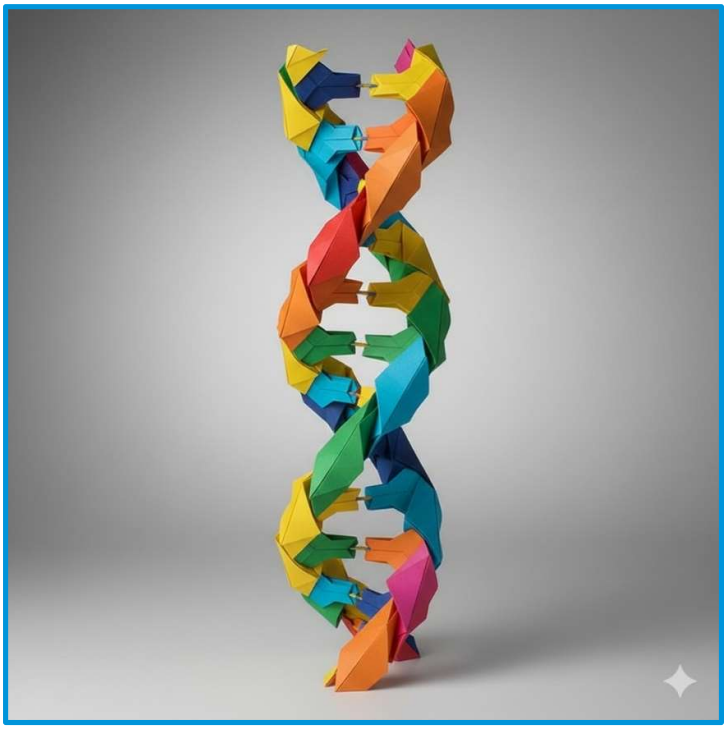
MEDISCH-GENETISCH CENTRUM ZUID-WEST NEDERLAND

33rd MGC Symposium

September 30, 2025 @ KINO Rotterdam



Program and abstract book

Erasmus MC University Medical Center Rotterdam

LUMC Leids Universitair Medisch Centrum

Universiteit Leiden The Netherlands

Coordination and editing:

Marike van Geest	m.vangeest@erasmusmc.nl	☎ 010-7043169
Thamar van Dijk	t.vandijk@erasmusmc.nl	☎ 010-7043937

Program 33rd MGC SYMPOSIUM

Tuesday 30 September, 2025 – KINO Rotterdam

8:30 Coffee and Registration

9:05 Opening: Joost Gribnau

Chairman: Joost Gribnau/ Sjaak Neefjes

9:10 **Eva Niggl** (Clinical Genetics, EMC)
Developing targeted therapy for TSC-Associated Epilepsy: From metabolic intervention to Antisense Oligonucleotides

9:30 **Fu Wei** (Anatomy and Embryology, LUMC)
Immune Cell Dynamics in Human Adult Ovary: Insights from Single-Cell Spatial Omics Analysis

9:50 **Ashutosh Choudhury** (Molecular Genetics, EMC)
Replication Stress-Induced Chromatin Loops Protect Forks and Maintain Genome Stability

10:10 **Jurjun van der Velde** (LACDR, UL)
Splicing factor SRSF7 as a critical regulator of breast cancer metastasis

10:30 Coffee/tea

Chairman: Ype Elgersma / Bob van de Water

11:00 **Marien van der Stel** (Developmental Biology, EMC)
Studying Diamond Blackfan Anemia Syndrome in iPSCs: genetic correction of RPS26 haploinsufficiency

11:20 **Pedro Sant'Anna Barbosa Ferreira** (Molecular Epidemiology, LUMC)
The Absence of Genetic Risk for Cardiovascular Diseases in Exceptional Survival (Longevity)

11:40 **Selçuk Yavuz** (OIC, EMC)
Chromatin organization by cohesin regulates compartmentalization and transcriptional activity of androgen receptors

12:00 **Mengyuan Li** (Anatomy and Embryology, LUMC) ***winner best talk MGC Workshop 2025***
Engineering Cas12 with an unbiased domain insertion library screening

12:20 **Josephine van Asbeck** (Clinical Genetics, LUMC)
The small vessel disease phenotype associated with monoallelic *NOTCH3* loss-of-function variants

12:40 Lunch

Chairman: Niels Geijsen

13:50 **Sam Leonard** (Epidemiology and Internal Medicine, EMC)
Immune Signatures of Smoking: Cytokine and Immunoglobulin Dysregulation and Partial Reversibility in a Population-Based Study

14:10 **Rosan Kuin** (Cell and Chemical Biology, LUMC)
Determinants of high fidelity DNA synthesis in the world's most deadliest pathogen *Mycobacterium tuberculosis*

14:30 **Héctor Tejeda** (Developmental Biology, EMC)
Vitamin K-Dependent Matrix Gla Protein Orchestrates Stromal Reprogramming to Fibrosis in Myeloproliferative Neoplasms

14:50 **Venda Mangkusaputra** (Human Genetics, LUMC)
Profiling BRCA1-BRCT interactions and their functional relevance at amino acid-resolution

15:10 **David Häckes** (Molecular Genetics, EMC)
Persistent and futile DNA repair can lead to severe Cockayne syndrome phenotypes

15:30 Coffee/tea

Chairman: Joost Gribnau

16:00 ***MGC Symposium Lecture***

Ana Pombo (Johns Hopkins University, Baltimore / Max Delbrück Centrum für Molekulare Medizin, Berlin)

Specialization of 3D genome structure in different cell types and stimulus responses

17:00 Drinks

17:30 Dinner buffet @ NIKO's Resto

Abstracts 33rd MGC Symposium

Eva Niggl

Developing targeted therapy for TSC-Associated Epilepsy: From metabolic intervention to Antisense Oligonucleotides (ASOs)

Tuberous sclerosis complex (TSC) is a neurodevelopmental disorder characterized by treatment-resistant epilepsy, caused by loss-of-function mutations in TSC1 or TSC2. These genes encode hamartin and tuberin, negative regulators of RAS homolog enriched in brain (RHEB), an upstream activator of the mammalian target of rapamycin complex 1 (mTORC1). mTORC1 controls diverse cellular functions, and we previously showed that either pharmacological inhibition with rapamycin or genetic deletion of Rheb in a Tsc1-deficient mouse model prevents both epilepsy and sudden unexpected death in epilepsy (SUDEP).

In this study, we tested antisense oligonucleotides (ASOs) targeting Rheb and interventions addressing mTORC1-associated metabolic dysregulation as potential therapeutic strategies for TSC. ASO treatment reduced Rheb expression and mTORC1 signaling, delaying seizure onset and extending survival, although a single administration did not completely prevent seizures or SUDEP. By contrast, metabolic interventions such as lowering glycine or increasing glutamine selectively reduced seizure frequency but had no effect on seizure onset or survival.

These findings highlight the promise of mechanism-based interventions for TSC and provide proof-of-concept that targeting downstream effectors in haploinsufficiency disorders can expand the therapeutic potential for rare genetic diseases.

Eva Niggl 1,2†, Claudia Milazzo 1,2†, Ilse Wallaard 1,2, Nils Meier 3,4, Edwin Mientjes 1,2, Fried Zwartkruis 3,4, Ype Elgersma 1,2*

1 Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands.
2 Erasmus MC Center of Expertise for Neurodevelopmental Disorders (ENCORE), Rotterdam, The Netherlands
3 Department of Genetics, UMC Utrecht, Utrecht
4 dLAB and Center for Molecular Medicine, UMC Utrecht, Utrecht
* Corresponding author: Y.elgersma@erasmusmc.nl
† These authors contributed equally to this work

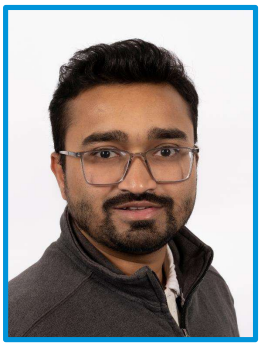
Fu Wei

I am a PhD candidate at the Department of Anatomy and Embryology at Leiden University Medical Center, working under the supervision of Prof. Susana Chuva de Sousa Lopes. My research focuses on ovarian follicle development and fertility preservation.

Immune Cell Dynamics in Human Adult Ovary: Insights from Single-Cell Spatial Omics Analysis

The adult human ovary is a dynamically remodeling organ, with its homeostasis maintained by interactions between ovarian and immune cells, though their dynamic changes during remodeling remain unclear. This study integrates single-cell spatial transcriptomics (Xenium In Situ) and Imaging Mass Cytometry (IMC) to map, for the first time, a single-cell resolution spatial atlas of ovarian cells at both transcriptomic and proteomic levels. Additionally, we chart the distribution dynamics of immune cells within the ovarian microenvironment, revealing their spatiotemporal roles in follicular atresia and tissue repair. Our findings show that macrophages, the predominant immune subtype in the ovary, exhibit remarkable functional plasticity, playing pivotal roles in both early and late remodeling stages. In early follicular atresia, macrophages drive inflammation and cell clearance; in later stages, they contribute alongside lymphocytes to promote tissue repair and vascular remodeling. Lymphocytes, including memory $CD4^+$ T cells, effector $CD8^+$ T cells, and NK cells, primarily contribute to tissue repair during the later phases of remodeling. This study provides a framework for understanding ovarian immune dynamics and showcases the potential of spatial omics technologies in deciphering complex ovarian ecosystems, offering new perspectives for future ovarian research.

Ashutosh Choudhury



Replication Stress-Induced Chromatin Loops Protect Forks and Maintain Genome Stability

The mammalian genome is intricately organized within chromatin, with distinct roles for heterochromatin and euchromatin regions in cellular processes. Despite its gene silencing role, the accumulation of heterochromatin in cancer cells suggests additional functions beyond transcriptomic changes. Recently, we demonstrated that cells experiencing replication stress accumulate de novo heterochromatin marks at stressed replication forks, leading to transient compaction crucial for replication fork stability*. However, the broader impact of this compaction on genome organization remains unclear.

High-throughput chromosome conformation capture (Hi-C) techniques have extensively explored genome organization globally. Yet, studying the organization of newly replicated DNA, particularly during replication stress, presents challenges. To address this, we developed Rep-Hi-C, enabling investigation of 3D organization of newly replicated DNA and capturing replication stress-induced genomic interactions. We find replication stress triggers local and spatial chromatin reorganization, enclosing stressed replicating regions within distinct chromatin loops. Using ForkDeg-Seq, a method we developed for genome-wide mapping of fork degradation sites, we show that these chromatin loops act as protective structures, safeguarding stressed replicated regions from degradation and maintaining genome stability. This study provides high-resolution insights into the three-dimensional spatial organization of replication forks under replication stress, emphasizing their critical role in preserving genome stability.

* Gaggioli V, et al. Dynamic de novo heterochromatin assembly and disassembly at replication forks ensure fork stability. (Nature Cell Biology 2023, DOI: 10.1038/s41556-023-01167-z)

Vincent Gaggioli 1,2†, Kaustav Sengupta 1,2†, Ashutosh Choudhury 1,2, Collin Bakker 1,2, Calvin S. Y. Lo 1,2, Alex Whale 7, Jeroen van den Berg 2,3, Sangavi Eswaran 4, Hina N. Khan 1, Estel Ferré 5, Gregorie Stik 5, Argyris Papantonis 6, Jon Houseley 7, Aleix Bayona-Feliu 8, Sriram Sridharan 9, Nitika Taneja 1,2#

1 Department of Molecular Genetics, Erasmus University Medical Center, Erasmus MC Cancer Institute; Dr. Molewaterplein 40, 3015GD Rotterdam, The Netherlands
2 Oncode Institute The Netherlands
3 Hubrecht Institute-KNAW & University Medical Center Utrecht, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands,
4 Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, India
5 Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain
6 Institute of Pathology, University Medical Center Göttingen, Göttingen, Germany
7 Epigenetics Programme, Babraham Institute, Cambridge, United Kingdom
8 Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain
9 Cancer Science Institute of Singapore, National University of Singapore, Singapore
Correspondence : n.Taneja@erasmusmc.nl

Jurjun van der Velde

Splicing factor SRSF7 as a critical regulator of breast cancer metastasis

Alternative splicing is increasingly recognized as a contributor to breast cancer progression, but the role of the spliceosome in triple-negative breast cancer (TNBC) cell motility is not well understood. We conducted an RNAi screen of spliceosome components in two TNBC cell lines, which identified a subset of factors influencing migration with limited impact on proliferation. Among these, the splicing regulator SRSF7 showed consistent effects across models. RNA sequencing revealed that SRSF7 modulates both gene expression and alternative splicing in pathways related to extracellular matrix organization and adhesion. Functional assays confirmed impaired migration upon SRSF7 depletion in vitro. In vivo, we used a tail-vein xenograft metastasis model, where inducible SRSF7 knockout delayed metastatic colonization. Clinical data further associated high SRSF7 expression with reduced metastasis-free survival. These findings support a role for SRSF7 in regulating metastatic behaviour in TNBC and provide a framework for further exploring splicing factors in cancer progression.

Jurjun J.S. van der Velde 1, Nasi Liu 1, Esmee Koedoot 1, Marcel Smid 2, Peter Stoilov 3, John W.M. Martens 2, Sylvia E. Le Dévédec 1, Bob van de Water 1

1 Division of Cell Systems and Drug Safety, LACDR, Leiden University, the Netherlands
2 Department of Medical Oncology and Cancer Genomics Netherlands, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, the Netherlands
3 Department of Biochemistry and Cancer Institute, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, USA

Marien van der Stel

RPS26-deficient patient derived iPSCs exhibit impaired processing of ribosomal RNA and upregulate ribosomal quality control proteins

Introduction: Diamond-Blackfan Anemia Syndrome (DBAS) is a rare and clinically heterogeneous disorder, most often caused by mutations in ribosomal protein genes. Affected individuals typically present with anemia, congenital malformations, growth retardation, and an increased predisposition to cancer. Mutations in RPS26 are the third most common genetic cause of DBAS, but the underlying pathogenic mechanisms remain poorly understood. To address this, patient-derived induced pluripotent stem cell (iPSC) models of RPS26-DBAS were established and used to study iPSC-derived hematopoiesis. These models provide insight into disease mechanisms and may support the development of iPSC-based therapies. It is hypothesized that RPS26 mutations disrupt ribosome biogenesis and hematopoietic differentiation through aberrant regulation of ribosomal RNA processing and associated protein factors.

Methods: iPSC lines were generated from a female patient with RPS26-DBAS (c.95-98dup, p.Asp33Glufs*6), enrolled through the Dutch DBAS registry. Erythroblasts isolated from peripheral blood mononuclear cells were reprogrammed using the CytoTune-Sendai-iPS2.0 system. The patient-specific RPS26 mutation was corrected by CRISPR/Cas9-mediated homology-directed repair. Healthy control, mutant, and corrected iPSC lines were compared during differentiation into embryoid bodies (EBs), hematopoietic organoids, and hematopoietic progenitors. All lines were characterized by whole genome sequencing, RNA sequencing, proteomics, western blotting, and pre-ribosomal RNA northern blotting.

Results: Whole genome sequencing confirmed successful correction of the RPS26 mutation. Mutant lines exhibited EB disaggregation and failed to support hematopoietic organoid development, whereas corrected lines displayed restored EB integrity and hematopoietic organoid development. Pre-ribosomal RNA northern blotting revealed accumulation of 26S and 18S-E pre-rRNA in mutant lines, consistent with RPS26 haploinsufficiency; these defects were fully rescued in corrected lines to healthy control levels. Proteomic analysis demonstrated increased abundance of ribosomal processing factors PNO1, NOB1, LTV1, and RIOK2 in mutant lines, despite no corresponding upregulation of their transcripts by RNA-sequencing.

Conclusion: RPS26-mutant iPSCs display molecular and developmental defects characteristic of the RPS26-DBAS subtype, including impaired EB and hematopoietic organoid formation and aberrant pre-ribosomal RNA processing. These defects were reversed upon genomic correction, validating iPSCs as a robust model for studying RPS26-associated ribosomopathy. Furthermore, proteomic analysis suggests that aberrant expression of ribosomal processing proteins, such as PNO1, may contribute to disease pathogenesis. Ongoing studies aim to clarify the role of these factors in RPS26-DBAS and further define the molecular mechanisms driving this disorder.

Marien van der Stel 1,2, Teun Slijkerman 1,3, Marie-Françoise O'Donohue 4, Pierre-Emmanuel Gleizes 4, Marieke von Lindern 1, Sjaak Philipsen 2, Chantal Clark 1,3, Marije Bartels 3, Emile van den Akker 1

1 Sanquin Research Amsterdam and Landsteiner Laboratory, Amsterdam, The Netherlands.
2 Erasmus MC, Department of Cell Biology, Rotterdam, the Netherlands.
3 University Medical Centre Utrecht, Utrecht University, Centre for Benign Hematology, Thrombosis and Hemostasis - Van Creveldkliniek, Utrecht, the Netherlands.
4 University of Toulouse, Centre de Biologie Intégrative (CBI), Molecular, Cellular and Developmental biology department (MCD), Toulouse, France.

Pedro Sant'Anna Barbosa Ferreira

The Absence of Genetic Risk for Cardiovascular Diseases in Exceptional Survival (Longevity)

Aging is the major risk factor for chronic diseases. Unlike the general population, members of long-lived families maintain exceptional health as they age. Healthy survival to extreme ages (longevity) clusters within families. However, research has not yet elucidated the underlying mechanisms of longevity. There are two important reasons for this: 1) the group with the highest heritability is often not studied and 2) the hypothesis-generating nature of most genetic studies requires larger study cohorts. In our previous work, we showed that members of the longest-lived families have a 10-year delayed onset of their first chronic diseases. We therefore hypothesize that the absence of genetic predisposition to chronic diseases is one of the key-mechanisms involved in longevity delayed disease onset. We investigated this hypothesis in the Leiden Longevity Study, a cohort with data from more than 400 long-lived families in 3-generations. To analyse our data, we constructed a set of PRSs covering the top 10 causes of death in the Netherlands. We observed that descendants of long-lived families have lower genetic risk for cardiovascular disease (CVD). Using accelerated failure time modelling, we further showed that around 20% of the delayed cardiovascular disease incidence in long-lived families is explained by CVD common genetic variants. We conducted gene-annotation enrichment analysis of the SNPs in the CVD PRS using DAVID and observed seven significantly enriched clusters. Finally, we constructed a novel cholesterol PRS based on the cholesterol metabolism cluster which significantly predicted time to all-cause mortality in a 90+ study population, covering 19 years of follow-up. Our study indicates that common variants related to cardiovascular diseases and cholesterol metabolism contribute to healthy aging. Furthermore, we demonstrate that investigating SNPs, identified in well-powered Genome Wide Association studies, associated with longevity-related endophenotypes can provide insight into the genetic architecture of the longevity phenotype itself.

Selçuk Yavuz

Chromatin organization by cohesin regulates compartmentalization and transcriptional activity of androgen receptors

The androgen receptor (AR) is a transcription factor that upon binding to testosterone regulates genes involved in the development and maintenance of the male phenotype. It binds to enhancers and promotor regions of those genes and recruits other transcription (co)factors and chromatin remodelling complexes, leading to the formation of nuclear foci which can be visualised by confocal microscopy. We have recently shown that these foci consist of a core of immobile macromolecular complexes on DNA surrounded by diffusive, phase-separated ARs and likely other transcription (co)factors (Yavuz et al., NAR 2023; Yavuz et al., Cells, 2024).
Recent findings also indicate a role for the cohesin chromatin structuring complex in AR-regulated gene transcription and foci formation, but its precise role remains elusive. To investigate this, we generated HCT116 cell lines that both express AR-HaloTag as well as one of the cohesion subunits RAD21, STAG1 or STAG2 tagged with an auxin-inducible degron. Quantitative high-resolution image analysis of AR condensates showed that the morphological characteristics of AR foci in the absence of cohesion subunits were significantly different in both size and fluorescence intensity, of which the STAG2 and RAD21 deficient cells showed the most prominent effect. Moreover, FRAP and single molecule tracking revealed that AR proteins are less stably bound on the DNA, showing that cohesin plays an important role in AR-regulated gene transcription by enhancing compartmentalisation and stabilizing AR-DNA interactions. In addition, AR ChIP-seq revealed a strong differential binding of ARs accompanied by differential expression of AR-target genes in absence of cohesin subunits, indicating the relevance of cohesin-mediated chromatin remodelling during AR-regulated gene transcription.

Selçuk Yavuz 1†, Emmanouela Epeslidou 2†, Wiggert A. van Cappellen 3, Tsion E. Abraham 3, Johan A. Slotman 3, Bart Geverts 3, Andrea Sacchetti 1, Ihor Smal 5, Kerstin Wendt 4, Martin E. van Royen 1, Stefan Prekovic 2 and Adriaan B. Houtsmuller 1,3*

1 Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands
2 Center for Molecular Medicine, UMC Utrecht, Utrecht, The Netherlands
3 Optical Imaging Center, Erasmus University Medical Center, Rotterdam, The Netherlands
4 Department of Cell biology, Erasmus University Medical Center, Rotterdam, The Netherlands
5 Department of Biology, Cell Biology, Neurobiology and Biophysics, Utrecht University, Utrecht, The Netherlands
† These author contributed equally to this work
* Corresponding author

Mengyuan Li

Engineering Cas12 with an unbiased domain insertion library screening

Fusing functional domains to host proteins is a powerful strategy in protein engineering for introducing novel activities. While conventional terminal fusions are widely applied, they can be restrictive when terminal regions are structurally or functionally essential, and they often fail to capture synergistic interactions between the fused domains and the recipient protein. Internal domain insertion offers a promising alternative with the potential for tighter structural and functional integration, yet the systematic identification of insertion-tolerant sites has remained a major challenge.

Here, we employed an established transposition-based, unbiased screening strategy to map insertion-tolerant positions across CRISPR-Cas12a, an RNA-guided DNA endonuclease. This comprehensive map highlights multiple internal regions that can accommodate foreign domains without disrupting recipient protein. These identified sites provide a valuable framework for engineering modular Cas12a variants with enhanced or novel functions.

As a proof of concept, we are inserting the SpyCatcher peptide into permissive positions of dCas12a to generate a docking platform capable of covalently recruiting SpyTag-fused effector proteins. Building upon this platform, we aim to engineer Cas12a variants with functional domains to create programmable editors with enhanced versatility and potential applications.

Josephine van Asbeck

The small vessel disease phenotype associated with monoallelic *NOTCH3* loss-of-function variants

Background and objectives

Monoallelic cysteine-altering *NOTCH3* (*NOTCH3^{cys}*) variants cause the adult-onset small vessel disease CADASIL, and biallelic *NOTCH3* loss-of-function (*NOTCH3^{lof}*) variants cause a rare, childhood-onset small vessel disease. Whether monoallelic *NOTCH3^{lof}* variants also cause a small vessel disease is subject of debate. This study aimed to delineate the small vessel disease phenotype of individuals with a monoallelic *NOTCH3^{lof}* variant, and to compare this to CADASIL.

Methods

In this observational study, monoallelic *NOTCH3^{lof}* cases were ascertained in gnomAD, UK Biobank, six clinical centres from Europe, Asia and the US, and literature. In gnomAD, *NOTCH3^{lof}* allele frequency was determined. In UK Biobank, normalized white matter hyperintensity volume (nWMHv), peak width of skeletonized mean diffusivity (PSMD), lacune count and stroke were compared between *NOTCH3^{lof}* cases, *NOTCH3^{cys}* cases and controls. In clinical *NOTCH3^{lof}* cases, white matter hyperintensities, lacune count and stroke incidence were assessed, and skin vessel wall pathology was analysed using immunohistochemistry and electron microscopy.

Results

In gnomAD, 306 *NOTCH3^{lof}* variants were identified (allele frequency 0.6/1000). In UK Biobank, 102 *NOTCH3^{lof}* cases were ascertained (median age 58 years, range 40-69, 55% female). *NOTCH3^{lof}* cases had an increased nWMHv (D0.44 mm^3, $p<0.001$) and PSMD (D0.19*10^{-4}, $p=0.017$) compared to controls. nWMHv and PSMD in *NOTCH3^{lof}* cases were comparable to *NOTCH3^{cys}* cases, but, in contrast to *NOTCH3^{cys}* cases, *NOTCH3^{lof}* cases did not have an increased stroke risk compared to controls. Clinically ascertained *NOTCH3^{lof}* cases (n=69, median age 50 years, range 20-94, 54% female) often had white matter hyperintensities (28/32, 88%), while lacunes (12/32, 38%) and stroke (11/69, 15%) were predominantly seen in cases with cardiovascular risk factors and at advanced age. Skin vessels of *NOTCH3^{lof}* cases more frequently showed abundant vessel wall collagen deposition compared to *NOTCH3^{cys}* cases and controls (37% vs 10% ($p=0.016$) and 5% ($p<0.001$) of vessels).

Discussion

We conclude that monoallelic *NOTCH3^{lof}* variants cause a small vessel disease that a) remains subclinical in most cases, but may be exacerbated by cardiovascular risk factors and aging, and b) is distinct from CADASIL in terms of vessel pathology and disease severity. These findings will guide counseling and management of individuals in whom a *NOTCH3^{lof}* variant is found.

Authors

Josephine Stephanie van Asbeck 1, Gido Gravesteijn 1, Minne Nicolas Cerfontaine 1, Jeroen P. Vreijling 1, Aat Mulder 2, Roman I Koning 2, Mark C. Kruit 3, Anna Bersano 4, Keith W. Muir 5, Sam J. Neilson 5, James F. Meschia 6, Md Manjurul Islam Shourav 6, Andreas Koupparis 7, Yi-Chung Lee 8,,9,10, Nicola Rifino 4, Remco J. Hack 1, Esther Nibbeling 1, Saskia A.J. Lesnik Oberstein 1, Julie Rutten 1

1 Department of Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands; 2 Electron Microscopy Facility, Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, the Netherlands; 3 Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands; 4 Cerebrovascular Unit, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Via Celoria 11, 20133, Milan, Italy; 5 Institute of Neuroscience and Psychology, University of Glasgow, Queen Elizabeth University Hospital, Glasgow, UK; 6 Department of Neurology, Mayo Clinic, Jacksonville, FL, USA; 7 The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; 8 Department of Neurology, Taipei Veterans General Hospital, Taiwan; 9 Department of Neurology and Brain Research Center, National Yang Ming Chiao Tung University, Taipei, Taiwan; 10 Center for Intelligent Drug Systems and Smart Bio-devices (IDS2B), National Yang Ming Chiao Tung University, Hsinchu, Taiwan

Sam Leonard

Immune Signatures of Smoking: Cytokine and Immunoglobulin Dysregulation and Partial Reversibility in a Population-Based Study

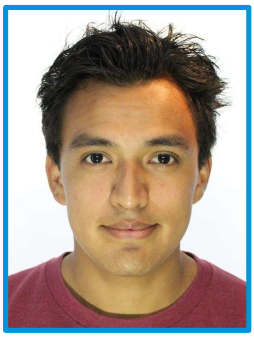
While smoking-induced epigenetic changes are increasingly recognized, the relationship between varying smoking history and immune protein profiles remains incompletely understood. This study investigates associations between smoking history and circulating inflammatory cytokines and serum immunoglobulins, and explores DNA methylation as a potential mediator. We analyzed 2,119 smoking participants from the Rotterdam Study, examining associations of smoking duration, pack-years, and cessation time with plasma inflammatory proteins (Olink Inflammation Panel) and serum immunoglobulins. Nine cytokines (six pro- and three anti-inflammatory) were selected based on prior evidence. Multivariable (non)linear regression models were applied, adjusted for age, sex, BMI, alcohol use, and socioeconomic status. A distinct immune signature emerged, with (z-transformed) IL-6, IL-18, IL-17C, and IL-10 levels increasing with pack-years and smoking duration (β range: 0.0029–0.015). IgA and IgG displayed reverse J-shaped associations with pack-years (β range: –0.050 to 0.048) and levels declined with smoking duration (β = –0.0067 and –0.028, respectively). IL-6 decreased with cessation time (β = –0.0064), and IgG increased (β = 0.018). TNF exhibited a U-shaped association, and IFN-γ declined until 20 years post-cessation (β = –0.010). IgM responses differed by sex. Mediation analysis using PCA-derived CpG components did not support a role for DNA methylation in these associations. These findings reveal dose-dependent, pro-inflammatory immune dysregulation with cumulative smoking exposure and partial reversibility of IL-6 and IgG following cessation. The absence of mediation by DNA methylation suggests other biological pathways are involved. These findings support the use of cytokines and immunoglobulins as potential biomarkers for immune surveillance and recovery monitoring.

Rosan Kuin

Determinants of high fidelity DNA synthesis in the world's most deadliest pathogen *Mycobacterium tuberculosis*

Drug resistance in Mycobacterium tuberculosis presents a major challenge in tuberculosis treatment, highlighting the need to understand the underlying mechanisms. DNA replication plays an important role in the acquisition of drug resistance and the expression of the DNA polymerase DnaE2 during adverse conditions has been associated with increased mutation rates. Here we investigate the functional differences between the high fidelity replicative DNA polymerase DnaE1 and the predicted error-prone DNA polymerase DnaE2, focusing on which amino acid changes affect polymerase fidelity. For this we identify potential fidelity-altering positions using a two-entropy sequence analysis combined with experimental validation to test whether changes of these positions affect the mutation rates. We find that a double mutation in the palm domain of DnaE1: D431S/R432D, increases mutation frequencies both in vivo and in vitro. The location of these two residues adjacent to the DNA backbone of the template strand suggests that the amino acid change results in a loser grip on the DNA, allowing for the incorporation of incorrect nucleotides. These insights improve our understanding of the mechanisms underlying drug resistance in M. tuberculosis and could help in the development of future strategies to combat it.

Héctor Tejeda



Vitamin K-Dependent Matrix Gla Protein Orchestrates Stromal Reprogramming to Fibrosis in Myeloproliferative Neoplasms

A subset of patients with myeloproliferative neoplasms (MPNs) develop myelofibrosis (MF), a fatal condition driven in part by excessive extracellular matrix deposition by stromal cells, yet the initiating events remain unclear. Although stromal cells are central drivers of fibrosis, the early events initiating their activation remain poorly defined. To investigate these initiating events, we performed scRNAseq of non-hematopoietic stromal cells across four patient-relevant MPN mouse models (transplantation of CALRdel52-, JAK2V617F-expressing cells, and TPO-overexpressing cells at early and late timepoints. We identified eight bone marrow mesenchymal stromal cell (MSC) populations and stratified our models by fibrosis grade. Comparative analysis of early versus late stages of fibrosis revealed gene expression programs linked to disease progression and identified Matrix Gla Protein (MGP), a vitamin K–dependent factor, as a biomarker of fibrosis. MGP expression was validated in MPN patient samples. In mice, vitamin K supplementation reduced the MPN phenotype, specifically thrombocytosis, and bone marrow fibrosis. Mechanistically, vitamin K reversed TGFβ-driven MSC activation and suppressed pro-inflammatory NF-κB signalling in malignant hematopoietic stem/progenitor cells. Colony assays from patient-derived CD34+ hematopoietic stem and progenitor cells confirmed that vitamin K inhibits the MPN clone and synergizes with ruxolitinib as the standard of care treatment. Our findings establish MGP as a fibrosis biomarker and support vitamin K supplementation as a readily translatable strategy to attenuate fibrosis and disease progression in MPN.

Adam Benabid*, Hélène F.E. Gleitz*, Hector Tejeda Mora, Marcelo Szymanski de Toledo, Ellen Sistemich, Jakob Kwitniewski, Inge A. M. Snoeren, Stijn N. R. Fuchs, Martin Grasshoff, Niklas Lutterbach, Niclas Flosdorf, Asim Cengiz Abkulut, Leon Schurgers, Ivan Costa, Steffen Koschmieder, Martina Crysandt, Rebekka K. Schneider

Venda Mangkusaputra

Profiling BRCA1-BRCT interactions and their functional relevance at amino acid-resolution

BRCA1 plays a central role in homologous recombination (HR) through interactions with multiple proteins across its various domains. The C-terminal BRCT domains bind HR regulators such as ABRAXAS1, CtIP, and BRIP1, each contributing to distinct, sometimes opposing, functions. While pathogenic mutations frequently cluster within the canonical BRCT phospho-binding pocket, the broader mutational landscape and its functional consequences remain poorly understood. Here, we used a site-saturation mutagenesis library of the BRCT domains to test >4,000 single-residue variants for their ability to bind ABRAXAS1 and CtIP. Using a yeast two-hybrid screen, we systematically assessed these interactions and validated key findings in mammalian cells. The resulting interaction map identified previously uncharacterized residues critical for partner binding and demonstrated their detrimental impact on HR. Importantly, we identified separation-of-function mutations that selectively disrupt individual protein interactions, enabling a more detailed analysis of each partner's contribution to HR. Functional assays suggested that disruption of CtIP binding had the most pronounced impact on HR. Furthermore, integration of our data with clinical variant data revealed a strong correlation between loss of protein binding and pathogenicity, highlighting the potential utility of our interaction map for clinical variant interpretation.

Venda Mangkusaputra 1,2, Andrea G. Murachelli 2,3, Zhengzhou Yu 1, Annouche den Hollander 1, Roberta Menafra 4, Anne Schreuder 1,2,5, Susan L. Kloet 4, Titia K. Sixma 2,3, Sylvie M. Noordermeer 1,2*

1 Leiden University Medical Center (LUMC), Department of Human Genetics, Leiden, The Netherlands;
2 Oncode Institute, Utrecht, The Netherlands;
3 Netherlands Cancer Institute (NKI), Amsterdam, The Netherlands;
4 LUMC, Leiden Genome Technology Center;
5 Current address: Institute of Molecular Cancer Research, University of Zurich, Zurich, Switzerland;
* Corresponding author: s.m.noordermeer@lumc.nl

David Häckes

Persistent and futile DNA repair can lead to severe Cockayne syndrome phenotypes

Nucleotide Excision Repair (NER) removes a wide range of bulky, helix-distorting DNA lesions. Central to this pathway is the multi-subunit complex Transcription Factor II H (TFIIH), which functions in both NER and transcription initiation. During NER, TFIIH utilizes the translocase/helicase activities of its XPB and XPD subunits to unwind the DNA duplex around the lesion, creating an open bubble for damage verification and subsequent incision.

Defects in this pathway give rise to severe photosensitive disorders, including xeroderma pigmentosum (XP), characterized by extreme cancer predisposition, and Cockayne syndrome (CS), characterized by severe progeroid and neurodevelopmental features. While the cancer risk in XP results from mutation accumulation due to defective DNA repair, the basis for the distinct developmental and neurodegenerative phenotypes in CS remains poorly understood. Therefore, we determined why different mutations in the TFIIH complex lead to the separate clinical outcomes of CS and XP.

We have previously shown that persistently stalled NER intermediates, such as RNA Polymerase II or TFIIH, rather than unrepaired DNA lesions, can drive the CS pathogenesis by causing a prolonged block of transcription. Here, we demonstrate in human cells that a repair-deficient TFIIH mutation may cause the severe features of CS by repeatedly inducing futile incisions in the DNA. This mechanism is also observed in C. elegans, where it leads to severe neuronal defects, which, remarkably, can be alleviated by preventing the recruitment of mutant TFIIH. Together, our findings reveal how CS can arise through multiple distinct mechanisms that all lead to persistent transcription blockage and open new possibilities for therapeutic strategies.

David Häckes 1*, Arjan F. Theil 1, Qing Yu 1, Anja Raams 1, Karen L. Thijssen 1, Marteijn Jurgen 1, Jun-Hyuk Choi 2, Wim Vermeulen 1, Hannes Lans 1

*d.hackes@erasmusmc.nl
1 Erasmus MC Cancer Institute, Molecular Genetics as department, The Netherlands
2 University of Science & Technology, Department of Bio-Analytical Science, Republic of Korea

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MGC secretariat
Department of Developmental Biology, Erasmus MC, Rotterdam
Marike van Geest m.vangeest@erasmusmc.nl ☎ 010-7043169
Thamar van Dijk t.vandijk@erasmusmc.nl ☎ 010-7043937
medgencentre.com