUS-ALS. We aimed to evaluate whether known negative modulators of the MCU complex, such as ruthenium red (RR), can prevent mitochondrial dysfunction and ferroptotic cell death in various ALS models. Specifically we investigated the effects of negative modulation of the mitochondrial calcium uniporter (MCU) by the ruthenium compound Ru265 on ferroptosis. Our study reveals mitochondrial disturbances and increased vulnerability to ferroptosis in cell models expressing mutated FUS. Targeting ferroptosis pathways, including iron chelation and modulation of mitochondrial calcium handling, alleviated cellular demise. This might pave the way for the development of novel therapeutic interventions aimed at slowing disease progression and preserving neuronal function in ALS patients.

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The impact of nucleocytoplasmic transport dysfunction on aging and neurodegeneration

Alexandra V. Jürs1, Christiane Hartmann1, Barbara Szewczyk1,2, Reymond Sutandy3, Christian Münch3, Andreas Hermann1,4,5

1 Translational Neurodegeneration Section “Albrecht Kossel”, Department of Neurology, Rostock University Medical Center, University of Rostock, Germany.
2 Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.
3 Institute of Biochemistry II, Goethe University Medical School, Frankfurt, Germany.
4 Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, Rostock, Germany
5 Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, Rostock, Germany.

Transferring macromolecules between the nucleus and cytoplasm requires a highly regulated nucleocytoplasmic transport (NCT) machinery consisting of a variety of nuclear pore complex (NPC) proteins. Revealing an age-dependency, NCT impairments thus reflecting a common feature in aging and age-associated neurodegenerative diseases like amyotrophic lateral sclerosis (ALS). This is accompanied by a leakage of the nuclear integrity and an altered cellular distribution of the NPC proteins while the origin of a dysfunctional NCT and the mechanism behind may vary between aging and neurodegeneration.

In this study, we therefore aimed to investigate the impact of aging on neurodegeneration and vice versa on NCT (dys-)function by using fibroblasts and induced pluripotent stem cell (iPSC)-derived spinal motor neurons of healthy young and aged as well as ALS-diseased donors. Mass spectrometric analyses of the nucleocytoplasmic fractioning were done and identified different age-associated clusters of protein expression. Especially nuclear proteins for the nuclear pore organization and transport proteins are decreased gradually with age. Moreover, some selected RNA-binding proteins revealed a nucleocytoplasmic shift in aged and ALS-diseased fibroblasts shown by immunofluorescence stainings. Further investigations of the expression and distribution of various NPC proteins, the nuclear shape as well as the determination of the nucleocytoplasmic shuttling via the 2Gi2R-Assay are ongoing.

Our results point out an age-dependent alteration of protein expression and distribution. Further examinations intend to contribute to our understanding on how aging and/or neurodegenerative mechanisms of different ALS types participate to NCT disruption and whether these might be amenable for general or individualized therapeutic approaches.

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Dissecting (nuclear) loss-of-function vs. (cytoplasmic) gain-of-function as the main driver of the FUS-ALS pathophysiology

Anže Karlek[1], Di Wu[1], Hannes Glaß[1], Andreas Hermann[1,2,3,4]

1 Translational Neurodegeneration Section "Albrecht Kossel", Department of Neurology, University Medical Center Rostock, 18147 Rostock, Germany
2 German Center for Neurodegenerative Diseases (DZNE) Rostock/Greifswald, 18147 Rostock, Germany
3 Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, University of Rostock, 18147 Rostock, Germany
4 United Neuroscience Campus Lund-Rostock (UNC), Rostock site.

Fused in sarcoma (FUS) is a protein that belongs to the FET/TET family. It is ubiquitously expressed and plays a role in various RNA metabolism processes. Under physiological conditions, FUS is localized to the nucleus by the proline-tyrosine nuclear localization signal (PY-NLS), which enables active transport of FUS through interaction with transportin-1 (TNPO1). Despite having a putative nuclear export signal, FUS is exported by passive diffusion rather than receptor-mediated. Mutations in the FUS NLS impair binding to TNPO1, leading to accumulation of FUS in the cytoplasm, where it forms insoluble aggregates that have been found in postmortem central nervous system and spinal cord tissue from patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In ALS, the transport defect is associated with a mutation in PY-NLS, whereas in sporadic FUS-FTD there is FUS aggregation without FUS mutations. A possible explanation for the pathology of FUS-FTD is arginine methylation in the RGG3 domain of FUS, which interferes with the binding of TNPO1 to PY-NLS. It has been proposed that phosphorylation of FUS by DNA-PK may affect the cellular localization of FUS.

Heat shock proteins (HSPs) are constitutively expressed in the cell, where they are induced by cellular stress. HSPs are part of a stress pathway called the heat shock response that regulates the formation of stress granules (SGs). It has been reported that severe mislocalization or overexpression of FUS can activate HSR. Another pathway responsible for the regulation of SG formation is the integrated stress response.

To investigate the role of gain or loss of function of FUS in the context of ALS, we first examined and compared the protein levels of HSPs between two isogenic pairs – WT/p.P525L – and – WT/FUS KO – of HeLa cells after heat shock. In the next step, we exposed the cell lines to a known oxidative stressor, sodium arsenite or heat shock to compare the assembly and disassembly of SGs.

As a next step, we plan to confirm the findings of FUS mislocalization after DNA-PK activation/inactivation with the proximity ligation assay and to further investigate the effect of FUS phosphorylation on cellular homeostasis. We also aim to generate hiPSC FUS KO lines and compare the gain of function with the current isogenic pair – WT/p.P525L – of hiPSC lines.

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Loss of the ipsilateral silent period in amyotrophic lateral sclerosis is associated with reduced white matter integrity in the motor section of the corpus callosum

Annaliis Lehto[a,b], Julia Schumacher[b,c], Elisabeth Kasper[b,c], Stefan Teipel[b,d], Andreas Hermann[a,b], Johannes Prudlo[b,c*]

[a] Translational Neurodegeneration Section "Albrecht Kossel", Department of Neurology, Rostock University Medical Center, Rostock, Germany,
[b] Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Rostock-Greifswald, Rostock, Germany,
[c] Department of Neurology, Rostock University Medical Center, Rostock, Germany,
[d] Department of Psychosomatic Medicine, Rostock University Medical Center, Rostock, Germany

Objective: Interhemispheric neurons in the motor section of the corpus callosum have an inhibitory effect on neurons of the contralateral motor cortex. Three quarters of patients with amyotrophic laterals sclerosis (ALS) show impaired transcallosal inhibition. We aimed to investigate whether structural changes co-occur with this functional impairment and to explore its phenotypic correlates.

Methods: The demographic, clinical, and neuropsychological data of 127 ALS patients were analysed. Transcallosal inhibition was assessed with an ipsilateral silent period (iSP) protocol using transcranial magnetic stimulation. Patients were categorised based on an iSP response or its loss, and the groups were characterised by demographic, clinical, and neuropsychological variables. Diffusion-weighted images from a subset of 63 patients were analysed using tractography, and white matter (WM) structural integrity metrics were compared across groups.

Results: 54% of patients displayed iSP loss. The average free-water-corrected fractional anisotropy values within the callosal tract between the primary motor cortices were lower for patients with iSP loss compared to patients with an iSP response. There were no group differences based on other diffusivity metrics. The groups did not differ regarding any of the demographic, clinical, or neuropsychological variables.

Interpretation: We found reduced WM integrity in the motor section of the corpus callosum that differentiated ALS patients with iSP loss from patients with an iSP response, but with a small effect size. Nevertheless, the underlying pathological substrate and potential genetic drivers for these structural and functional changes in a subset of ALS patients remain to be satisfactorily investigated

Glutamatergic Argonaute2 controls retina development

Yunxiao Li[1], Xiaojing Han[1], Andreas Hermann[1,2,3], Jiankai Luo[1,2], and Xin Yan[1]

1 Translational Neurodegeneration Section "Albrecht Kossel", University Medical Center Rostock, University of Rostock, Rostock, Germany
2 Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, University of Rostock, 18147 Rostock, Germany
3 Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, 18147 Rostock, Germany

During development, the mammalian retina originates from the diencephalon and shares both anatomical and functional similarities with the central nervous system (CNS). Retinal ganglion cells (RGCs), which are Vglut2-positive glutamatergic neurons, exhibit typical properties of CNS neurons. In present study, we demonstrated that the conditional deletion of Argonaute2 (Ago2), an RNA-binding protein crucial for microRNA (miRNA)-mediated gene silencing, in Vglut2^{+} neurons led to impaired RGCs development and retinal vascularization. Moreover, Ago2-deficient mice exhibited morphological defects of horizontal and amacrine cells, as well as increased glial activation in the retina. These findings highlight the critical role of Ago2 in retinal development, where it regulates neuron-blood vessel interactions through the microRNA signaling pathway.

Microglia limit the initial number of layer V neurons perinatal

Franz Markert[1], Jürke Hartz[1] and Alexander Storch[1,2]

[1] Department of Neurology, University of Rostock, Germany
[2] German Center for Neurodegenerative Diseases (DZNE), Rostock / Greifswald, Germany

Microglia are known for phagocyting neural stem cells during prenatal brain development, but also for secreting neurotrophic factors that are essential for neuronal survival postnatal. This functional switch goes along with the change of their shape from amoeboid to ramified. However, some amoeboid microglia already enter the cortical layers prenatal, where their function is not clear. To investigate whether microglia phagocyte neurons during this time period we have used untreated and hyperoxia treated (75% pO_2 from E14.5 to E16.5) C57BL6 mice, which are known to have an enlarged cortical layer V. Immunohistochemistry was used to detect active microglia (Iba1/CD68), layer V cells (Ctip2), top layer cells (Satb2) and cortical volume in perinatal mice at E16.5, P0.5 and P3.5. Intriguingly, we could show that an increase number of active microglia in layer V specifically targeted $Ctip2^+$ neurons and, consequently, normalized the cortex of hyperoxia treated mice in size and morphology. However, there were no signs of apoptotic layer V cells or altered apoptosis in general within the cortical plate. In summary, we could show that microglia are able to limit the number of layer V neurons during perinatal brain development. This previously unknown function of microglia is potentially of great interest for neurodevelopmental diseases.

RIG-1 but not STING mediated neuron-specific IFN type 1 signaling in FUS-ALS induces neurodegeneration and offers new biomarker potential

Marcel Naumann(1*), Stefanie Kretschmer(2), Kevin Peikert(1), Hannes Glaß(1), Dajana Großmann(1), Rene Günther(3), Susanne Petri(4), Annekathrin Rödiger(5), David Brenner(6), Francisco Pan-Montojo(7), Eleonora Aronica(8), Markus Kipp(9), Anand Goswami(10), Jared Sterneckert(11), Torsten Grehl(12), Tobias Böckers(13), Patrick Oeckl(6,14), Min Ae Lee-Kirsch(2), Andreas Hermann(1,15,16)

1 Translational Neurodegeneration Section "Albrecht Kossel", Department of Neurology, University Medical Center Rostock, University of Rostock, Rostock, Germany.
2 Department of Pediatrics, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany.
3 Department of Neurology, Technische Universität Dresden, Dresden, Germany; Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Dresden, Dresden, Germany.
4 Department of Neurology, Hannover Medical School, Hannover, Germany.
5 Department of Neurology, Jena University Hospital, Jena, Germany.
6 University Hospital Ulm, Department of Neurology, Ulm, Germany.
8 Amsterdam UMC, University of Amsterdam, Department of (Neuro)Pathology, Amsterdam Neuroscience, Amsterdam, The Netherlands.
7 Munich Cluster for Systems Neurology, SyNergy, Department of Neurology, Ludwig-Maximilian University Hospital, Munich, Germany.
9 Rostock University Medical Center, Institute of Anatomy, Rostock, Germany.
10 Department of Neurology, Eleanor and Lou Gehrig ALS Center, Columbia University, New York, NY, USA.
11 Center for Regenerative Therapies TU Dresden (CRTD), Technische Universität Dresden, Dresden, Germany.
12 Department of Neurology, Alfried Krupp Hospital, Essen, Germany.
13 Institute of Anatomy and Cell Biology, University of Ulm, Ulm, Germany.
14 German Center for Neurodegenerative Diseases e.V. (DZNE) Ulm, Ulm, Germany.
15 Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, Rostock, Germany.
16 Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, University of Rostock, Rostock, Germany.

Recent research demonstrated a significant contribution of aberrant innate immune system activation to the ALS pathology spectrum due to mutations in SOD1, TARDBP, and C9orf72 with a focus on the cGAS-STING-TBK1-IRF3 pathway. This pathway gets activated by e.g. cytosolic DNA accumulation. In FUS-ALS, accumulation of damaged DNA is evident in hiPSC-derived motoneurons (sMN) and post-mortem sMN, which was recently reported by us and others. We hypothesized that such defects might result in cytosolic DNA accumulation similarly triggering a neuroinflammatory phenotype.

Assessment of innate immune pathways in 4 different FUS mutant iPSC-derived sMN cultures including isogenic lines and in HeLa cells was conducted by qPCR for interferon-stimulated genes (ISG) and western blot. This was validated by post-mortem IHC of FUS-ALS patient spinal tissue. Structural damage was measured by repeated live cell imaging of axons and neurofilament light chain (NfL) in medium supernatant. Finally, we screened a cohort of FUS-ALS patients for their upregulation of ISGs in peripheral blood samples normalized to 10 healthy controls.

The expression of a set of ISGs was significantly upregulated in sMN with c-terminal FUS mutations in contrast to FUS mutant HeLa cells. Western blot indicated a stimulation of the TBK1-IRF3 pathway in mutant sMN. However, there was no significant difference in the level of STING and treatment with the established STING inhibitor H151 did not alleviate the upregulated ISGs in mutant sMN. However, cytosolic dsRNA – measured by J2 antibody ICC – was markedly increased in mutant sMN. In line with this, RIG-1, a major cellular dsRNA detector, was found to be significantly upregulated in mutant sMN and siRNA-mediated knockdown resulted in ISG reduction. In post-mortem analysis, RIG-1 was highly expressed in the remaining sMN. Furthermore, treatment with the EMA-approved JAK-STAT

inhibitor Ruxolitinib alleviated the upregulated ISGs, improved axonal outgrowth, and reduced NfL levels in mutant sMN. Finally, we analyzed ISG expression in blood samples from 15 FUS-ALS patients. Six patients showed an ISG upregulation above the 2xSD of a healthy control group. Importantly, those patients had a c-terminal and clinically more severe mutation.

Innate immune activation in FUS-ALS is evident in blood samples and selectively found in iPSC-derived sMN. Pharmacological Inhibition of the JAK-STAT1 pathway poses a putative novel individualized therapeutic strategy.

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Electromagnetic field stimulation (EMS) leads to a distinct, harmonized gene expression profile across all wild type and mutant lines that rescues clinically relevant phenotypes in amyotrophic lateral sclerosis (ALS)

Arun Pal[1,2], Jonas Gronemann[1], Marc Uhlarz[1], Wonphorn Kandhavivorn[1], Julia Japtok[2], Hannes Glaß[3], Andreas Hermann[3,4,5], Jens Pietzsch[6], Richard Funk[7,8], Jochen Wosnitza[1,9], Björn Falkenburger[2], Andreas Dahl[10] and Thomas Hermannsdörfer[1]

1: Dresden High Magnetic Field Laboratory (HLD), Helmholtz-Zentrum Dresden-Rossendorf, D-01328 Dresden, Germany
2: Division for Neurodegenerative Disease, Dept. Neurology, Technische Universität Dresden, D-01307 Dresden, Germany
3: Translational Neurodegeneration Section "Albrecht-Kossel", Department of Neurology, University Medical Center Rostock, University of Rostock, D-18147 Rostock, Germany
4: Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, D-18147 Rostock, Germany
5: Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, University of Rostock, D-18147 Rostock, Germany
6: Institute of Radiopharmaceutical Cancer Research, Zentrum für Radiopharmazeutische Tumorforschung (ZRT), Helmholtz-Zentrum
Dresden-Rossendorf, D-01328 Dresden, Germany
7: Institute of Anatomy, Medizinisch Theoretisches Zentrum, Technische Universität Dresden, D-01307 Dresden, Germany
8: Dresden International University, D-01067 Dresden, Germany
9: Institute Of Solid State And Materials Physics, Technische Universität Dresden, D-01307 Dresden, Germany
10: Dresden Concept Genome Center, c/o CRTD - Center for Regenerative Therapies TU Dresden, Technische Universität Dresden, D-01307 Dresden, Germany

Electromagnetic stimulations (EMS) with alternating fields (ACMS) can have a beneficial impact on cellular events. In our *ThaXonian* project, we develop a prototype of a patient stretcher with multiple arrayed built-in magnetic coils that allow to expose all body parts to vectorized electromagnetic fields of any spatiotemporal modulation for the treatment of neurodegenerative diseases. The coils can be operated either in a continuous mode with alternating current (AC) or with repetitive pulses by discharging a bank of capacitors. Through our accompanying *in vitro* experiments on cultured iPSC-derived spinal motoneurons from familiar ALS patients, we have empirically established the optimal magnetic field configuration with respect to the AC frequency, wave form, amplitude, and duration. Moreover, the specific architecture of our compartmentalized neuron cultures has enabled us to test different orientations of the magnetic field lines to the axons. Following ACMS, we analyzed its impact on axonal organelle trafficking, regeneration after axotomy and DNA damage response (DNA-DR) in the nucleus by live-cell imaging. All three readout assays are clinically relevant for neurodegeneration and revealed clear defects in our untreated ALS neurons. Beyond a critical threshold of field strength, we found a sustained rescue of the motility of axonal mitochondria and lysosomes along with increased outgrowth speed of growth cones after axotomy and a re-activated DNA-DR through AC sine waves at perpendicular field orientation to the axonal plane with a special frequency optimum. Remarkably, these phenotypic rescues clearly corresponded to systemic transcriptomic alterations revealed by genome-wide deep RNA sequencing: whereas the untreated pool of mutant ALS-FUS lines showed diverse gene expression profiles distinct from the wild type pool, our ACMS protocol led to a distinct, harmonized gene expression profile across all wild type and mutant lines. Our preliminary analysis suggests that ACMS impacts on diverse transmembrane neurotransmitter receptors and ion channels, thereby triggering kinase signaling cascades that eventually alter transcription and other DNA binding factors in the nucleus. Moreover, several hotly debated therapeutic ALS targets such as Rock1, UNC13 and PARP isoforms were greatly altered in their gene expression through ACMS. Our

results point to a powerful non-invasive and non-pharmacological novel treatment of neurodegenerative diseases.

Identification of Non-Motor Symptoms in an α-Synuclein-Overexpressing Parkinson's Rat Model

Hanna Weber[1], Meike Statz[1], Sarah Pfeisinger[1], Alexander Storch[1,2,3], Mareike Fauser[1]

[1]Department of Neurology, University of Rostock, Gehlsheimer Straße 20, 18147 Rostock, Germany
[2]German Centre for Neurodegenerative Diseases (DZNE) Rostock/Greifswald, Gehlsheimer Straße 20, 18147 Rostock, Germany
[3]Department Ageing of Individuals and Society, University of Rostock, Gehlsheimer Straße 20, 18147 Rostock, Germany

Correspondence: hanna.weber@med.uni-rostock.de

Introduction:

Parkinson's disease is the second most prevalent progressive neurological disorder in humans with accumulation of the α-Synuclein protein as a critical initiation of the disease. The development of a novel transgenic rat model expressing the entire human SNCA sequence in Sprague-Dawley rats provides an opportunity to expedite research in the realms of symptomatic progression and symptom control. Prior to embarking on additional experiments, it is imperative to thoroughly characterize this animal model. My project aims to characterize non-motor symptoms in the α-Synuclein-overexpressing Parkinson rat model (SNCA rats) to identify the symptomatic onset of Parkinson's disease in this model. These results contribute to selecting a specific age for subsequent experiments.

Methods:

Specific behavioral tests were conducted to identify various non-motor symptoms in SNCA rats. The Light Dark Box Test was employed to investigate anxiety-like behavior, the Sucrose Preference Test examined (an)hedonic behavior. The buried pellet test assessed olfactory function in SNCA rats. Additionally, histological studies were conducted to determine the degeneration in the dopaminergic system in the animals.

Results:

Significant differences between wild-type and SNCA animals were observed starting at the age of 9 months. For instance, increased anxiety in the animal model was discerned. Histological analysis remained controversial to the gained behavioral experiments.

Conclusion:

Rats aged 9 months will be utilized for future planned experiments based on the findings of this study.

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The role of altFUS in the context of neurodegeneration

Di Wu[1], Anže Karlek[1], Hannes Glaß[1], Andreas Hermann[1,2,3,4]

1 Translational Neurodegeneration Section “Albrecht Kossel”, Department of Neurology, University Medical Centre Rostock, 18147 Rostock, Germany
2 German Centre for Neurodegenerative Diseases (DZNE) Rostock/Greifswald, 18147 Rostock, Germany
3 Centre for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Centre Rostock, University of Rostock, 18147 Rostock, Germany
4 United Neuroscience Campus Lund-Rostock (UNC), Rostock site, 18147 Rostock, Germany

FUS (fused in sarcoma) is a multifunctional DNA/RNA binding protein associated with neurodegeneration. FUS proteinopathy is characterized by nuclear loss and cytoplasmic aggregation of FUS in affected neurons. Over-expression of wild type FUS protein and/or ALS-linked mutation can activate toxic mechanisms in cell models. Heterozygous mutations in the FUS gene cause FUS-ALS. Recently, an alternative open reading frame (altORF) camouflaged in the *FUS* gene encoding a 170 amino acids protein named alternative FUS (altFUS) was identified, which is endogenously expressed in human tissues including motor neurons. Previous studies suggested that the suppression of altFUS expression in a *FUS*-related toxicity *drosophila* model protects against neurodegeneration. This led us to wonder how altFUS might play a role in neurodegeneration and how much altFUS contributes to FUS proteinopathy. Therefore, we generated HeLa cells overexpressing altFUS. The overexpression was confirmed by using specific antibody against altFUS. Our results of immuno-staining of altFUS in HeLa cells overexpressing altFUS have presented a co-localization of altFUS on mitochondria.

To further ascertain the function of the altFUS protein and distinguish it's individual impact from FUS, a HeLa cell line with a FUS knockout was obtained. The knockout was confirmed using western blot and immunostaining. After confirmation, we generated HeLa cells with a FUS knockout that overexpress altFUS, and this overexpression was confirmed by immunofluorescence. To further characterize the differences between wild-type HeLa cells and the FUS knockout, live-cell imaging for mitochondrial membrane potential (ΔΨm) was conducted, revealing a reduction of ΔΨm in FUS knockout cells. We are completing the characterization of the FUS knockout and investigating the individual impact of altFUS in various aspects, including DNA damage, mitochondria, mitophagy, and autophagy. These findings may provide new insights into the mechanisms and offer a deeper understanding of FUS proteinopathy.

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