## Project A1: Electrophysiological evaluation of sweat gland barrier properties (Dorothee Günzel, Jörg Scheffel)

**Background:** Hypohidrosis/anhidrosism is a local or general reduction in, or even complete lack of, sweat production and may be life-threatening through inadequate body temperature control. One of the genetic causes for hypohidrosis is the lack of functional claudin-10b in HELIX syndrome. However, mislocalization of claudin-10b in sweat gland tight junctions has also been observed in other conditions associated with hypohidrosis.

**Hypotheses:** Claudin-10b deficiency causes a reduction in $Na^+$ secretion in the secretory coil of the sweat gland. In part, apical $K^+$ channels may functionally compensate at the price of an altered sweat composition.

**Aims:**
- To establish sweat gland organoid culture from human skin.
- To apply and further develop electrophysiological techniques for measurements on 2D and 3D cell and organoid cultures.
- To test the effects of disease-related sweat secretion stimulators/inhibitors on TJ protein expression and distribution.

**Thesis project:** The PhD doctoral researcher will build on the work of the previous PhD researchers and continue to further develop, optimize and apply impedance spectroscopy to investigate TJ properties in epithelial model systems. Initially, the project will concentrate on 2D and 3D cultures of a standard cell line (MDCK C7 cells with and without stable transfection with claudin-10b). In parallel the development and use of sweat gland organoids from *ex vivo* skin samples obtained from plastic surgery will be pursued, following published protocols. Sweat gland organoids will be characterized with respect to the expression levels of key ion transport proteins, morphologically (optical methods) and functionally (electrophysiology). Different approaches will be tested to simulate pathological conditions leading to hypohidrosis in HELIX syndrome or cholinergic urticaria and to pharmacologically treat these conditions by activating alternative routes for cation secretion.

**Methods:** 2D and 3D cell culture and organoid techniques will be employed and advanced. Standard molecular biological methods will be used for genetic engineering and transfection of established cell lines. Ussing-chamber-based as well as “static” impedance spectroscopy will be applied and electrode miniaturization will be attempted. Data will be evaluated by fitting to various equivalent circuit models. Morphological conclusions from impedance measurements will be cross-validated with optical methods (confocal laser-scanning microscopy, CLSM; super-resolution STED microscopy).

**Requirements:**
Affinity for wet lab work as well as for mathematics and physics (electrochemistry).

**For more information please contact:**

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**Project-related publications:**

Alzahrani AS, Hussein M, Alswailem M, Mouna A, Albalawi L, Moria Y, Jabbar MA, Shi Y, Günzel D, Dasouki M (2021) A novel claudin-10 mutation with a unique mechanism in two unrelated families with HELIX syndrome. Kidney Int. 100: 415-429.

Günzel D, Zakrzewski S, Schmid T, Pangalos M, Wiedenhoeft J, Blasse C, Ozboda C, Krug SM (2012) From TER to trans- and paracellular resistance: Lessons from impedance spectroscopy. Ann. N.Y. Acad. Sci. 1257: 142-151

Klar J, Piontek J, Milatz S, Tariq M, Jameel M, Breiderhoff T, Schuster J, Fatima A, Asif M, Sher M, Mäbert K, Fromm A, Baig SM, Günzel D, Dahl N (2017) Altered paracellular cation permeability due to a rare CLDN10B variant causes anhidrosis and kidney damage. Plos Genet. 13: e1006897.

Mannweiler R, Bergmann S, Vidal S, Brandner JM, Günzel D (2021) Direct assessment of in-dividual skin barrier components by electrical impedance spectroscopy. Allergy 76: 3094-3106

Schmid T, Bogdan M, Günzel D (2013) Discerning apical and basolateral properties of HT-29/B6 and IPEC-J2 cell layers by impedance spectroscopy, mathematical modeling and machine learning. PLOS One 8: e62913

Sewerin S, Piontek J, Schönauer R, Grunewald S, Rauch A, Neuber S, Bergmann C, Günzel D, Halbritter J (2022) Defective claudin-10 causes a novel variation of HELIX syndrome through compromised tight junction strand assembly. Genes & Dis. 9: 1301-1314.

Wang Y, Scheffel J, Vera CA, Liu W, Günzel D, Terhorst-Molawi D, Maurer M, Altrichter S (2022) Impaired sweating in patients with cholinergic urticaria is linked to low expression of acetylcholine receptor CHRM3 and acetylcholine esterase in sweat glands. Front Immunol. 13: 955161.

## Project A2: Food poisoning *C. perfringens* enterotoxin (CPE): Pore structure & molecular mechanism of CPE-claudin complex formation (Jörg Piontek, Daniel Roderer)

**Background:** Clostridium perfringens enterotoxin (CPE) causes one of the most common food-borne illnesses. CPE binds to tight junction (TJ) proteins (for instance claudin-3 and -4) and subsequently forms pores in the membrane leading to damage of epithelial cells. The cytotoxicity of CPE is exploited to target tumor cells that overexpress CPE-binding claudin subtypes. The non-toxic recombinant claudin binding domain of CPE (cCPE) is used as a TJ modulator to improve paracellular drug delivery. Previously, we investigated the pathomechanism of CPE-mediated cell damage, elucidated the molecular mechanism of the CPE-claudin interaction and applied this knowledge to design cCPE/CPE variants with shifted claudin subtype specificity. These enabled targeting of carcinoma cells expressing claudin-1 or -5 in xenograft mice models (CPE), TJ-opening for drug delivery across the blood-brain barrier or the skin in *in vitro* models (cCPE) and high-resolution imaging of claudins under physiological and pathophysiological conditions. Moreover, we applied cryogenic electron microscopy (cryo-EM) to analyze the structural background of bacterial toxin binding to their receptors and toxin integration into target cell membranes.

**Aim** of this project is to understand the structure-function relationship of CPE pore formation and CPE pore-claudin association. These data will (i) improve our structural and mechanistic understanding of CPE toxicity and (ii) guide the design of cCPE/CPE modular biologicals targeting claudins for (a) *TJ* super resolution imaging, (b) improved drug delivery by TJ modulation, (c) molecular diagnosis or (d) cytotoxic treatment of carcinomas.

**Thesis project:** The structure of the CPE pore complex will be analysed by cryo-EM. Claudin-expressing cells will be cultured and incubated with recombinant CPE. CPE pore complexes formed after CPE-binding to cell surface claudins will we purified from cell lysates. Initially, the purification will be optimized using different CPE constructs, cells and conditions. High quality preparations will be investigated by cryo-EM and single particle analysis and/or cryo-electron tomography (cryo-ET), followed by modeling of the CPE-claudin pore complex structure into the experimentally derived density maps. In parallel, model-derived claudin mutants will be expressed in HEK293 cells and tested for their CPE binding and pore formation abilities by cytotoxicity assays. Mutants still preserving CPE-claudin interaction but preventing toxic pore formation will verify critical protein-protein interfaces to guide our understanding of the molecular mechanism of formation of the prepore and functional pore. Imaging of claudins in CPE-claudin complexes and TJs by confocal and super resolution microcopy will complement the functional and mechanistic investigation of CPE action. cCPE/CPE constructs generated in this work will be propagated as modular biologicals targeting claudins.

**Methods:** Cryo-EM (high-priority access to state-of-the art research infrastructure Titan Krios cryo-TEM equipped with a Gatan K3 camera and an energy filter), single particle analysis, cryo-ET, site-directed mutagenesis, protein biochemistry, cell culture, cytotoxicity assay, confocal and super resolution (e.g. STED) microcopy, immunocytochemistry.

**Requirements:** We search for a highly motivated candidate with an excellent Master degree in Biochemistry, Biophysics, Biology or a related field and a genuine interest in molecular details of protein-protein interactions. Experience in protein biochemistry, cell biology or structural biology are of advantage.

**For more information please contact:** joerg.piontek@charite.de and roderer@fmp-berlin.de

**Project-related publications:** 1. Beier et al.(2022) **Claudin targeting as an effective tool for directed barrier modulation of the viable epidermis**. Ann N Y Acad Sci. 1517(1):251-265. 2. Pahle et al (2021) **Effective Oncoleaking Treatment of Pancreatic Cancer by Claudin-Targeted Suicide Gene Therapy with Clostridium perfringens Enterotoxin (CPE).** Cancers (Basel) 13(17): 4393. 3. Piontek et al (2020) **Targeting claudin-overexpressing thyroid and lung cancer by modified Clostridium perfringens enterotoxin**. Mol Oncol. 14(2):261-276. 4. Roderer et al. (2020). **Glycan-dependent two-step cell adhesion mechanism of Tc toxins**. Nat Comm 11 (2694). 5. Roderer et al. (2019). **Structure of a Tc holotoxin pore provides insights into the translocation mechanism**. PNAS 116 (45). 6. Neuhaus et al. (2018) **Reversible opening of the blood-brain barrier by claudin-5-binding variants of Clostridium perfringens enterotoxin's claudin-binding domain.** Biomaterials. 161:129-143. 7. Eichner et al. (2017) **In Colon Epithelia, Clostridium perfringens Enterotoxin Causes Focal Leaks by Targeting Claudins Which are Apically Accessible Due to Tight Junction Derangement.** J Infect Dis. 217(1):147-157. 8. Protze et al (2015) **Directed structural modification of Clostridium perfringens enterotoxin to enhance binding to claudin-5.** Cell. Mol. Life Sci. 72: 1417-1432.

**Project A3: Functional-structural relationship of paracellular channels formed by claudin-10b and claudin-15 (Michael Fromm, Jörg Piontek)**

**State of the art**: Claudins are tetraspan membrane proteins of the bicellular tight junction (TJ) that form a paracellular barrier against solutes and water passage across epithelia. However, a subset of claudins not only build a barrier against large molecules but also form paracellular channels through the tight junction. Of these, claudin-10b and claudin-15 form cation-selective homopolymeric channels. They differ in permeability for various cations depending on their respective size and hydration shell (Eisenman sequence) as well as for water. These differences result in specific transport functions, for instance in kidney tubules mutations of claudin-10b lead to diseases caused by altered reabsorption, and in small intestine loss of claudin-15 leads to malabsorption. The channel structure of claudins is yet unknown, although models of their molecular architecture have been suggested based on the claudin-15 crystal structure. The amino acids of the extracellular segments that are widely conserved between claudin-10b and claudin-15 have been shown to be critical for charge selectivity.

**Aims**: Clarifying the molecular determinants of cation and water channel function for claudin-10b and claudin-15.

**Hypotheses:** The permeability properties of claudin-10b and of claudin-15 channels are determined by specific sequence differences in their extracellular segments.

**Methods**: Mutagenesis, culture of epithelial cell clones, transfection, immunostaining, confocal and STED microscopy, dilution potentials, water transport, and structural bioinformatics.

**Thesis project**: We will study molecular determinants regulating the function of claudin-10b and -15 channels. Comparison of molecular models of claudin-10b and -15 channels are used to select regions and individual pore-lining amino acid residues in the extracellular domain that differ between both. Candidate chimeric mutations predicted to convert one claudin functionally into the other claudin (Eisenman sequence, water permeability) will be designed, generated by site-directed mutagenesis and stably expressed in epithelial cell lines with different claudin background (MDCK-C7 with, quinKO MDCKII without endogenous TJs). Cell membrane transport and TJ incorporation will be tested by immunostaining (claudin mutant, TJ markers), confocal and STED microscopy. Mutants lacking TJ localization provide information about claudin polymer assembly. Mutants with TJ localization will be further analyzed functionally: Barrier formation against small and large molecule permeation by tracer flux assays; selective ion channel formation by electrophysiology and water transport assays. Iterative combination of (i) channel structure modelling, (ii) candidate mutant selection, (iii) mutant generation/testing, and (iv) evaluation concerning model consistency will be performed, (i) and (ii) largely by other team members. Goal is to elucidate the mechanism underlying regulation of paracellular cation and water transport.

**Requirements**:
Skills in molecular / cell biological standard techniques, interest in structure-function studies.

**Contact for more information**: michael.fromm@charite.de and joerg.piontek@charite.de

**References**:


Conrad MP*, Piontek J* (*shared 1st authorship), Günzel D, Fromm M, Krug SM (2016) Molecular determinants of anion channel properties of claudin-17. *Cell. Mol. Life Sci.* 73: 185-200.

Günzel D, Fromm M (2012) Claudins and other tight junction proteins. *Compr. Physiol.* 2: 1819-1852.

Hempel C, Protze J, Altun E, Riebe B, Piontek A, Fromm A, Lee IM, Saleh T, Günzel D, Krause G, Piontek J (2020) Assembly of tight junction strands: Claudin-10b and claudin-3 form homo tetrameric building blocks that polymerise in a channel-independent manner. *J. Mol. Biol*. 432: 2405-2427.

Hempel C, Rosenthal R, Fromm A, Krug SM, Fromm M, Günzel D, Piontek J (2022) Tight junction channels claudin-10b and claudin-15: Functional mapping of pore-lining residues. *Ann. N.Y. Acad. Sci.* 1515: 129-142.

Piontek J, Krug SM, Protze J, Krause G, Fromm M (2020) Molecular architecture and assembly of the tight junction backbone. *Biochim. Biophys. Acta Biomembr.* 1862: 183279

Rosenthal R, Milatz S, Krug SM, Oelrich B, Schulzke JD, Amasheh S, Günzel D, Fromm M (2010) Claudin-2, a component of the tight junction, forms a paracellular water channel. *J. Cell Sci.* 123: 1913-1921

Rosenthal R, Günzel D, Krug SM, Schulzke JD, Fromm M, Yu AS (2017) Claudin-2-mediated cation and water transport share a common pore. *Acta Physiol.* 219: 521-536.

Rosenthal R, Günzel D, Piontek J, Krug SM, Ayala-Torres C, Hempel C, Theune D, Fromm M (2020) Claudin-15 forms a water channel through the tight junction with distinct function compared to claudin-2. *Acta Physiol.* 228: e13334.

**Project A4: Nanophysiology of Tight Junctions (Volker Haucke, Martin Lehmann, FMP Berlin)**

**Background:** The tight junction (TJ) connects neighboring epithelial or endothelial cells and acts as a barrier for solutes, water and pathogens. The coassembly of TJ proteins, e.g. claudins controls TJ tightness and permeability and is regulated by expression, interactions, signaling and endocytosis [1]. Previously we have found that a) only half of the claudins can form polymers when expressed individually in TJ free cell lines and b) novel nanoscale organization principles of claudins and c) segregation of barrier and channel claudins enables paracellular ion flux [2].

**Hypotheses:** The structural differences, nanoscale organization and variable interaction partners of claudins change TJ barrier properties. The TJ nanostructure and claudin copolymers are altered by toxin exposition, hypoxia and inflammatory responses.

**Aims:** The central aim of project A4 is to resolve the nanoscale molecular organization of TJs using advanced super-resolution and live cell microcopy under physiological and pathological conditions.

**Thesis project:** We will combine molecular cell biology techniques with live cell and super-resolution microscopy to characterize the organization and function of claudins and other TJ proteins. Findings will be verified in tissues and primary cells and functionally tested in CrispR knock-in and knock-out epithelial cell lines using 3D cyst, novel Lipid/ion flux assays or standard electrophysiological measurements. Our analysis will provide a better understanding of molecular mechanisms of disease, permeability defects, pathological conditions and inspire new pharmacological treatments. The work will be conducted at the FMP that offers access to state of the art technology platforms (Microscopy, Drug screening, NMR, Mass Spectrometry, CryoEM), additional Graduate school courses and great scientific interaction with FMP, MDC and BIH research. Close collaborations with other basic and clinical research groups within TJTrain for example A1 (Electrophysiology), A2 (Structural Modelling) and B3 (Organoid) are planned.

**Methods:** Molecular and Cell biology techniques (Cloning, CrispR knock-in and knock-out, lentiviral transduction, 2D and 3D cell culture, immunostaining of cells and tissues, protein biochemistry), Advanced Microscopy techniques (liveSTED, STORM, FRET, automated & spinning disc confocal microscopy), Quantitative Image Analysis, Ussing chambers ion flux measurements

**Requirements:**

- Experience with Molecular Cell Biology, Cell culture and Fluorescence Microscopy
- Computational and data management skills as well as previous experience with Image Processing are advantageous

**For more information please contact:**

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**Project-related publications:**

1. Gonschior H, Haucke V, Lehmann M. Super-Resolution Imaging of Tight and Adherens Junctions: Challenges and Open Questions. Int J Mol Sci. 2020, 21(3). pii: E744. https://doi.org/10.3390/ijms21030744
2. Gonschior, H., Schmied, C., Van der Veen, R.E., Eichhorst, J., Himmerkus, N., Piontek, J., Günzel, D., Bleich, M., Furuse, M., Haucke, V. and Lehmann, M., August 2022. Nanoscale segregation of channel and barrier claudins enables paracellular ion flux. Nature Communications, 13(1), pp.1-20. https://doi.org/10.1038/s41467-022-32533-4

## Project B1: Receptor-mediated endocytosis in directing neural stem cell versus neural crest cell fate (Annette Hammes, MDC, Berlin)

**Background:**

The low-density lipoprotein receptor-related protein 2 (LRP2) is a multifunctional **endocytic receptor** localized at the apical surface of **polarized epithelia**. **Patients** with mutations in the *LRP2* gene suffer, amongst other congenital anomalies, from **neural tube defects and craniofacial anomalies**. LRP2-deficient mice consistently reflect disorders seen in humans. Our lab tries to understand the mechanisms underlying the brain and head defects caused by LRP2 loss of function. Our recent results indicate a crucial role of LRP2 as a central hub at the base of the primary cilium of neuroepithelial cells for establishing cell polarity, specification of neuronal progenitors, and ultimately morphogenesis of the neural tube and brain. Endocytosis mediated by LRP2 includes two cooperating processes in the dorsolateral neuroepithelium. 1st LRP2-mediated folate and morphogen uptake triggers signalling processes pivotal for neural tube closure and 2nd LRP2-dependent subapical intracellular scaffold formation and endocytosis are essential for plasma membrane/cargo trafficking to control cell and tissue shape changes during morphogenesis. We showed that LRP2 functionally interacts with intracellular adaptor scaffold proteins and actin-binding proteins that are involved in dynamic cell shape changes, apical constriction, and cell-cell contact modulation, processes that are crucial for neural tube closure. Moreover, we identified LRP2 in **neural crest stem cells**. Interestingly, LRP2 deficient embryos show significant defects in collective directed neural crest cell migration suggesting an important role of the receptor not only in neuroepithelial cell specification but also in neural crest cell dynamics. Neural crest cells (NCCs) are a multipotent and migratory cell population that contributes to the formation of a wide range of tissues during embryonic development.

**Hypotheses:**

Receptor-mediated endocytosis is involved in directing **neural stem cell versus neural crest cell fate** at the neural plate border - the niche for neuronal and neural crest cells.

**Aims:**

Epithelial integrity and **epithelial-to-mesenchymal transition (EMT)** seem to be incompatible features but yet have to cooperate at the neural plate border to allow proper neuronal specification - essential for neural tube closure - and proper neural crest development via EMT. Identifying the molecular mechanisms underlying the balance between epithelial integrity and EMT will be the focus of the project.

**Thesis project:**

Changes in cell behaviour require major reorganization of plasma membrane and its proteins; yet, how receptor-mediated endocytosis controls EMT during development is incompletely understood. We will extend our current data on this question moving onto single cell level by proteomics and transcriptomics. Identifying the transcriptome and proteome signature of the NPB cells at several developmental stages in mutants and controls will allow us to identify disease-specific signatures predicting the phenotype. **Identifying (patho)mechanisms for EMT** not only helps to understand processes during embryonic development but also contributes to understanding EMT in disease and tissue remodelling in the adult organism. Based on the outcome of the omics data we will also use our established *ex vivo* cephalic cultures from *Sox10$^{gfp}$; Lrp2* mutant and wild-type mouse embryos to analyse their neural crest delamination and migration dynamics under various conditions including incubation with **folate** and with folate receptor antagonists. The question of whether the function of LRP2 in **NCC dynamics** could also be influenced by folate availability is of particular clinical relevance in the view of birth defect prevention.

**Methods:**

Molecular biology, mouse models, tissue and stem cell culture models, high- and super-resolution imaging, single-cell transcriptomics, and proteomics

**Requirements:**

Master's degree in Biology, Biochemistry, or Biotechnology

**For more information please contact:**

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**Project-related publications:**

Kowalczyk I, Lee C, Schuster E, Hoeren J, Trivigno V, Riedel L, Görne J, Wallingford JB, Hammes A*, Feistel K*. Neural tube closure requires the endocytic receptor Lrp2 and its functional interaction with intracellular scaffolds. Development 2021 Jan 26;148(2): dev195008. doi: 10.1242/dev.195008. PMID: 33500317 *joint senior and corresponding authors

Mecklenburg N*, Kowalczyk I*, Witte F*, Görne J, Laier A, Mamo TM, Gonschior H, Lehmann M, Richter M, Sporbert A, Purfürst B, Hübner N, Hammes A. Identification of disease-relevant modulators of the SHH pathway in the developing brain. Development 2021 Sep 1;148(17): dev199307. doi: 10.1242/dev.199307. Epub 2021 Aug 31. PMID: 34463328 *joint first authors

Szabó A, Mayor R. Mechanisms of Neural Crest Migration. Annu Rev Genet. 2018 Nov 23;52:43-63. doi: 10.1146/annurev-genet-120417-031559. PMID: 30476447.

**Project B2: Identifying IBD patients at risk for colitis-associated cancer (Michael Schumann, Michael Hummel)**

**Background:**

Colitis-associated cancer (CAC) is a risk to patients suffering from inflammatory bowel disease (IBD). In contrast to sporadic colon cancer, which is the major neoplastic disorder of the large intestine and affects mostly older individuals (age at diagnosis males 68 yrs, females 72 yrs in Germany), CAC often hits young individuals. As it is triggered by chronic inflammation, the steps to colitis-associated carcinogenesis are distinct from sporadic carcinogenesis. Thus, it is of importance to identify markers that will contribute recognizing the subgroup of IBD patients that are at high risk for developing CAC. Previously, we have uncovered a crucial role for the cytokine osteopontin (OPN) in CACs and have described the pathways OPN activates to trigger CAC. Preliminary results point to a role for OPN in modulating tumor checkpoint inhibition.

**Hypotheses:**

(i) IBD patients at risk for CAC can be identified by means of gene expression analysis.

(ii) OPN affects the interaction of T-cells and epithelial cells and thereby modulates checkpoint inhibition in colon epithelial cells.

**Aims:**

(i) Identifying individuals at risk for CAC using an oligo-gene-expression analysis. (ii) Further elucidating the signalling pathway that leads from OPN secretion to CAC development.

**Thesis project:**

Identifying IBD patients at high risk for developing colitis-associated carcinoma.

**Methods:**

We are going to make use of the large population of IBD patients in the Benjamin Franklin´s IBD clinic, who are regularly examined by endoscopy in our department. Mucosal biopsy specimen will be collected and processed to formalin-fixed, paraffin-embedded material. Expression analysis will be carried out at the level of RNA expression using the Nanostring technique. Expression on protein level will be performed in such a way that it allows for spatial resolution. Organoids will be deployed to elucidate OPN signaling in colon epithelial cells.

**Requirements:**

Nanostring gene expression. Multiplex protein expression system by Akoya.

**For more information please contact:**

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**Project-related publications:**

Sehn, Cardoso, Manna et al., Osteopontin in colitis-associated carcinogenesis. *Manuscript in preparation*

**Project B3: The Magnesiome – how claudin-10 affects magnesium levels (Dominik Müller, Tilman Breiderhoff)**

**Background:**

The kidney is essential for the homeostasis of body fluids and electrolytes. This is achieved by secretion and reabsorption of water and solutes across epithelia. Tight junctions (TJs) connect neighboring epithelial cells and form the seal and the pores. Members of the claudins protein family form the functional units of the TJ.

The importance of claudins in renal (patho-) physiology has been underscored by the identification of mutations in patients with genetic renal salt wasting disorders. Patients with mutations in *CLDN16* or *CLDN19* suffer from hypomagnesemia, hypercalciuria, and nephrocalcinosis while patients with mutations in *CLDN10* present with hypermagnesemia, salt wasting and the absence of nephrocalcinosis (HELIX-Syndrome). By using mice with targeted deletion of *Cldn16* showed that claudin-16 mediates paracellular reabsorption of calcium and magnesium in the thick ascending limb[1,2]. Mouse models of claudin-10 deficiency were instrumental to show that claudin-10a mediates the paracellular uptake of chloride in the proximal tubule[3] and that claudin-10b forms a sodium pore in the thick ascending limb[2,4].

**Hypotheses:**

So far, the analysis of claudin-10 was limited to the individual claudin-10 isoforms, claudin-10a and claudin-10b. A mouse model that lack both claudin-10 isoforms would mimic the situation in most of the patients, since the majority of mutations affect both isoforms. We expect a more severe salt wasting in combination with hypermagnesemia.

**Thesis project:**

The successful candidate will characterize mice with a kidney specific deletion of *Cldn10.* This is achieved using the Cre-lox-system and a mouse line expressing Cre recombinase under control of a *Pax8* promotor, which is already present in the lab. The candidate will analyze renal function and energy metabolism of these mice using biochemical, molecular biological, electrophysiological and histological techniques. Additionally, the candidate will use state-of the-art proteomic and NGS techniques to characterize pathology in this model and elucidate compensatory mechanisms.

**Requirements:**

The candidate should be willing to perform animal experimentation

**For more information please contact:**

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Dr. Tilman Breiderhoff tilman.breiderhoff@charite.de

**Project-related publications:**

1. Will, C., et al. (2010). Targeted deletion of murine Cldn16 identifies extra- and intrarenal compensatory mechanisms of $Ca^{2+}$ and $Mg^{2+}$ wasting. Am. J. Physiol. Renal Physiol. *298*, F1152-1161. 10.1152/ajprenal.00499.2009.
2. Breiderhoff, T., et al. (2018). Deletion of claudin-10 rescues claudin-16-deficient mice from hypomagnesemia and hypercalciuria. Kidney Int. *93*, 580–588. 10.1016/j.kint.2017.08.029.
3. Breiderhoff, T., et al. (2022). Claudin-10a Deficiency Shifts Proximal Tubular Cl- Permeability to Cation Selectivity via Claudin-2 Redistribution. J. Am. Soc. Nephrol. JASN *33*, 699–717. 10.1681/ASN.2021030286.
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**Project B4: Characterization of kidney disease-associated tight junction gene regulation (Dorothee Günzel, Kai Schmidt-Ott)**

**Background:** The kidney coordinates key physiological processes, including blood pressure control, volume homeostasis, excretion and retention of electrolyte-free water, and acid-base homeostasis. Tight junctions (TJ) are critically involved in these processes. Acute and chronic kidney diseases are common and result from a diversity of causes, including metabolic disease (e. g. diabetes), autoimmune disease (e. g. vasculitis), or critical illness with hypoxia or ischemia. However, little is known about the dysregulation of TJs in kidney diseases. This PhD project will focus on the role of transcription factors (TFs) in the expression control of genes encoding TJ components, and how their function is altered in kidney disease. Our laboratory has established methods to obtain single cell gene expression profiles and single cell chromatin accessibility in kidney cells from patients with kidney diseases and in mouse models of kidney disease. The PhD student will utilize an existing and steadily growing dataset, use bioinformatics strategies to predict mechanisms of TJ deregulation in disease, and validate these predictions in collaboration with other groups from the graduate school "TJ-Train".

**Hypotheses:**

1. We hypothesize that co-regulated TF-encoding genes and TJ component-encoding genes across kidney cells comprise regulatory units.
2. We hypothesize that dysregulation of TF/TJ component encoding regulatory units occurs in kidney disease.
3. We hypothesize that TFs and co-regulated TJ components functionally participate in kidney disease pathogenesis

**Aims:** Through bioinformatic data integration, the PhD candidate will aim to:

1. identify cell type-specific disease-related changes in gene regulation.
2. identify candidate regulators of tight junction genes and associated expression signatures.
3. predict and validate disease-related changes in candidate regulators in existing and newly generated locally available datasets as well as in public repositories.
4. establish the basis for perturbation experiments in model organisms and isolated cells.

**Thesis project:** In the proposed PhD project, single cell profiles of the transcriptome (snRNA-seq) and the epigenome (snATAC-seq) of kidney cells from patients and model organisms with acute and chronic kidney diseases will be analyzed bioinformatically. The goal of the analyses is to identify disease-related changes in chromatin accessibility and expression levels of genes encoding tight junction components, and to validate and further characterize them in collaboration with other members of our research consortium.
This project is designed to elucidate the impact of chronic disease on TJ composition in the kidney, predicting that regulation of TJ composition and regional heterogeneity of TJ gene expression differs between healthy and diseased kidney tubules.

**Methods:** We have set up pipelines to generate single nuclei mRNA-seq and ATAC-seq data from healthy and diseased mouse kidneys and from human kidneys. Bioinformatic data integration of existing and newly generated datasets will be used to identify and validate disease- and cell type-specific dysregulation of TF/TJ component-encoding regulatory units.

**Requirements:** Master in Bioinformatics, Biology or Biochemistry or similar qualification, good knowledge of R.

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**Project-related publications:**

Hinze C, Kocks C, Leiz J, et al. Single-cell transcriptomics reveals common epithelial response patterns in human acute kidney injury. Genome Med. 2022;14:103.

Markó L, Vigolo E, Hinze C, et al. Tubular Epithelial NF-κB Activity Regulates Ischemic AKI. J Am Soc Nephrol. 2016;27:2658..

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Hinze C, Karaiskos N, Boltengagen A, et al. Kidney Single-cell Transcriptomes Predict Spatial Corticomedullary Gene Expression and Tissue Osmolality Gradients. J Am Soc Nephrol. 2021;32:291.

Breiderhoff T, Himmerkus N, Meoli L, et al. Claudin-10a deficiency shifts proximal tubular Cl- permeability to cation selectivity via claudin-2 redistribution. J. Am. Soc. Nephrol. 2022;33: 699.

**Project C1: Bacterial toxins' impact on epithelial tight junctions (Jörg Schulzke, Roland Bücker)**

**Background:**

Intestinal pathogens as *Clostridioides difficile* and *Campylobacter species* cause diarrhea and intestinal inflammation.

**Hypotheses:**

Bacterial toxins such as TcdA, TcdB and CDT from *Clostridioides difficile* or zonula occludens toxins (ZOT) from *Vibrio cholerae* or *Campylobacter concisus* can affect the tight junctions of the colonic epithelium.

**Aims:**

To identify pathogen-dependent mechanisms of tight junction modification.

**Thesis project:**

The central part of this project deals with the extra- and intracellular signaling either directly at the epithelium or via the mucosal immune system, which will be investigated in co-cultures of epithelial cell and organoid models together with immune cells such as M1 macrophages. The pro-inflammatory functionality is examined using an inflammation-prone mouse model. The results are compared with data from human intestinal biopsies. A screening of barrier-protecting food components and pharmaceuticals will be performed.

**Methods:**

The PhD student will study the structure and function of tight junctions and their proteins using electrophysiological and molecular methods, including confocal STED microscopy and RNA sequencing (with pathway analysis).

**Requirements:**

No specific requirements.

**For more information please contact:**

Roland F. Bücker (roland-felix.buecker@charite.de)

**Project-related publications:**

Heils L, Schneemann M, Gerhard R, Schulzke JD, Bücker R (2023) CDT of *Clostridioides difficile* induces MLC-dependent intestinal barrier dysfunction in HT-29/B6 epithelial cell monolayers. ***Toxins (Basel)*** 15(1): 54

Bücker R, Schulz E, Günzel D, Bojarski C, Lee IM, John LJ, Wiegand S, Janßen T, Wieler LH, Dobrindt U, Beutin L, Ewers C, Fromm M, Siegmund B, Troeger H, Schulzke JD (2014) α-Haemolysin of *Escherichia coli*: a potentiator of inflammatory activity in the colon. ***Gut*** 63(12): 1893-1901

**Project C2: Role of the tricellular tight junction in inflammatory bowel diseases (Susanne Krug)**

**Background:** In inflammatory bowel disease (IBD), the epithelial barrier is impaired, leading on one hand to loss of ions and water, on the other hand to enhanced luminal antigen uptake supporting the inflammatory process further (Krug et al., 2014, Martini et al., 2017). Expression of tight junction (TJ) proteins is linked to barrier disturbance and is regulated by pro-inflammatory cytokines released from various types of immune cells. While the bicellular TJ (bTJ) is located between two cells, the tricellular TJ (tTJ) is formed at the meeting points of three cells. The tTJ is distinct in molecular composition and serves as a regulating site for macromolecule passage. Tricellulin (Ikenouchi et al., 2005, Krug et al., 2009a) and the members of the angulin family (Higashi et al., 2013) are specialized TJ proteins located at the tTJ and are regulatory factors for the barrier properties of the tTJ.
Alterations of the tTJ protein expression and localization are crucially involved in IBD. While downregulation of tricellulin has direct effects on the paracellular macromolecule barrier in ulcerative colitis (Krug et al., 2018), angulins are mainly responsible for its tricellular localization (Higashi et al., 2013) and angulin-1 has been shown to be affected Crohn's disease (Hu et al., 2020), where tricellulin is mislocated. Besides the main forms of angulins, a new variant has been recently found in intestinal tissues, which might be involved in the regulatory processes of the tTJ and is unknown in its functional properties.
**Hypotheses:** The newly discovered angulin isoform is involved in regulation of the intestinal tTJ. By this, it affects barrier properties as well as immune regulatory processes in both, normal intestinal function and in inflammatory diseases.
**Aims:** The new angulin isoform will be analyzed: (i) functionally: Effects on barrier properties, effects on tTJ proteins, (ii) structurally: E.g. specific differentiation from other isoforms, interaction with other tTJ proteins, (iii) regarding expression profiles in different tissues and sample types
**Thesis project:** Mechanistic characterization of a new angulin isoform.
For this, the angulin on one hand will be overexpressed on the other hand downregulated or knocked out in suitable cell culture or intestinal organoids. The resulting clones will be characterized in detail using electrophysiological techniques and functional assays as well as standard molecular biological techniques to clarify the role of this angulin isoform in context of the TJ barrier properties with special focus on the tTJ. Interaction with TJ proteins will be analysed to bring the observed properties and structural particularities in functional and mechanistic context. Besides this functional characterization on molecular level, the physiological and potential pathophysiological role will be elucidated for this angulin isoform. Expression profiles in different tissues apart from the intestine will be analysed and may be connected to the functional findings. In addition, different conditions of the intestine will be of further interest as inflammatory or other pathological conditions affecting the intestinal epithelium might be linked to expression changes of this angulin isoform, which might contribute essentially to the resulting barrier impairment.
**Methods:** Standard and advanced molecular biological techniques, e.g. cloning, cell culture, transfections, CRISPR/Cas; Western blotting, real-time quantitative PCR, immunofluorescent staining; electrophysiological techniques to characterize the effects on epithelial barrier properties (Ussing chamber-based measurements of dilution potentials, two-path impedance spectroscopy (Krug et al., 2009b), flux measurements;. development of isoform-specific probes for RT-PCR, co-immunoprecipitation, Förster resonance energy transfer measurements
**Requirements:**
• Experience in molecular biological standard techniques.
• Skills in handling cell cultures and sterile working would be beneficial.
**For more information please contact:** susanne.m.krug@charite.de

**Project-related publications:**

HIGASHI, T. et al. 2013. Analysis of the 'angulin' proteins LSR, ILDR1 and ILDR2--tricellulin recruitment, epithelial barrier function and implication in deafness pathogenesis. *J Cell Sci,* 126**,** 966-77.
HU, J. E. et al. 2020. Leptin Downregulates Angulin-1 in Active Crohn's Disease via STAT3. *Int J Mol Sci,* 21.
IKENOUCHI, J., et al. 2005. Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. *J Cell Biol,* 171**,** 939-45.
KRUG, S. M et al. 2009a. Tricellulin forms a barrier to macromolecules in tricellular tight junctions without affecting ion permeability. *Mol Biol Cell,* 20**,** 3713-24.
KRUG, S. M., et al. 2018. Tricellulin is regulated via interleukin-13-receptor α2, affects macromolecule uptake, and is decreased in ulcerative colitis. *Mucosal Immunol,* 11**,** 345-356.
KRUG, S. M., et al. 2009b. Two-path impedance spectroscopy for measuring paracellular and transcellular epithelial resistance. *Biophysical journal,* 97**,** 2202-2211.
KRUG, S. M., et al. 2014. Tight junction, selective permeability, and related diseases. *Semin Cell Dev Biol,* 36**,** 166-76.
MARTINI, E. et al. 2017. Mend Your Fences: The Epithelial Barrier and its Relationship With Mucosal Immunity in Inflammatory Bowel Disease. *Cell Mol Gastroenterol Hepatol,* 4**,** 33-46.

**Project C3: *Candida albicans* induces epithelial polarity changes contributing to celiac disease development (Michael Schumann, Britta Siegmund)**

**Background:**

Celiac disease (CeD) is a small intestinal malabsorptive enteropathy triggered by the ingestion of the wheat protein gluten. Except for its peptide trigger i.e., gluten/gliadin, and a set of genes having been linked to the pathogenesis of CeD, further etiologic factors are lacking. This becomes obvious as many CeD patients develop their disease only later in life (age 35 to 50). We followed the hypothesis that alterations in the small intestinal microbiome are an additional factor and identified *Candida albicans* as a potential disease trigger. Nevertheless, the mechanism, by which *C. albicans* contributes to disease development is unclear.

**Hypotheses:**

(iii) CeD patients have an altered fungal microbiome in the duodenum.

(iv) *C. albicans* interacts with duodenal epithelial cells to increase mucosal-to-submucosal gliadin translocation.

**Aims:** (i) Characterizing the duodenal microbiome of CeD patients. (ii) Understanding the mechanisms of *C. albicans*-triggered gliadin translocation.

**Thesis project:** Mechanisms of *Candida albicans*-triggered gliadin uptake.

**Methods:**

Human duodenal organoids of CeD patients and control individuals are generated in such a way that mucosa-associated T-cells, B-cells and monocytes are preserved. In the presence and absence of *C. albicans* or adherence-defective *C. albicans* mutants gliadin peptides are exposed to these organoids and examined for (i) gliadin translocation, (ii) alterations of epithelial polarity and (iii) epithelial expressional changes using methodology that enables spatial resolution of RNA expression.

**Requirements:** Human duodenal organoids, spatial transcriptomics.

**For more information please contact:** michael.schumann@charite.de

**Project-related publications:**

Branchi F, Wiese JJ, Heldt C, Manna S, Dony V, Loddenkemper C, Bojarski C, Siegmund B, Schneider T, Daum S, Hummel M, Moos V, Schumann M. The combination of clinical parameters and immunophenotyping of intraepithelial lymphocytes allows to assess disease severity in refractory celiac disease. Dig Liver Dis. 2022 Dec; 54(12): 1649-1656. doi: 10.1016/j.dld.2022.06.024.

Delbue D, Lebenheim L, Cardoso-Silva D, Dony V, Krug SM, Richter JF, Manna S, Muñoz M, Wolk K, Heldt C, Heimesaat MM, Sabat R, Siegmund B, Schumann M. Reprogramming Intestinal Epithelial Cell Polarity by Interleukin-22. Front Med (Lausanne). 2021 Apr 12; 8: 656047. doi: 10.3389/fmed.2021.656047.

Schuppan D, …, Schumann M, …, CEC-3 Trial Group. A Randomized Trial of a Transglutaminase 2 Inhibitor for Celiac Disease. N Engl J Med. 2021 Jul 1; 385(1): 35-45. doi: 10.1056/NEJMoa2032441.

Delbue D, Cardoso-Silva D, Branchi F, Itzlinger A, Letizia M, Siegmund B, Schumann M. Celiac Disease Monocytes Induce a Barrier Defect in Intestinal Epithelial Cells. Int J Mol Sci. 2019 Nov 9; 20(22): 5597. doi: 10.3390/ijms20225597.

**Project C4: The role of glucagon-like peptide-2 for macromolecule uptake and immune activation in HIV enteropathy (Nina Hering, Hans-Jörg Epple)**

**Background:** HIV-associated enteropathy is structurally characterized by villus atrophy and surface loss of the small intestinal mucosa. Our previous data indicate an increased macromolecule translocation across the gut mucosa of HIV-infected patients causing elevated serum levels of microbial components. Whereas the latter is currently considered a key mechanism of HIV immunopathogenesis, the mechanisms of HIV enteropathy and the related pathological macromolecular uptake are poorly defined. In a previous study, we demonstrated increased mucosal production of inflammatory cytokines in HIV-infected patients. It seems plausible, that these could affect the expression, regulation and/ or activity of growth factors. Glucagon-like-peptide-2 (GLP2) is an intestinotrophic peptide hormone that is mainly secreted in the ileum. It enhances crypt cell proliferation, improves intestinal expansion and nutrient absorption. In HIV enteropathy, the cellular turnover is very low resulting in epithelial hypoproliferation and dysmaturation. This suggests that GLP2 therapy might be beneficial to improve HIV enteropathy and barrier dysfunction.

**Hypotheses:** We hypothesize that GLP2 can reverse intestinal atrophy and surface loss in HIV enteropathy. An improved structure and function of the epithelium subsequently reduces macromolecule translocation and enhances the uptake of nutrients in the ileum.

**Aims:** This project aims to elucidate the role of GLP-2 in HIV enteropathy and its consequences for macromolecular uptake and immune activation. The following questions are going to be answered: (i) Does GLP2 affect intestinal barrier function and the transport of macromolecules (FD4000, HRP, flagellin, LPS)? If so, then what are the underlying mechanisms? (iii) What is the impact of HIV cytokines on the mucosal expression of GLP2 and its receptor? Does GLP2 affect cytokine expression of monocytes?

**Thesis project:** The candidate will study the putative impact of GLP2 on growth, morphology, barrier function and transport processes or cytokine expression in HIV enteropathy using patients derived 2D and 3D organoid cultures and an in vitro atrophy model (“Impact of GLP2 on growth, morphology, barrier function, transport processes and cytokine expression in HIV enteropathy”).

**Methods:** Methods employed will be: (i) generating/ culturing of 3D and 2D organoids and performing co-culture experiments with cell lines or mucosal immune cells, (ii) immunohistochemistry, light/ immunofluorescence and confocal microscopy, (iii) electrophysiological and physiological analysis of epithelial macromolecular transport and barrier function, (iv) quantification of epithelial protein expression and apoptosis by standard assays, (v) subset analysis of mucosal immune cells and their impact on modulation of GLP-2 expression by HIV-cytokines

**Requirements:** Experiences in working with cell cultures and organoids is a plus.

**For more information please contact:** nina.hering@charite.de

**Project-related publications:**

Blumenstein, I. (2019). "GLP-2 Analogues as First Specific Treatment of Intestinal Failure." Visc Med 35(5): 320-323.

Brenchley, J. M. et al. (2006). "HIV disease: fallout from a mucosal catastrophe?" Nat Immunol 7(3): 235-239.

Croteau, J. D. et al. (2017). "Marked Enteropathy in an Accelerated Macaque Model of AIDS." Am J Pathol 187(3): 589-604.

Dandekar, S. (2007). "Pathogenesis of HIV in the gastrointestinal tract." Curr HIV/AIDS Rep 4(1): 10-15.

Epple, H.-J. et al. (2009) Impairment of the intestinal barrier is evident in untreated but absent in suppressively treated HIV-infected patients. Gut 58: 202-207

Epple, H. J. and M. Zeitz (2012). "HIV infection and the intestinal mucosal barrier." Ann N Y Acad Sci 1258: 19-24.