Mendel...

Mendel Genetics Conference

Book of Abstracts

A tribute to Gregor Johann Mendel on the bicentennial of his birth

GREGOR JOHANN MENDEL

The humble genius was born on **20 July 1822** in Hynčice. Proved to be a talented child he attended the Piarist school in Lipník nad Bečvou, then enrolled at the grammar school in Opava. In 1840 Mendel applied to study at the Philosophical Institute in Olomouc and in 1843 entered the Augustinian Order at the monastery in Old Brno and took the monastic name of Gregor.

By joining the order, Gregor Mendel received new opportunities of education and research. His superior, Abbot Napp, sent him to study in Vienna, which was a key factor in his later experiments with the common pea and with other plants. Through mathematics and physics, he learnt to make statistical analyses, to plan experiments, and in general to apply the scientific method.

He made experiments for 9 years. The results of his research led to the formulation of three principles, which we now know as **Mendel's laws**. In 1865 he lectured on his findings, but he did not receive recognition until 1900, when his discoveries were confirmed and Gregor Mendel was dubbed the **"Father of Genetics"**.

In 1868 Mendel was elected head of the Augustinian abbey in Old Brno, which left him with little time for experiments. However, he did retain his two hobbies – beekeeping and meteorology. He had an apiary of his own design built in the monastery garden and he regularly made meteorological measurements in several parts of the monastery while keeping meticulous records.

Gregor Johann Mendel died on **6 January 1884**. He is buried in the Augustinian tomb in the Central Cemetery in Brno.

CYRIL NAPP Scholarship for researchers from Ukraine

In response to the war in Ukraine, the Organizing Committee of the conference decided to support Ukrainian researchers by announcing the scholarship. The registration fee has been waived for scholarship recipients. Cyril Napp was an Augustinian Abbot who recognized Mendel's scientific talent, supported him in his university studies, and created the conditions for his experiments on pea crossing.

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200th anniversary of the birth of Gregor Johann Mendel Celebrated in association with UNESCO

Mendel Genetics Conference

A tribute to Gregor Johann Mendel on the bicentennial of his birth

20–23 July, 2022

Conference Organisers:
Masaryk University
Mendel University in Brno
Moravian Museum
Augustinian Abbey in Brno
Společně, o.p.s.

MASARYK UNIVERSITY
Mendel University in Brno
MORAVIAN MUSEUM
OPATSTVÍ AUGUSTINŮ V BRNĚ
SPOLEČNĚ obecně prospěšná společnost

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Mendel Genetics Conference

A tribute to Gregor Johann Mendel on the bicentennial of his birth

20–23 July, 2022 Brno, Czech Republic

Augustinian Abbey in Brno & Hotel Passage **

CONTENT

Masaryk University
Masaryk University, founded in 1919, is the bearer of the democratic traditions and intellectual legacy of T. G. Masaryk, the first Czechoslovak president, and at the same time is a progressive modern European university. It has 10 faculties, over 300 departments, 30,000 students in almost 500 study programmes from bachelor's to doctoral and 3,300 teachers and researchers. Masaryk University deals with many fields of human knowledge, supports research and regularly invests in its growth. It is involved in European and non-European projects, thanks to which students can travel to all the corners of the globe. One of the priorities of MU is science and research – it has a modern cyber polygon, the most up-to-date unique simulation centre of the Faculty of Medicine, the CEITEC and RECETOX interdisciplinary research centres, the Teiresiás centre, which is dedicated to helping students with special needs and, last but not least, the award-winning MUNI HELPS volunteer centre.

Mendel University in Brno
Mendel University in Brno is a public institution with a long tradition of excellence in teaching and research that has driven new ways of thinking since 1919 and proudly bears the name of G. J. Mendel, the founder of modern genetics. Mendel University comprises five faculties: AgriSciences, Forestry and Wood Technology, Business and Economics, Horticulture and Regional Development and International Studies.

Moravian Museum
The Moravian Museum is the second-largest and second-oldest museum in the country. Besides its other activities, the Moravian Museum operates the Mendelianum, an interactive museum and center of G. J. Mendel founded on a strong historical basis and equipped with many modern components.

Augustinian Abbey in Brno
Gregor Johann Mendel was allowed by the Order of Saint Augustine to study and conduct experiments for many years. The Abbey has long cared for Mendel's legacy and thanks to its activities, science and faith meet under one roof.

Společně, o.p.s.
Společně, o.p.s. is a non-profit organization, one of its most important objectives is to promote G. J. Mendel and cooperate closely with the Augustinian Abbey and other Mendel-related institutions.

Welcome to Brno and the South Moravian Region
The South Moravian region ranks among the 50 most innovative regions of Europe. Thanks to its growing business in computer technology, telecommunications, software development, and other hi-tech sectors, the place became a Central European innovation hub therefore, it is an imagined technological center of the country.

Brno is the second-largest city in the Czech Republic. It was voted 23rd best place to live in Europe – Brno is a city of the 21st century with all the technologies and services you need, it is also a village where everybody knows everyone, everything is nearby and you always have time to enjoy a great cup of coffee. Within a radius of 200 km from Brno, there are other important European capitals: Prague, Vienna, and Bratislava. Despite being called the suburbs of Vienna, Brno has its own style and authentic feeling.

It is a modern, dynamic, and fast-growing center of industry, trade, science, and information technology. Thanks to the many international companies there are more than 50 000 expats living here which makes Brno very multicultural and open to visitors from all over the world as well.

A lot of the expats came to Brno for education before getting a job here. With more than 65 000 students in 10 different universities, Brno is a student city and center of knowledge. Well and also never-ending parties, concerts and other happenings which keep the city young, hip and fresh.

Brno is the capital of the Moravia region and the South Moravia district. It is surrounded by picturesque towns and villages full of wine and traditions. That is why it is a great opening gate to the region and together creates the 2nd most popular tourist region in the Czech Republic.

GREETINGS

Welcome to the Mendel Genetics Conference
We are very honoured to welcome you in Brno, Czech Republic, at the international Mendel Genetics Conference dedicated to the celebration of Gregor Johann Mendel's birth bicentennial. Mendel spent a substantial part of his life in Brno where he also formulated the Mendel's laws of inheritance and, in addition to his scientific work, Mendel also served as a priest and later on abbot of the Augustinian Abbey.

The conference officially begins on 20th July 2022, exactly 200 years after Mendel was born, and the final session on 23rd July 2022 symbolically takes place in the Augustinian Abbey in Brno, where Mendel realized his hybridizing experiments on pea plants, kept bees and established a meteorological station.

Mendel Genetics Conference is focused on different genetic fields, such as human genetics, plant and animal genetics or genetics of microorganisms. However, the sessions focus also on the history and future of genetics, novel emerging technologies and specific topics related to ethical issues in genetics. Keynote speakers are world-leading researchers inspiring future generations of scientists and they cover the most important biological questions touching the origin of life from a genetic perspective, discuss both inherited and somatic genetic variability and also consider an impact of genetics and genomics on personalized medicine.

This premier meeting brings together not only researchers, academicians and students, but also professionals, representatives of genetic patients and even the general public, as the meeting is directly followed by the Festival Mendel for a broad audience. Genetics are closely associated with many other scientific fields, forming one of the central pillars of modern biology, but are highly relevant for the whole society, as we have witnessed during COVID-19 pandemic.

We would like to thank all speakers for very interesting and inspiring speeches and discussions and all participants for the presentations and fruitful debates. We thank all of you for your participation on the celebrations of Gregor Johann Mendel's heritage.

Brno, July 2022

prof. RNDr. Šárka Pospíšilová, Ph.D.
Vice-Rector for Research and Doctoral Studies at Masaryk University
doc. Ing. Svatopluk Kapounek, Ph.D.
Vice-Rector for Research at Mendel University in Brno
ThLic. Ing. Jozef Ržonca, Ph.D. et Ph.D.
Pastor in Augustinian Abbey
Mgr. Jiří Mitáček, Ph.D.
Director General of the Moravian Museum
Ing. Jakub Carda
Director of Společně, o.p.s.

GENERAL INFORMATION

Internet Facilities
Wi-Fi internet connection MENDEL is available at the hotel free of charge.
No password needed.

Time Zone
The local time in the Czech Republic at the time of the conference will be GMT +2 due to Summer Daylight Saving Time.

Electricity
The Czech Republic uses a 230 volt 50 Hz system.

Emergency Telephone Number
The emergency phone number is 112.

Insurance
The organizers of the conference do not accept liability for any injury, loss or damage, arising from accidents or other situations during the conference. Participants are therefore advised to arrange insurance for health and accident prior to travelling to the conference.

Taxi Service
We recommend using Liftago, Bolt or taxi service of the following reliable company:
City taxi plus s. r. o., +420 542 321 321.

Venue

Hotel Passage ****
Lidická 23, 602 00 Brno-střed, Czechia
Phone: +420 530 352 100
GPS: 49°12'7.37"N 16°36'25.12"E

Augustinian Abbey
907/1A, Mendlovo nám. 1/4,
603 00 Brno-střed, Czechia
Phone: +420 543 424 011
GPS: 49.1979047N, 16.6085639E

REGISTRATION AND INFORMATION DESK

All participants must be registered before attending the lectures.
Registration will be open from 8:00 am (one hour before the start of the first lecture) on Thursday, Friday and Saturday.

Registration and information desk in the hotel Passage will be open for the whole duration of the conference program.

CONFERENCE POLICY

Badges
Participants and accompanying persons will receive a name badge upon registration. Everyone is kindly requested to wear their name badge when attending the conference. Only participants who are wearing their name badge will be admitted to the lecture halls.

Name badges have been colour-coded as follows:

- Purple: Speakers
- Yellow: Sponsors, Press
- Green: Participants
- Blue: Organisers

Official Language
The official language of the conference is English.

Program Changes
The organizers cannot assume liability for any changes in the program due to external or unforeseen circumstances.

Mobile Phones
Participants are kindly requested to keep their mobile phones in the off position in the meeting room while the session is being held.

Social Media
For the latest information, visit www.mendel22.cz/conference and you can follow the Twitter account @MendelBrno.

CONFERENCE OPENING CEREMONY

19, 23 July 2022: Trip to Mendel's birth house in Vražné
20 July 2022: Conference Opening Ceremony and Welcome Reception in the Augustinian Abbey
21 July 2022: Dinner for invited guests in the Moravian Museum
22 July 2022: Janáček's Glagolitic Mass in the Basilica and Kool & The Gang in the Augustinian Abbey
23 July 2022: Mendel Festival in the Augustinian Abbey
20, 23 July 2022: Visit of Villa Tugendhat

POSTER SESSION

The posters will be exhibited on 21 and 22 July 2022 in the conference venue at Hotel Passage. Posters will be available to the conference participants from 8 am to 7 pm, and there will be the possibility of a poster discussion on Thursday 21 July from 6 to 7 pm.

Requirements
The participant is responsible for making sure that the poster display fits on the display board, and is completely responsible for attaching the individual elements to the display board according to our instructions.
We will provide on-site assistance with installation which will take place on Thursday 21 July 2022 from 8 to 9 am.

Poster Session program and information for presenting authors
Poster Session – Thursday 21 July from 6 to 7 pm. Presenting authors are kindly requested to present throughout the official Poster Sessions time in order to explain their research and to answer the questions. There will be no guided formal discussion.

Book of Abstracts
Each participant will receive the printed version. The Book of Abstracts is available online at the conference website www.mendel22.cz/conference.

DNAnexus Best Poster Award
The best poster will be selected by the Scientific Committee members and a representative of the conference's sponsor DNAnexus. The winner will be announced on Friday, 22 July, ahead of the Roundtable on genetics and environment session, at 5 pm.

AUSPICES GRANTED TO THE CONFERENCE

The conference is organized under the auspices of

President of the Czech Republic
Senate of the Parliament of the Czech Republic
Vlastimil Válek – Deputy Prime Minister and Minister of Health
Mikuláš Bek – Minister for European Affairs
Jan Lipavský – Minister of Foreign Affairs
Zdeněk Nekula – Minister of Agriculture
Ministry of Education, Youth and Sports
Jan Grolich – Governor of the South Moravian Region

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PROGRAM

Day 1 — Wednesday 20 July 2022, Augustinian Abbey

16:00 – 16:20	**Conference Introduction**
16:20 – 16:30	**Opening Speeches of Šárka Pospíšilová and Jiří Sekerák**
16:30 – 16:50	**Daniel Fairbanks:** Gregor Mendel at his Bicentennial: Highlights of the Life and Legacy of a Scientific Genius
16:50 – 17:10	**Nils Christian Stenseth:** Gregor Johann Mendel's scientific and cultural impact
17:15 – 18:00	**Concert of the music of Leoš Janáček and his contemporaries**
18:00 – 22:00	**Welcome reception in the Augustinian Abbey, Guided Tours of Mendel Museum, Moravian Folk Music**

Day 2 — Thursday 21 July 2022, Hotel Passage

KEYNOTE SPEAKERS

09:00 – 09:30	**Šárka Pospíšilová,** *Masaryk University, CZ*	Opening presentation – Ways to the Genome of Gregor Johann Mendel
09:30 – 10:15	**Thomas R. Cech,** *University of Colorado Boulder, US*	Noncoding RNAs in Catalysis and as Regulators of Epigenetic Gene Silencing
10:15 – 11:00	**Sir Paul Nurse,** *The Francis Crick Institute, GB*	What is life?
11:00 – 11:20	**Coffee Break**	
11:20 – 12:05	**Diana W. Bianchi**, *National Institute of Child Health and Human Development, NIH, US*	From peapods to pregnancy: Mendel's influence on prenatal genomic medicine
12:05 – 12:50	**Kim Nasmyth,** *University of Oxford, GB*	Cohesin and its roles in organising the topology of chromosomal DNAs
12:50 – 14:00	**Lunch Break**	
14:00 – 14:45	**Jan Korbel,** *EMBL Heidelberg, DE*	Structural Variation in the Human Genome
14:45 – 15:30	**Sir Mike Stratton,** *Wellcome Sanger Institute, GB*	Mutations in normal human cells
15:30 – 16:15	**Sir David Lane**, *Karolinska Institutet, SE*	Tumor Suppressor Genes and Genetic Stability
16:15 – 16:35	**Coffee Break**	
16:35 – 17:20	**Jakub Tolar,** *University of Minnesota, US*	Reaping Mendel's Harvest: Genome editing and the era of precision medicine
17:20 – 17:50	**Ada Hamosh,** *The Johns Hopkins University, US*	Online Mendelian Inheritance in Man (OMIM), a catalog of human genes and genetic disorders and traits
18:00 – 19:00	**Poster Session & Discussion**	

Day 3 — Friday 22 July 2022, Hotel Passage

OVERVIEW

	Section A	Section B	Section C
09:00 – 10:30	**Human Genetics – Inherited Diseases**	**Plant Genetics**	**History of Genetics***
10:30 – 10:50	**Coffee Break**		
10:50 – 12:20	**Human Cancer Genetics & Genetics of Microorganisms**	**Animal Genetics**	**History of Genetics***
12:20 – 13:20	**Lunch Break**		
13:20 – 14:50	**Novel Emerging Technologies in Genetic Research**		**History of Genetics***
14:50 – 15:10	**Coffee Break**		
15:10 – 16:40	**Genetics and Society**		**History of Genetics***
16:40 – 17:00	**Coffee Break**		
17:00 – 18:00	**ROUNDTABLE ON GENETICS AND ENVIRONMENT**		

**History of genetics: more than a century of international research into the life and legacy of Gregor Johann Mendel, the origin of genetics, and its development*

Day 3 — Friday 22 July 2022, Hotel Passage
DETAILED PROGRAM

	SECTION A	
09:00 – 10:30	**HUMAN GENETICS – INHERITED DISEASES**	
	Maurizio Genuardi **Milan Macek**	Session Chairs
09:00 – 09:20	**Wayne W. Grody**	Twenty Thousand Genes, Infinite Traits: Grappling with Mendel's Legacy in the Age of Genomic Medicine
09:20 – 09:40	**Johannes Zschocke**	Dominance and Recessiveness in Medical Genetics
09:40 – 10:00	**Olaf Riess**	Beyond the exome: Solving the unsolved rare diseases
10:00 – 10:20	**Martin Bareš**	The cerebellum in movement disorders in the era of neurogenetics
10:20 – 10:30	**Discussion**	
10:30 – 10:50	**Coffee Break**	
10:50 – 11:35	**HUMAN CANCER GENETICS**	
	David Lane **Šárka Pospíšilová**	Session Chairs
10:50 – 11:10	**Michael Doubek**	Genetic Background of Leukemia in the context of clinical practice
11:10 – 11:30	**Waseem Qasim**	Human applications of genome editing through cancer immunotherapies
11:30 – 11:35	**Discussion**	
11:35 – 12:20	**GENETICS OF MICROORGANISMS**	
	David Lane **Šárka Pospíšilová**	Session Chairs
11:35 – 11:55	**Ece Kartal**	Microbiome in human cancer and bacterial genomes
11:55 – 12:15	**Pavel Plevka**	Impact of genetic variability on enterovirus infection
12:15 – 12:20	**Discussion**	
12:20 – 13:20	**Lunch Break**	
13:20 – 14:50	**NOVEL EMERGING TECHNOLOGIES IN GENETIC RESEARCH**	
	Vladimír Beneš **Karla Plevová**	Session Chairs
13:20 – 13:40	**Ivo Gut**	What is new in sequencing technology?
13:40 – 14:00	**Žaneta Andrusivová**	Overview of spatial transcriptomics
14:00 – 14:20	**Fredrik Edfors**	Next generation plasma proteome profiling of a pan-cancer cohort
14:20 – 14:40	**Eva Hasel de Carvalho**	Optogenetics: manipulating life and death of cells in space and time
14:40 – 14:50	**Discussion**	
14:50 – 15:10	**Coffee Break**	
15:10 – 15:50	**KEYNOTE LECTURE**	
	Pavel Tomančák, *CEITEC, CZ*	Chairs
15:10 – 15:50	**Ada E. Yonath,** *Weizmann Institute of Science, IL*	The proto-ribosome as the kernel of origin of life
15:50 – 16:50	**GENETICS AND SOCIETY**	
	Nils Chr. Stenseth **Věra Franková**	Session Chairs
15:50 – 16:10	**Guido de Wert**	Genomics and personalized medicine: ethical reflections
16:10 – 16:30	**Mons. Alberto G. Bochatey**	Human Person and Genetics Studies
16:30 – 16:50	**John Mulvihill**	Solidarity in Genetics: Every Citizen a Researcher?
16:50 – 16:55	**DNA Nexus award for the best poster**	
16:55 – 17:15	**Coffee Break**	
17:15 – 18:15	**ROUNDTABLE: GENETICS AND ENVIRONMENT**	
17:15 – 17:30	**Dirk Inzé**	Opening Speech
Moderators of the debate	**Dirk Inzé** **Karel Říha**	
Debaters	**Petr Blížkovský, Richard Sťahel, Milada Šťastná, Ortrun Mittelsten Scheid**	

	SECTION B	
09:00 – 10:30	**PLANT GENETICS**	
	Dirk Inzé **Jaroslav Doležel**	Session Chairs
09:00 – 09:30	**Liam Dolan**	How Gregor Mendel helped us understand plant development and evolution
09:30 – 10:00	**Crisanto Gutiérrez**	Stem cell regulators and cell proliferation control during organogenesis
10:00 – 10:30	**Brande Wulff**	Harnessing the power of disease resistance in wild grasses – the case for GM wheat
10:30 – 10:50	**Coffee Break**	
10:50 – 12:20	**ANIMAL GENETICS**	
	Michael Lampson **Petr Hořín**	Session Chairs
10:50 – 11:20	**Jiří Forejt**	Darwin, Mendel and the origin of species
11:20 – 11:50	**Michael Lampson**	Violation of Mendel's First Law: centromere competition in meiosis
11:50 – 12:20	**Cesare Galli**	Gametes and embryo research: assisted reproduction and beyond
12:20 - 13:20	**Lunch Break**	

	SECTION C **History of genetics: more than a century of international research into the life and legacy of Gregor Johann Mendel, the origin of genetics, and its development**	
09:00 – 10:30	**ANTHROPOLOGICAL AND GENOMIC ANALYSIS OF MENDEL'S REMAINS**	
	Daniel J. Fairbanks **Uwe Hoßfeld**	Session Chairs
09:00 – 09:20	**Dana Fialová**	Multidisciplinary Approach to Identification of Gregor Johann Mendel's Skeletal Remains
09:20 – 09:40	**Eva Drozdová**	Body Remains of the Founder of Genetics Gregor Johann Mendel – a Case Study
09:40 – 10:00	**Filip Pardy**	Reconstructing the genome of Gregor Johann Mendel using state-of-the-art molecular and bioinformatics tools
10:00 – 10:20	**Eva Chocholová**	Metagenomic and Proteomic Analysis of Dental Calculus of Abbot Gregor Johann Mendel
10:20 – 10:30	**Discussion**	
10:30 – 10:50	**Coffee Break**	
10:50 – 12:20	**MENDEL'S HISTORY IN THE NINETEENTH CENTURY**	
	Daniel J. Fairbanks **Uwe Hoßfeld**	Session Chairs
10:50 – 11:10	**Silvia Eckert-Wagner**	Johann Gregor Mendel - His family and origin
11:10 – 11:30	**Jiří Sekerák**	Mendel's birth date
11:30 – 11:50	**Peter Van Dijk**	A New Reconstruction of Mendel's 1865-lectures and a Content Comparison with the 1866 Paper
11:50 – 12:10	**Johann Vollmann**	Mendel's Contemporaries: Convergence and Strategies in 19th Century Plant Breeding
12:10 – 12:20	**Discussion**	
12:20 – 13:20	**Lunch Break**	
13:20 – 14:50	**MENDEL'S LEGACY IN THE TWENTIETH AND TWENTY-FIRST CENTURIES**	
	Daniel J. Fairbanks **Uwe Hoßfeld**	Session Chairs
13:20 – 13:40	**Michael Mielewczik**	New insights from a new critically commented edition of Mendel's classic article on plant-hybridization and its role in the transformation of science and agriculture
13:40 – 14:00	**Gregory Radick**	The Role of the Cold War in Transforming a Statistical Puzzle about Mendel's Pea Data into a Scientific Scandal
14:00 – 14:20	**Toshiyuki Nagata**	The fate of Mendel's grapevine
14:20 – 14:40	**Milan Macek sr.**	Development of medical genetics in the Czech Republic
14:40 – 14:50	**Discussion**	
14:50 – 15:10	**Coffee Break**	

15:10 – 16:40	MENDEL'S THEORY AND ITS IMPLICATIONS	
	Daniel J. Fairbanks **Uwe Hoßfeld**	Session Chairs
15:10 – 15:30	**Pablo Lorenzano**	An Analysis of Mendel's Two Hybridist Theories and of Their Relationships
15:30 – 15:50	**Jaroslav Nešetřil**	Genius Loci: Mendel in Context of Central and Peripheral Categories
15:50 – 16:10	**Hui Zhang**	On the Bicentennial of Mendel's Birth, Attempting to Recover Mendel's Inheritance Principles with Mendel's Eyes
16:10 – 16:30	**Petr Dostál**	Genetic Algorithms Optimize Problems in Business and Economics
16:30 – 16:40	**Discussion**	

Day 4 — Saturday 23 July 2022, Augustinian Abbey

10:00 – 12:00	ROUNDTABLE: ETHICAL QUESTIONS IN GENETICS
Moderators of the debate	**Věra Franková, Milan Macek**
Debaters	**Eva Zažímalová, Tomáš Machula, Jan Konvalinka, Dominik Opatrný, Marek Svoboda, Hynek Latta**

13:15 – 18:00	Satellite workshop
	EARLY DIAGNOSIS OF PATIENTS WITH RARE DISORDERS IN THE EU: CRUCIAL ROLE OF THE NEWBORN SCREENING For more info visit: https://www.mzcr.cz/wp-content/uploads/2022/07/Technical-meeting_newborn-screenings.pdf This satellite workshop organised within the CZ EU PRES activities is without pre-registration. Participation is based on a first come/first serve basis for already fully registered participants to the Mendel Genetics Conference up to the capacity of the Mendel´s refectory.

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ABSTRACTS OF SPEAKERS

KEYNOTE SPEAKERS

S01 Gregor Mendel at his Bicentennial: Highlights of the Life and Legacy of a Scientific Genius

Fairbanks D. J.[1]
[1]*Utah Valley University*
Email of the presenting author: daniel.fairbanks@uvu.edu

As we celebrate the bicentennial of Gregor Mendel's birth, a few highlights of his life and legacy illustrate the breadth of his contributions and his genius. Born into poverty, he excelled in education in his youth. He entered the St. Thomas Monastery as a friar where he joined an extraordinary community of scholars. At the University of Vienna, he studied with some of the world's finest scientists, especially in mathematics, physics, botany, and evolution, and published his first research papers there. His famous experiments led him to an enduring theory thatis more expansive than often portrayed. It includes not only the well-known laws of segregation and independent assortment, but also definitive evidence of the nature of fertilization and accurate interpretations of aspects often portrayed as exceptions to his theory, such as pleiotropy, incomplete dominance, and epistasis. His subsequent genetic research was elaborate and extensive, including hybridization experiments in at least twenty plant genera as he sought to broaden his theory. As Darwin's contemporary, his annotations in Darwin's books and Darwinian comments in letters reveal much about his understanding of the role of hybridization in evolution. Although he published several scientific papers, most in meteorology, much of his genetic research remained unpublished, partially evident to us now in his preserved correspondence with Nägeli. The neglect and rediscovery of his theory constitute one of the most intriguing stories in the history of science. At the bicentennial of his birth, his theory endures essentially unchanged as the foundation of genetics.

Acknowledgement: Mendelianum, Moravian Museum

KEYNOTE SPEAKERS

S02 Gregor Johann Mendel's scientific and cultural impact

Stenseth N. C.[1]
[1]*Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo*
Email of the presenting author: n.c.stenseth@mn.uio.no

Nothing in biology make sense except in the light of evolution and Mendelian genetics. Darwin developed the theory for evolution through natural selection; a theory which require a mechanism for heredity, which Darwin never came to understand. Mendel was the one that provided such a mechanism – what has come to be called Mendelian genetics. Thus, the contributions of Darwin and Mendel are the cornerstones of modern biology and our quest to understand human nature. Human culture and rituals are not part of human nature – but products of it. Similarly, art is not a part of our nature – but the ability to enjoy and appreciate art is. Hence, the celebration of Gregor Johann Mendel and the theory of evolution through natural selection is a celebration of science as well as the humanities.

KEYNOTE SPEAKERS

S03 Ways to the Genome of Gregor Johann Mendel

Pospisilova S.[1, 2, 3]
[1]*Central European Institute of Technology (CEITEC), Masaryk Univerzity, Brno, Czech Republic,*
[2]*Faculty of Medicine, Masaryk Univerzity, Brno, Czech Republic,*
[3]*Univerzity Hospital Brno, Czech Republic*
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Gregor Johann Mendel – a versatile and multitalented scientist, considered the "father of modern genetics", was born in 1822 and the bicentennial of his birth in July 2022 provides a unique opportunity to remind the legacy of this great scientist. Mendel formulated the principles of heredity based on his experimental work on a hybridization of about 28,000 pea plants, using application of his interdisciplinary knowledge of breeding, plant biology, mathematics, statistics and combinatorics. The Mendelian laws, published in 1865, described for the first time the presence of two versions of each element (allele) in a dominant or recessive form thatis inherited from both parental organisms into the filial generation (known as the laws of segregation, independent assortment, and dominance of alleles). The disorders with Mendelian inheritance, named also monogenic disorders, appear as a result of a single-gene mutation present in one or both alleles of a gene.
Currently, 200 years from Mendel's birth and 138 years from his death, there persisted an uncertainty about the place of his grave. Therefore, on the occasion of his anniversary, the scientists, in close collaboration with representatives of the Order of Augustinians, initiated a project on an archeological research of the Augustinian tomb followed by anthropological and genetic research of the found remains. Very exciting novel findings on Gregor Johann Mendel personality have been discovered and, symbolically, the genome of the father of genetics has been obtained. In addition, the geneticists analyzed whether Mendel was a carrier of any disease with Mendel's mode of inheritance, also called Mendelian disorders. Finally, the project brought novel findings on Mendel's physique, health problems, genetic predispositions or ancient ancestors. We believe that the story of Mendel's life could further highlight his legacy, inspire young researchers and also attract society's attention to genetics and science.

Acknowledgement: I would like to thank to all colleagues and collaborators, who realized the interdisciplinary archeological, anthropological and genetic research project on G. J. Mendel.

KEYNOTE SPEAKERS

S04 Noncoding RNAs in Catalysis and as Regulators of Epigenetic Gene Silencing

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Noncoding RNAs have diverse and essential functions in biology. Some noncoding RNAs participate directly in catalysis, as exemplified by ribozymes, the ribosome, and the spliceosome. More recently, it has been found that nascent transcripts in the nucleus act as epigenetic regulators. This was surprising, because we considered RNA to be the product of transcription, not the start of a gene-regulatory circuit. I will describe one example, Polycomb Repressive Complex 2 (PRC2), in some detail. PRC2 binds nuclear long noncoding RNAs and pre-mRNAs broadly. Using CRISPR genome-editing of induced pluripotent stem cells, we found that RNA provides an initial step for PRC2 recruitment to sites of action (Long et al., Nature Genetics 52, 931-938, 2020). To understand the mechanisms in more detail, we have now applied biophysical measurements and cryo-EM structural analysis. Given that many DNA-binding proteins also bind RNA, these mechanisms may be more generally applicable.

KEYNOTE SPEAKERS

S05 The proto-ribosome as the kernel of origin of life

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The site for peptide bond formation, the PTC, is located in all ribosomes, within an almost fully structural conserved internal region made exclusively of rRNA. The high structural conservation of this region, called by us the proto-ribosome, implies its existence irrespective of environmental conditions and indicates that it may represent a prebiotic RNA machine, which could be the kernel around which life originated. Lab constructs designed according to these pocket were shown to possess stereochemical capabilities for peptide bond formations, thus indicating that a molecular prebiotic bonding entity still exists and functions within all living cells of all organisms.

KEYNOTE SPEAKERS

S06 "Whatis Life?"

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In this lecture I consider the question "Whatis Life?" by discussing five great ideas of biology, ranging from the 'Cell' to 'the Logic of Life'. By considering these concepts a direction of travel is set towards a definition of life.

KEYNOTE SPEAKERS

S07 Mendel's reciprocal segregation of gene variants depends on association sister DNAs by cohesin and its subsequent dissolution by separase. Whatis the molecular basis of this process thatis unique to eukaryotic cells?

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Mendel's reciprocal segregation of gene variants depends on association sister DNAs by cohesin and its subsequent dissolution by separase. Whatis the molecular basis of this process thatis unique to eukaryotic cells?
To explain the ratios of types of progeny produced by hybrid peas, Mendel proposed that traits such as the shape or colour of their seeds are determined by variants of specific elements, which segregate reciprocally in germ cells so that gametes inherit one and only one variant. His concept of variant segregation predated the subsequent discovery of chromosomes and their longitudinal division into two chromatids during mitosis by at least two decades. A proper description of the meiotic process responsible for Mendelian segregation had to wait another three or more decades. Sister chromatid cohesion is fundamental for both types of segregation and is a feature unique to eukaryotic cells that enables them to segregate DNAs long after they have been replicated. It has been proposed that cohesion is mediated by co-entrapment of sister DNAs inside a tripartite cohesin ring created by a pair of rod-shaped proteins (Smc1 and Smc3) whose two ends are connected through dimerization of their hinges at one end and by association of their ATPase domains at the other end with the N- and C-terminal domains of a kleisin subunit (Scc1). The ring model explains how Scc1 cleavage triggers anaphase but has hitherto only been rigorously tested using small circular mini-chromosomes in yeast, where crosslinking the ring's three interfaces, creating a covalent circular molecule, induces catenation of individual sister DNAs. If the model applies to real chromatids, then the ring must have a DNA entry gate essential for mitosis. My talk will address the identity and physiological importance of this gate.

KEYNOTE SPEAKERS

S08 Structural Variation in the Human Genome

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My laboratory's main interest centers around understanding determinants for the formation and selection of genetic variation. Genomic structural variants (SVs) are a major class of genetic variation, thought to be responsible for the majority of nucleotide-level DNA variation existing between as well as within individuals. We are developing novel methods based on single cell and long read sequencing technologies to obtain insights into SV classes that have remained largely elusive in the past. Unlike copy number variants (CNVs), inversions have remained an underexplored genetic variation class. By integrating multiple genomic technologies, we discover 729 inversions in 41 human genomes. Approximately 85% of inversions <2 kbp form by twin-priming during L1 retrotransposition; 80% of the larger inversions are balanced and affect twice as many nucleotides as CNVs. Balanced inversions show an excess of common variants, and 72% are flanked by segmental duplications (SDs) or retrotransposons. Since flanking repeats promote non-allelic homologous recombination, we developed complementary approaches to identify recurrent inversion formation. We describe 40 recurrent inversions encompassing 0.6% of the genome, showing inversion rates up to 2.7 × 10-4 per locus per generation. Recurrent inversions exhibit a sex-chromosomal bias and co-localize with genomic disorder critical regions. We propose that inversion recurrence results in an elevated number of heterozygous carriers and structural SD diversity, which increases mutability in the population and predisposes specific haplotypes to disease-causing CNVs.

KEYNOTE SPEAKERS

S09 Mutations in normal human cells

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Mutations in DNA/RNA cause the phenotypic variation upon which natural selection acts during evolution. Mutations arise and are transmitted between generations of organisms and between generations of cells in the somatic tissues of multicellular organisms. However, our knowledge of the biological processes that generate mutations and the rates at which they occur in different cell types has been rudimentary until recently. Cancer is a recognized outcome of somatic mutations. However, we have limited understanding of the wider consequences of somatic mutations. I will review our current understanding of the landscape of somatic mutations, and their consequences, in normal human cells.

KEYNOTE SPEAKERS

S10 From peapods to pregnancy: Mendel's influence on prenatal genomic medicine

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Mendel's interests were more aligned with nature than with medicine. In 1863, however, he was nominated as the curator of the Institute for the Deaf and Dumb in Brno, so presumably he was familiar with children who had special needs. At that time prenatal testing did not exist. My talk will focus on noninvasive prenatal testing (NIPT), using sequencing of the cell-free DNA (cfDNA) that circulates in the plasma of pregnant women. It is the most mature example of the global implementation of genomics into clinical medicine. Tens of millions of tests have been performed to date. The testing has been predominantly used to screen for the common fetal aneuploidies (trisomies 13, 18, and 21) and for determination of fetal sex. Since 2011, the testing has transformed prenatal care, replacing serum screening tests that have lower positive predictive values (PPVs), which has significantly reduced the number of invasive diagnostic procedures and cytogenetic analyses performed globally.
This testing also analyzes maternal DNA. Maternal DNA abnormalities sometimes provide the underlying biological explanation for false positive or non-reportable results. When the NIPT result is abnormal and the fetal karyotype or chromosome microarray results are normal, the differential diagnosis includes confined placental mosaicism, twin demise, and maternal findings, including autoimmune and metabolic conditions, benign and malignant tumors, hematologic abnormalities, autosomal and sex chromosome aneuploidies, and clinically significant copy number variants. In early 2020, our research team at the National Human Genome Research Institute began the Incidental DEtection of maternal Neoplasia Through non-Invasive cell Free DNA analysis (IDENTIFY) study to prospectively determine the underlying causes for non-reportable or false positive NIPT results. Results thus far have demonstrated a high proportion of clinically asymptomatic maternal malignancies. IDENTIFY is a crucial study that will provide evidence to inform antenatal practice recommendations for subsequent management.

Acknowledgement: Amy Turriff, GC, National Human Genome Research Institute Christina Annunziata, MD, National Cancer Institute
Ashkan Malayeri, MD, Department of Radiology, Clinical Center, NIH

KEYNOTE SPEAKERS

S11 Tumor Suppressor genes and Genetic Instability

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A striking feature of human cancer is the enormous extent of genetic alteration found in cancer cells. Many cancers show massive chromosome loses and gains , extreme aneuploidy, extraordinary copy number variations and in up to 30% of cancers circular extrachromosomal DNA. How are such cells able to escape detection by the internal mechanisms that rigorously maintain the diploid state in normal cells and recognize foreign or damaged DNA as a potent signal for specific immune and inflammatory reactions? Loss of tumor suppressor genes that both detect and repair DNA damage are crucial mechanisms but other processes that resist the promotion of the growth of normal cells containing driver mutations must also be in play. Recent insights suggest that inhibition of centrosome loss , inhibition of transposition, inhibition of cell fusion and maintenance of the integrity of the nuclear membrane are all crucial processes that depend upon the activity of p53, one of the most important tumor suppressors known. How p53 is able to achieve such diverse activities is still unclear but may involve novel functions of the protein distinct from its activity as a transcription factor.

KEYNOTE SPEAKERS

S12 Reaping Mendel's Harvest: Genome editing and the era of precision medicine

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Gregor Mendel's detailed study of heredity in the humble garden pea was the foundation of the future of medicine. He described dominant and recessive traits, hypothesized the "elementen" contributed by each parent, and demonstrated a reproducible mathematical theory that could predict the patterns of inheritance. Two centuries after his birth, with the knowledge of the genome, DNA, chromosomes, and alleles, we are able to flesh out, confirm, and exploit his discoveries.

The science of exploring the genome, assigning specific traits to specific allelic locations, has given us a better understanding of inherited disorders and predisposition to certain diseases. Development in the field of genome editing has opened the possibility of correcting those disorders and intervening before a disease causes suffering.

There is a symmetry in the fact that Mendel's work resulted from a large amount of data and brilliant mathematical modeling, the same tools we use now to look at an individual's genome and predict what we need to do to prevent or treat their diseases.

Beginning in the 1940s with increasing understanding of DNA and paused briefly in the 1970s to examine the ethical implications of altering it, genome editing began its modern evolution in 1985 with the discovery of zinc finger nucleases and their ability to "read" DNA and target specific DNA sequences. TALEN (Transcription Activator-Like Effector Nucleases) developed for use in plant biology, lowered the cost and increased the accuracy of the targeting.

The next developments, CRISPR (Clustered regularly interspaced palindromic repeats) genome engineering, base editing, and prime editing have provided increasingly accurate, benign, and multi-functional tools in correcting the "misspellings" of DNA. With the completion of the human genome sequence in 2003, we now have the ability to build on Mendel's discovery and genome engineering to create the next generation of personalized, precision medicine.

SPEAKERS

S13 Twenty Thousand Genes, Infinite Traits: Grappling with Mendel's Legacy in the Age of Genomic Medicine

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Most beginning biology students learn of Mendel's laws of unit inheritance through examples of his initial monohybrid crosses in pea plants, assaying one trait at a time (green vs. yellow, etc.). But, ever curious as he was, Mendel did not stop with those simple experiments; he went on to perform multi-hybrid crosses, involving two, three or more traits being passed on simultaneously. He was able to characterize his results mathematically using ever-expanding algebraic equations, while also deducing the phenomenon of independent assortment. How would he have reacted if a soothsayer told him that one day his scientific descendants would be tracking tens of thousands of these "units", all in a single assay taking 2-3 days, across species from viruses to humans?

The elucidation of the structure of DNA not only reconfigured Mendel's far-reaching concepts of heredity into physicochemical form, but also led to powerful tools to dissect these phenomena at the molecular level. Most recently, the advent of massively parallel ("next-generation") DNA sequencing has brought into clinical practice the precepts of Mendel's multivariate crosses, allowing fine-structure analysis of all the genes of an organism – in the case of Homo sapiens, about 20,000.

In the field of Medical Genetics, NGS has availed practitioners of a form of genetic testing unprecedented in its scope, revealing novel disease mechanisms and ending the long "diagnostic odyssey" for patients with ultra-rare or unknown genetic disorders. Nevertheless, it fails to identify the molecular cause in many patients, while at the same time unmasking other sequence changes of uncertain significance or unexpected consequences, raising profound ethical dilemmas. This presentation will consider these issues based on our 12 years' experience with clinical NGS and the development of practice guidelines through the American College of Medical Genetics, all in the context of the fertile ground of Gregor Mendel's discoveries.

SPEAKERS

S14 Gregor Mendel, medical genetics, and the concepts of dominance and recessiveness

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Gregor Mendel's observation that some physical traits are inherited as discrete units was a crucial step in the understanding of genetic inheritance mechanisms. Key to the success of his research, presented in 1865, was the focus on discontinuous traits — clearly distinguishable pairs of 'differentiating characters' that always occurred in an either/or constellation — and the specific exclusion of traits that showed an intermediate form. Mendel introduced the terms dominant and recessive to describe the presence or complete absence of a specific trait in the hybrid (in the current term: heterozygote). He did not know or discuss the physical basis of this observation, which he described mathematically in the format A+2Aa+a rather than AA+2Aa+aa, and he did not make a conceptual distinction between the manifesting trait and the heritable material. Mendel did not have the "genetic information" concept of two separate alleles that code for potentially different phenotypes. Rather, he may have thought of the organism as something of an alloy of two components that are separated into distinct elements in germ cells. Soon after the rediscovery of Mendel's work in 1900 it was recognized that the principles identified by him also apply to some rare human diseases. It is interesting to note that the definition of dominance and recessiveness in medicine differs from Mendel's original concept, as it does not usually consider the phenotype in the homozygous state. Most "dominant diseases" in humans do not fulfil the original Mendelian criteria but show an intermediate – in current terminology semidominant – inheritance. The homozygous phenotype is usually much more severe, and often unknown. Understanding the pathomechanisms by which heterozygous variants cause – or do not cause – clinically noticeable manifestations, is at the core of understanding monogenic inheritance in medical genetics.

Reference: Zschocke J, Byers PH, Wilkie AOM. Gregor Mendel and the concepts of dominance and recessiveness. Nat Rev Genet 2022;23:387-388; PMID: 35508637.

SPEAKERS

S15 Beyond the exome: Solving the unsolved rare diseases

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About 80% of all rare diseases are caused by genetic changes in the genome. Wit the best standard of care, which is not widely implemented throughout Europe yet, whole exome diagnostic is applied to find these genetic causes. However, diagnostic sensitivity is barely above 40% of all patients analysed, even if clear family information indicated a genetic cause of the underlying disease. Thus, other disease mechanisms have to be considered "beyond the exome" which need to be implemented into the diagnostic process. First step in this direction is the sequencing of the entire genome in diagnostics, but despite of lack of knowledge and due to limited bioinformatic tools we increase diagnostic sensitivity only by about 6% in total. Complementary transcriptome sequencing by RNASeq further delivers data sets to identify potential genetic alterations. With my talk I will give an overview on sequencing several thousand RD patients in the diagnostic setting and provide examples of solving cases. Also, the European research network SOLVE-RD developed structures which are excellent to be rolled out in even larger European initiatives.

SPEAKERS

S16 The cerebellum in movement disorders in the era of neurogenetics

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Although movement disorders like Parkinson's disease (PD) or dystonia have traditionally been regarded as a basal ganglia (BG) dysfunction, evidence has emerged of cerebellar involvement in the pathophysiology of these movement disorders.

Methods: Patients with PD and dystonia, Spinocerebellar ataxia (SCA), Essential tremor, and healthy controls (HC) underwent a series of functional magnetic resonance imaging (fMRI) studies during an interception of a moving target. Transcranial magnetic stimulation (TMS) was used, too. Brain connectivity and its modulation by the behavioral outcome in a network comprised of the cerebellum, BG, and other cortical areas were studied, too.

Results: (i) In patients with PD, despite having similar hit ratios, the PD failed more often than the HC to postpone their actions until the right moment and to adapt their behavior from one trial to the next. We found more activation in the right cerebellar lobule VI in HC than in PD during successful trials. Successful trial-by-trial adjustments were associated with higher activity in the right putamen and lobule VI of the cerebellum in HC.

(ii) in the dystonia group we have found evidence for altered activation in the posterior cerebellar lobules, premotor areas, the associative parietal cortex, and visual regions as well as novel evidence of decreased cerebellar connectivity with bilateral basal ganglia structures and the dorsolateral prefrontal cortex (1).

Discussion: The studies bring new insights into the brain regions essential for the development of some movement disorders and the cerebellum might be considered the target for therapeutics in PD or dystonia.

References:
(1) Filip P, Gallea C, Lehéricy S, Bertasi E, Popa T, Mareček R, Lungu OV, Kašpárek T, Vaníček J, Bareš M. Disruption in cerebellar and basal ganglia networks during a visuospatial task in cervical dystonia. Mov Disord. 2017; 32(5):757-768.

SPEAKERS

S17 Genetic Background of Leukemia in the context of clinical practice

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Leukemia develops when the DNA in blood and bone marrow cells mutate or change, disabling their ability to control growth and division. In some cases, these mutated cells escape the immune system and grow out of control, crowding out healthy cells in the bloodstream and bone marrow.
Many genetic changes are found in leukemic cells; these changes affect cell division, cell self-renewal, cell dissemination, DNA repair or cause changes in the cell cycle. Plenty of these changes are somatic mutations, but in some patients, also germline mutations are found. Germline mutations can significantly increase the risk of developing hematopoietic malignancies in the individual.
In this review lecture, number of these mutations and their clinical features are described. These predispositions can be broadly classified as those leading to bone marrow failure, those involving tumor suppressor genes, DNA repair defects, immunodeficiencies or other congenital syndromes associated with hematologic disorders. While leukemia can develop as a secondary event in the syndromes, there are also several syndromes that specifically lead to the development of leukemia as their primary phenotype. Many of the genes discussed in this review can also be somatically mutated in other cancers, highlighting the importance of understanding shared alterations and mechanisms underpinning syndromic and sporadic leukemia.
The lecture will also mention a case of young patient with germline mutation predisposing to the development of leukemia. Now, the patient has no problems, but her risk of developing leukemia is very high. And this leukemia will certainly have a bad prognosis if it does occur. Thus, treatment options for this patient and options for preventing the transmission of this predisposition to offspring will also be discussed.

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SPEAKERS

S18 Human applications of genome editing through cancer immunotherapies

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Cellular immunotherapies using engineered T cells are being investigated widely, and successful trials against haematological malignancies have led to market authorisations and more routine availability of autologous cell products. Most therapeutic applications have employed gamma-retroviral or lentiviral vectors for ex-vivo gene-addition before re-infusion of cells expressing chimeric antigen receptors (CARs), and long-term safety data has accumulated over two decades. The emergence of genome editing technologies has enabled more complex manipulations, including efficient multiplexed gene knockouts and site-specific transgene insertion. Transcription Activator-Like Effector Nucleases (TALENs), Homing endonucleases, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 and cytidine deaminase base editors have been used to generate 'universal' donor CAR-T cells for use without the need for matching. While early phase trials have provided first in human data in the context of advanced malignancies, experience and confidence in the technologies is setting the scene for wider applications, including the correction of defined single gene disorders.

Acknowledgement: NIHR, MRC, Wellcome Trust

SPEAKERS

S19 The microbiome and its role in human cancer

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Microbiomics is a very active research field and the microbiome is now firmly established as an important factor in human health and disease, although mechanistic understanding still remains limited. However, recent technological advances have transformed classical microbiology towards more high throughput studies, allowing the investigation of more complex communities an towards a deeper understanding of the true underlying microbiome complexity.
Several studies found a strong association between microbial dysbiosis and cancer, suggesting that specific bacterial species can directly and indirectly modulate tumorigenesis by producing toxins or tumorigenic molecules and by causing severe inflammation or immune suppression. Colorectal and pancreatic cancers have been associated with community-wide changes in gut microbiome composition, and for some species there is evidence of causal effects. We explored the fecal and salivary microbiota as potential diagnostic biomarkers using shotgun metagenomic and 16S rRNA amplicon sequencing for Colorectal and PAncreatic cancer screening.

Acknowledgement: We are thankful to members of the Bork, Malats and Zeller groups for inspiring discussions and all contributions. Additionally, we thank the EMBL Genomics Core Facility and funding resources.

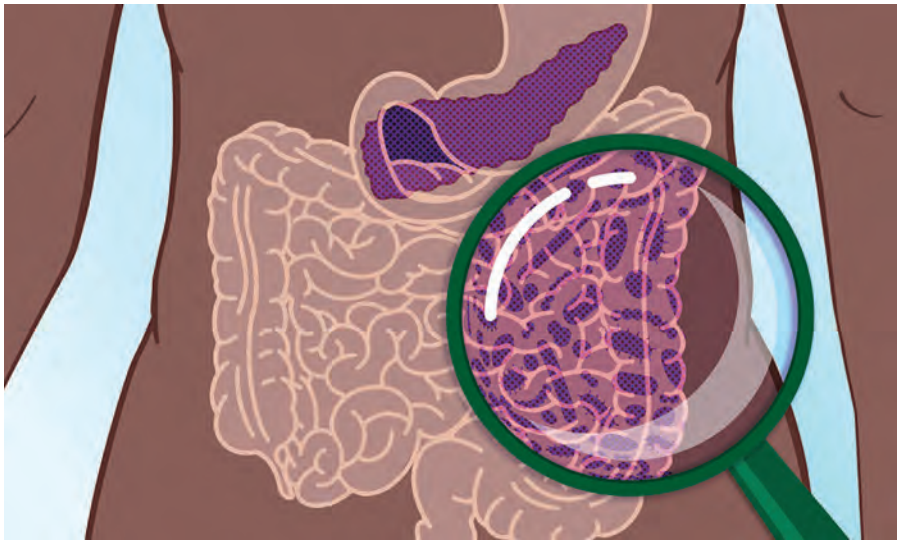

Fig. 1: Fecal microbiome-based detection may provide a non-invasive, cost-effective and robust approach to cancer diagnostics.

SPEAKERS

S20 Impact of genetic variability on enterovirus infection

Ishemgulova A.[1], Mukhamedova L.[1], Trebichalská Z.[1], Šmerdová L.[1], Moravcová J.[1], Hrebík D.[1], Buchta D.[1], Škubník K.[1], Füzik T.[1], Nováček J.[1], Plevka P.[1]

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Single-stranded RNA genomes of enteroviruses are replicated by viral RNA-dependent RNA-polymerases without proofreading activity, which results in frequent mutations that fuel the evolution of new species. Enteroviruses have diversified into numerous variants with different serological properties that circulate in the human population. The high prevalence of enteroviruses is enabled by variability in surface-exposed residues of enterovirus capsids. The distinct capsid surface properties allow the viruses to utilize different cell-entry receptors. However, in spite of the divergent antigenic properties, the viruses retain the common principle of capsid structure, cell entry, and genome release. Despite the use of various receptors, all enteroviruses detach from receptors soon after endocytosis and then reach the host cell cytoplasm by triggering a cellular mechanism to disrupt virus-containing endosomes. Subsequently, pressure from the genome induces enterovirus capsids to open. Comparative analysis of enteroviruses and related single-stranded RNA viruses provides another example that structures and mechanisms of biological functions are much more conserved than protein sequences.

Acknowledgement: The research leading to these results received funding from the Czech Science Foundation grant GX19-25982X to PP.

SPEAKERS

S21 Whatis new in sequencing technology?

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Sidney Brenner famously said that "Progress in science depends on new techniques, new discoveries and new ideas, probably in that order". The term Next Generation Sequencing (NGS) joins a series of disruptive new technologies, particularly for research and application in biology and medicine. NGS has passed through several distinct generations. 2nd Generation DNA sequencing, that followed Sanger sequencing, brought us the ability to sequence a human genome for less than 1000 Euros. In addition to using it to sequence entire genomes, many different methods have been developed by the research community to capture other levels of biological information such as transcriptomes, epigenomes and even proteomes. It was followed by 3rd and 4th generation sequencing technologies that extended into direct reading of DNA molecules, long-reads and the analysis of nucleic acid molecules in situ. Hardly anybody dreamt of these tools twenty years ago when the sequence of the human genome was published. However, now NGS is omnipresent and modern biological research is unthinkable without it. There are also major efforts to include NGS technologies into healthcare.
In this presentation I will review the current status of next-generation sequencing technology and show the impact they have in different domains such a personalized medicine and new technological applications. I will describe issues the introduction of NGS into personalized medicine cause and will also show recent technology developments, such as to carry out full-length sequencing of native DNA molecules of the mitochondrial genome to capture low level heteroplasmy and physically determine phase.

SPEAKERS

S22 Spatially Resolved Transcriptomics

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The spatial organization and structure of cells are essential elements in the regulatory as well as functional processes of multicellular organisms. In the past years, technologies like single cell RNA-seq (scRNA-seq) have emerged as important tools to facilitate the study of cellular heterogeneity in tissues due to their ability to profile each individual cell's gene expression. While scRNA-seq plays a major role in cell type discovery the original tissue structure is lost during sample handling and, therefore, the spatial context is missing. Several new technologies have been developed to overcome this issue and enable the study of spatial gene expression patterns. One such approach is capturing mRNA using barcoded poly(dT) capture probes organized as gridded areas of a solid slide. This method, originally published in 2016, called Spatial Transcriptomics has gained widespread popularity within the field and is, as of 2019, distributed by 10x Genomics as a commercial product under the name Visium.

SPEAKERS

S23 Next generation plasma proteome profiling of a pan-cancer cohort

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Cancer is the second-highest cause of mortality today and accounts for more than ten million deaths worldwide. It has a significant health burden on society and more precise methods for early detection and diagnosis are vital to improve the chances for long-term survival. Here, we describe an effort to generate a comprehensive map of the plasma protein levels in the context of cancer by profiling fifteen different cancer types.

Methods: Blood plasma samples from approximately 50-250 patients per cancer type were collected and analyzed using state-of-the-art omics platforms to interpret the plasma proteome sensitively and quantitatively. Altogether 1,800 samples have been studied to create a knowledge resource to identify shared molecular features for specific cancer types. Samples were collected as EDTA-plasma from patients and analyzed using two fundamentally different technology platforms
(1) Proximity Extension Assays based on Olink Explore 1536 and (2) a targeted proteomics workflow based on mass spectrometry.

Results: A regularized generalized linear model (glmnet) trained on 1,470 protein profiles measured with the PEA platform was used to identify protein panels that can distinguish between the studied cancer types with high precision (AUC: 1-0.87). The targeted proteomics dataset could identify unique patterns within the plasma proteome of multiple myeloma (MM) (AUC = 0.96).

Discussion: By utilizing data-driven systems, we can identify and classify novel biomarkers distinguishing different cancer types from each other using a pan-cancer approach. The study demonstrates the usefulness of "next-generation plasma protein profiling" to identify novel molecular signatures of importance for specific cancer types. Ultimately, this strategy can be used to stratify cancer patients based on a panel of biomarkers.

SPEAKERS

S24 Optogenetics: manipulating life and death of cells in space and time

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Being able to precisely control cellular processes in time and space allows a variety of experimental approaches in many disciplines from neuroscience to developmental and synthetic biology, biomechanics and immunology. Optogenetics provides a versatile method to control protein function from the sub-cellular to tissue scale with high temporal precision. This talk gives examples of how researchers at EMBL use optogenetics to control cell morphology and behavior, signaling and cell death in vivo.
To study innate immunity in the zebrafish skin we developed optogenetic tools based on the Cry-2olig system to induce inflammasome formation, as well as inflammatory and apoptotic caspases in single cells. We induce inflammatory reactions in specific cells within minutes to look at immediate cellular and tissue responses. To our surprise we found that the same molecular stimulus can elicit different forms of cell death when we compare morphology of dying cells and signals sent to neighboring cells.

Acknowledgement: Petr Broz & Kateryna Shkarina, University Lausanne

SPEAKERS

S25 Online Mendelian Inheritance in Man (OMIM), a catalog of human genes and genetic disorders and traits

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Online Mendelian Inheritance in Man (OMIM®) is the outgrowth of the book, Mendelian Inheritance in Man, by Victor A. McKusick, published in 12 editions starting in 1966, with 1600 entries, as a catalog of autosomal dominant, autosomal recessive, and X-linked disorders and transitioning in the last published edition in 1998 with 8400 entries, as a catalog of human genes and genetic disorders. OMIM has been freely available and searchable on the internet since 1987. OMIM is a continuously updated, comprehensive, authoritative compendium of human genes and genetic phenotypes (disorders and traits) with full-text, referenced overviews of over 8,300 phenotypes and over 16,700 genes. OMIM focuses on the relationships between phenotypes and genes (see figure). In addition to the descriptive entries, OMIM.org provides additional unique displays of information including the Clinical Synopses, Gene Map, and Phenotypic Series. OMIM has responsibility for naming and classifying genetic disease, as this was a necessary outgrowth of our work and MIM numbers are required by journals and other databases as unique identifiers for Mendelian phenotypes. At the core of OMIM are the expert biocurators and MD and PhD science writers who review, evaluate, and summarize prioritized relevant articles from the peer-reviewed biomedical literature into structured entries. OMIM has an unparalleled breadth and richness of description of human phenotypic variation that directly facilitates clinical care, disease-gene discovery, and translational science. OMIM is accessed by almost 3 million users annually from every country in the world because it is an essential resource not only for clinical geneticists but for other health care professionals who care for patients with genetic disorders and for researchers in many fields including molecular biology, genetics, genomics, bioinformatics, and drug discovery.

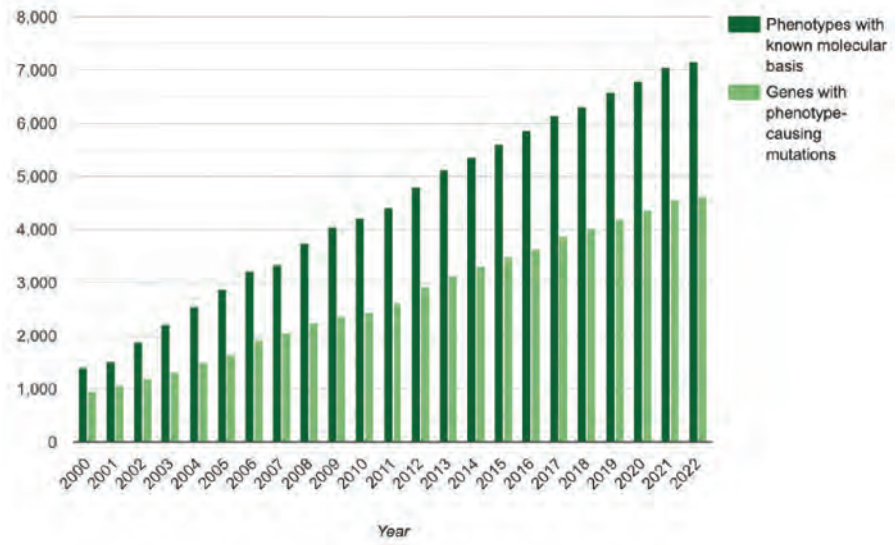

Fig. 1: The Growth of Gene-Phenotype Relationships in OMIM (as of 7 May 2022)

SPEAKERS

S26 Genomics and personalized medicine: ethical reflections

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While progress in genetics is impressive, this at the same time raises a conundrum of normative questions and challenges. This presentation sketches an overview of issues linked with present and future applications of human genetics in health care, taking account of a series of relevant documents and recommendations of the European Society of Human Genetics (ESHG). The focus will be, firstly, on conceptual, ethical and policy issues of reproductive and non-reproductive genomic screening, using prenatal screening, neonatal screening and 'opportunistic' screening for later-onset disorders as major examples. Secondly, the ethics of non-reproductive and reproductive (human) germline genome editing will be scrutinized.

SPEAKERS

S27 Human Person and Genetics Studies

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It is a fact that our Mendel is well known for his laws of genetic inheritance, and there has been little insight into the humanist approach that how he did and persevered in his research was part of his quest for truth and science.
Pope Francis describes Fratelli Tutti, as a "Social Encyclical" who used these words to "address his brothers and sisters and proposed to them a way of life marked by the flavor of the Gospel". The Encyclical aims to promote a universal aspiration toward fraternity and social friendship. In the background of the Encyclical there are the conviction that "no one can face life in isolation" and that the time has truly come to "dream, then, as a single human family" in which we are "brothers and sisters all".
Mendel searched for new things in nature and discovered the laws of genetic inheritance in it, taking the first step in the modern era to know how we relate to each other and how we inherit our genes, from one generation to the next. Global Bioethics provides us with an ethical framework for the large family that inhabits the common home: we are all shaped by the same natural laws. In order to follow, know and love these laws, we must be educated in them and in the scientific method.
The Church does not restrict her mission to the private sphere. While not engaging in politics she does not, however, renounce the educational dimension of life itself, attention to the common good and concern for integral human development, according to evangelical principals.Educational work is a great gift, first of all for those who do it, like Gregor: it demands a lot but also gives a lot. The constant relationship between educators, young people and researchers is an ever-living source of humanity, despite all the hardships and problems that it entails.

SPEAKERS

S28 Solidarity in Genetics: Every Citizen a Researcher?

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To many, "solidarity" means the Polish labor union that helped end the Soviet Union. Now the word appears in discussions of inequities in wealth, conflicts, and access to employment and education. One definition is "unity (as of a group) that produces or is based on community of interests, objectives, and standards." An imperfect synonym is "the common good." An antonym is "autonomy," which, since the Nuremberg Trials and the United Nation's Universal Declaration of Human Rights, has become a major principle of the biomedical ethics. Progress in medical genetics illustrates the tension between autonomy and solidarity. The fact that human and bonobo DNAs differ by only 5% supports the biochemical concept on one human family. Can (or must) a medical geneticist disclose to relatives a patient's newly discovered variant for a potentially lethal illness? The answer is "NO," based on the patient's autonomy, and "YES," based on solidarity and the common good. Another example is screening of all newborns for harmful disorders: To help one out of 300 newborns, the parents (or society, on their behalf) of the 299 unaffected babies allow a painful heel stick on the first day of life. Clinical genetics research likewise illustrates solidarity: patients give up some prlvacy and autonomy for the common good. Such research is expanding from cases series at academic health centers, to retrospective case-control protocols, to prospective population-based cohorts, some with genotyping. Examples are the UK Biobank, the US All-of-Us Cohort, the International Hundred Thousand Plus Cohort Consortium, and the massive linkage of national registries, e.g., Danish studies of neurofibromatosis 1. Increasing also is participation of members of the target populations in the design, goals, methods, conduct, analysis, and dissemination and benefits of results. So, yes, one can predict a future where every citizen is a contributing researcher, and, I think, Mendel would agree.

Acknowledgement: I thank many colleagues for contributing ideas by conference calls and emails for global celebrations and commemoration of the Bicentennial

SPEAKERS

S29 How Gregor Mendel helped Us Understand Plant Development and Evolution

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Mendel discovered the fundamental principles of inheritance using peas. Since Mendel numerous fundamental discoveries about the molecular mechanisms of life have been discovered using genetics in plants. Genetics has provided the tools to investigate the molecular mechanisms of plant growth and development. Combining knowledge from development, phylogenetics and evolution has led to the formulation of hypotheses about the mechanism that evolved when plants exploded in morphological diversity at and, or soon after plants colonized the continents and rapidly radiated into a diversity of forms that transformed the planet.
We used genetics to discover genes that controlled the formation of the first rooting structures to evolve among plants around the time they colonized the continental surfaces of the planet around 500 million years ago. Rooting structures give land plants access to water and nutrients and form a surface at which plants and microbes interact. Comparative morphology suggests that the ancestors of land plants did not form rooting structures. Tip-growing filamentous cells – rhizoids and root hairs – develop at the interface between the plant and the soil in the two monophyletic lineages of extant land plants, bryophytes and vascular plants. This suggests that filamentous rooting cells developed at the plant-soil interface in their last common ancestor. The presence of rhizoids at the plant-soil interface among fossil plants from the Devonian Period (258 – 301 million years ago) is consistent with this hypothesis.
We show that the ROOT HAIR DEFECTIVE SIX-LIKE (RSL) class I genes likely acted in common ancestor of the land plants. Then RSL class II genes contributed to the development of root hairs in the vascular plant lineage while RSL class III genes contributed to rhizoid development in the bryophyte lineage. These data demonstrate the nature of a molecular mechanism that acted in the last common ancestors of the land plants that are long since extinct. They also demonstrate how this mechanism evolved independently in different land plant lineages.

Acknowledgement: Research is funded by the European Research Council (EVO500 and DENOVO-P) and the Austrian Academy of Sciences.

SPEAKERS

S30 Stem cell regulators and cell proliferation control during organogenesis

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Organogenesis in plants is largely postembryonic and occurs in a continuous manner. New plant organs initiate in meristems where the stem cell niche remains active over long periods of time. Organ growth depends on the production of new cells, the stem cell derivatives, coordinated with their differentiation into various cell types. The functional links between stem cell regulators and the cell cycle machinery remains unclear. We have tackled this question in the root meristem of Arabidopsis thaliana by combining live imaging, genetics and computational modelling. A key step in this study is to determine precisely the duration of cell cycle phases during root development. This has been facilitated by the PlaCCI tool, an Arabidopsis line that expresses three cell cycle factors, labeled with different fluorescent markers (Desvoyes et al., Nat Plants, 2020, 6: 1330). Using live-imaging strategies we have measured cell cycle phase duration along the longitudinal axis, from the more distal region where the stem cell niche is located up to the proximal meristem region where cells arrest cell cycle and enter differentiation. We found that the G1 duration is strikingly different along the root meristem and shows a positional gradient ranging from ~2 h near the meristem boundary to >20 h in stem cells and early derivatives. Mutants in the PLETHORA (PLT) genes shortened G1 length, flattening its gradient. Computer modeling predicted the inference of a negative regulatory pathway. We identified the RETINOBLASTOMA-RELATED (RBR1) and the CDK inhibitor KRP5, a PLT target, as part of this negative branch. We propose that PLT genes play opposing roles, maintaining meristem and stem cell activity and inhibiting G1 progression by prevening RBR1 phosphorylation. This establishes a previously undescribed developmental feature of the root meristem in which a G1 duration gradient is shaped by stem cell and meristem maintenance regulators.

Acknowledgement: Funding: Spanish Ministry of Science and Innovation and Fondo Europeo de Desarrollo Regional FEDER, grant RTI2018-094793-B-I00 and European Union, grant 2018-AdG_833617.

SPEAKERS

S31 Sustainable Control of Disease Resistance – The Case For GM Wheat

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Worldwide wheat yields are reduced by 20% every year due to pest and disease. Safeguarding our wheat crop with pesticides is expensive, environmentally unfriendly and unsustainable. The wild grassy relatives of wheat represent a treasure trove of genetic resistance, however, introducing this resistance into domesticated wheat through traditional breeding is like crossing a racehorse with a donkey; it takes many years to combine the best of both worlds. However, if we could clone enough disease resistance genes from the wild relatives, then these could be delivered as transgenes into elite cultivars. A stack of multiple resistance genes holds great promise for long-lasting, i.e. durable disease resistance.
Faced with this task, we have developed fast and efficient methods for gene discovery and cloning which use mutant and natural populations followed by sequence alignment to locate genes. We also co-developed a method for halving the generation time of wheat and other crops, in a controlled environment, dramatically speeding up capabilities for research and breeding purposes.
Our focus is on wheat and its major diseases, in particular wheat rusts which throughout history have been associated with crop failure and famine. Our long-term aim is to engineer pyramids of rust resistance genes. I will present our enabling technologies and a roadmap for sustainable, disease resistant GM wheat.

Acknowledgement: This work was funded principally by the BBSRC-UK, 2Blades Foundation, and KAUST.

SPEAKERS

S32 Darwin, Mendel and the origin of species

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All living forms on Earth are characterized by discontinuity in their phenotypic variation. Charles Darwin's theory of 'Evolution by Natural Selection' explained variations within species, but not the discontinuity between them. Gregor Mendel uncovered the genetic origins of phenotypic variations, but it was not until Theodosius Dobzhansky merged the theory of evolution with Mendelian genetics in the 'Modern Synthesis of Evolutionary Theory.' Dobzhansky recognized the crucial importance of reproductive isolation between related taxa for speciation and proposed the genetic mechanism, known as Dobzhansky-Muller Incompatibility, that can explain the evolution of this barrier. One of the most studied reproductive isolation barriers between closely related species is sterility of their hybrids. I will summarize what we know about the genetic architecture of hybrid sterility in various species, with particular focus on hybrid sterility between closely related subspecies of the house mouse (*Mus musculus)*.

SPEAKERS

S33 Violation of Mendel's First Law: centromere competition in meiosis

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The concept that gametes are equally likely to carry either allele of a gene is at the core of Mendelian genetics. Selfish genetic elements can cheat by violating this principle to increase their representation in the gametes (and therefore in the progeny) by meiotic drive. Examples from a range of eukaryotes show that meiotic drive is a widespread phenomenon that impacts many aspects of reproduction, genetics, and evolution. Female meiosis provides the most straightforward way to cheat because only one egg is produced from two rounds of cell division, so selfish elements compete for transmission to the egg. I will discuss how selfish centromeres drive in female meiosis by subverting the chromosome segregation machinery. We developed hybrid mouse model systems and defined the underlying cell biological mechanisms, including functional differences between centromeres of homologous chromosomes and asymmetry within the meiotic spindle. We also showed that selfish centromeres exploit the same microtubule destabilizing activities that correct errors in every cell division. Finally, we established a conceptual model explaining both drive by selfish centromere DNA sequences and suppression by rapidly evolving centromere proteins, based on modulating destabilizing activities while maintaining essential centromere functions.

SPEAKERS

S34 Gametes and embryo research: assisted reproduction and beyond

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Assisted reproduction techniques are now well developed both in the human and animal field for the treatment of infertility or for the selections of desired genotypes in animals. The importance of this are of research was witnessed by the award of the Nobel prize for Physiology and Medicine to the pioneers of human in vitro fertilization that lead to the birth of Louise Brown, the first test tube baby, in 1978. The animal field contributed significantly in the last 50 years to this area by unravelling the requirements of oocyte growth and development as well as technologies for collection, culture and cryopreservation of gametes and embryos. These technologies have implications in several areas including medical treatment of human infertility, pre-implantation genetic diagnosis, advanced breeding of livestock species for agricultural and leisure, the rescue of endangered species like the Northern White Rhinoceros. Oocytes and sperm are also the totipotent cells present in mammals. The studies in nuclear transfer and cloning of animals since Dolly the sheep, generated the scientific basis and the fundamental knowledge that lead to the discovery of the induced pluripotent stem cells (iPSC). iPS hold promises that are at the basis of the new developing field of regenerative medicine. The ability to master and access in vitro the very early stages of mammalian pre-implantation development is also crucial for editing the mammalian genome. Genome editing can be performed on somatic cells that are later used in somatic cell nuclear transfer to generate animals carrying the targeted mutation as well as on zygotes around the time of in vitro fertilization. The integration of embryology with cellular and molecular biology with genomic is offering great opportunities for medical, biotechnology and agriculture applications.

SPEAKERS

S35 Multidisciplinary Approach to Identification of Gregor Johann Mendel's Skeletal Remains

Fialová D.[1], **Drozdová E.**[1], **Chocholová E.**[1], **Brzobohatá K.**[1], **Pardy F.**[2], **Šenovská A.**[1], **Svobodová H.**[1], **Tichý B.**[2], **Peška M.**[3], **Zůbek A.**[3], **Doubek M.**[4,5], **Pospíšilová Š.**[4,5]
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During archaeological excavation in June 2021 at the Central Cemetery in Brno, skeletal remains of five men from the Augustinian tomb were found. The aim was to identify Gregor Johann Mendel. Archaeological, anthropological, and genetic approaches were used for this purpose.
Methods: Coffin and grave goods were dated by archaeological investigation. For anthropological identification, methods for determining sex, age, body height, pathologies, and superprojection technique were used. The genetic analysis compared mitochondrial DNA from skeletal remains of all five men and twenty DNA samples from Mendel's personal belongings (e.g., hair from his books and swabs). Ancient DNA was extracted by two protocols in the specialized facility for ancient biomolecules at the Laboratory of Biological and Molecular Anthropology. Libraries for next-generation sequencing (NextSeq Illumina) were enriched by the myBaits Mito panel.
Results: The deepest coffin was dated archaeologically between 1883 and 1885. The coffin lay under the foundation of the Augustinian tomb (built in 1885), and inside were newspapers dated to October 1883. The anthropological analysis determined more than 60 years old man with average height stature and positively identified the skull with two Mendel's portraits and one photograph using the superprojection technique. Genetic analysis found the same 12 polymorphisms in the mitogenome from one hair from Mendel's book (Populäre Astronomie by Littrow) and in the skeletal remains.
Discussion and conclusion: Identification of Mendel's skeletal remains was successful using all three different approaches. The deepest coffin was dated to Mendel's funeral. Anthropological data had a good match with Mendel's characteristics. Finally, genetic analysis of mitogenome confirmed the same polymorphisms in skeletal remains and hair from Mendel's book. This paved the way for proceeding with the project and reading his entire genome.

Acknowledgement: This work was supported by project ROZV/FPP/01/2021 and CERIT Scientific Cloud LM2015085. The CF Genomics is supported by the NCLG research infrastructure (LM2018132 funded by MEYS CR).

SPEAKERS

S36 Body Remains of the Founder of Genetics Gregor Johann Mendel – a Case Study

Drozdová E.[1], Fialová D.[1], Chocholová E.[1], Novotná K.[1], Šenovská A.[1], Peška M.[2], Zůbek A.[2], Doubek M.[3,4], Pardy F.[5], Pospíšilová Š.[3,4]

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On the occasion of the 200th anniversary of his birth, interdisciplinary research of the skeletal remains of Gregor Johann Mendel was carried out by a team of researchers coming from Masaryk University. The aim of the research is a complex survey of skeletal remains found in the Augustinian tomb. The main focus is concentrated on the identification of the person of Gregor Mendel, on evaluation of biological traits of his body, and on obtaining and analysing his genetic information.
Case summary: The archaeological and anthropological field research at the Central Cemetery of Brno took place during the period from June 6th to June 30th, 2021, including the exhumation of the body remains from the uncovered coffins. A complex archaeological situation was discovered during the excavations that brought to light the skeletal remains of five monks buried in very diverse situations. All excavated skeletons and mummified tissues were further examined in the laboratory by anthropological and genetic methods.
Conclusions: The evaluation of the skeletal remains of abbot Mendel brought new information about his body parameters. His body was gracile with a relatively strong muscle system. The skull has characteristics typical for the members of modern period populations, it is broad and short. Mendel's face was gracile with a broad forehead and relatively small jaws. The cranial capacity of his skull was 1580 cm3 which is above the average cranial capacity of contemporary males. Despite his death at the age of 62, his biological age was 65, slightly surpassing his calendar age. Interesting is that he was approx. 168 cm tall, which was a moderate stature height in his time. From today's point of view, his stature is rather short. Most of the body characteristics can be confirmed in his photographs. Mentioned body traits are only an example of the results of anthropological analysis. The research brought more interesting findings of his body and mummified body parts and new knowledge of his phenotypic features or physiological traits.

Acknowledgement: The authors express their gratitude to the members of the Abby of St. Augustine in Brno who were very cooperative and supportive to realization of the research Project ROZV/FPP/01/2021.

Fig. 1: Coffin with body remains of Gregor Johann Mendel

Fig. 2: Skull of Gregor Johann Mendel

Fig. 3: Photo of Augustinians from Brno Abby. Gregor Johann Mendel is standing, is second from the right side

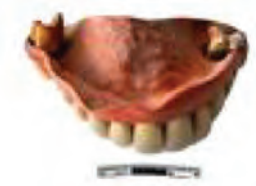

Fig. 4: Dental prothesis of Gregor Johann Mendel

SPEAKERS

S37 Reconstructing the Genome of Gregor Johann Mendel Using State-of-the-art Molecular and Bioinformatics Tools

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Reconstruction of the genome from ancient DNA (aDNA) is difficult process, hampered by low amount of available DNA, post-mortem damage of nucleobases and contamination risks. However, we applied state-of-the-art NGS methods and bioinformatics to inspect genomic features of Gregor Johann Mendel, founder of genetics.

Methods: aDNA was isolated from tooth using column-based isolation and concentrated with Amicon filter unit. After QC, Illumina Sequencing library was created using Swift 2S flexible kit (IDT), using molecular barcode oligo (IDT) and PreCR repair mix (NEB). Library was amplified with EvaGreen dye to optimize the cycling conditions. Libraries were sequenced using Illumina NovaSeq instrument with S4 chemistry in paired-end 150 cycles. The eager bioinformatics pipeline, freely available in the nf-core pipelines repository,was used to retrieve SNV/Indel variants and aDNA quality control metrics. Computationally demanding analysis was performed on cluster using Kubernetes technology.

Results: In total, we managed to reconstruct 91% of Mendel's genome, 99% of exome, with 9,26x coverage in average. We detected 4,1 million highly confident SNV/Indel variants genome-wide. Of them, 57308 occurred in the gene coding regions.

Discussion and conclusion: Using the latest approaches in the molecular biology and bioinformatics, we reconstructed Mendel's genome and identified genetic variants that might have shaped his life and health. Variants impact to phenotype is currently under investigation by biologists and physicians. In the opportunity of Mendel's bicentennial anniversary, we considered this as an important contribution and a gift to this genius.

Acknowledgement: We acknowledge the CERIT Scientific Cloud (LM2015085) and NCMG research infrastructure (LM2018132) for their support with obtaining scientific data presented on this poster.

SPEAKERS

S38 Metagenomic and Proteomic Analysis of Dental Calculus of Abbot Gregor Johann Mendel

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As part of the interdisciplinary research of abbot and founder of genetics, Gregor Johann Mendel, his dental calculus was sampled to gain insight into his health that could not be obtained by mere genome sequencing. Ancient dental calculus as a calcified dental plaque is a rich source of information. This precious material is used to study the diet, health, or habits of past populations based on the biomolecules trapped inside.
Methods: Sampling, decontamination, ancient DNA extraction and amplification, and the first stages of protein extraction were carried out in the specialized facility for ancient biomolecules in the Laboratory of Biological and Molecular Anthropology. DNA and proteins were extracted with protocols modified for ancient biomolecules. LC-MS/MS analysis performed on timsTOf Pro combined with nanoElute (Bruker). The prepared metagenomic libraries of variable regions V1 and V6 were sequenced on the NextSeq Illumina platform. Scanning electron microscopy with energy dispersive X-ray analysis was performed on Magellan 400/L (FEI).
Results: A total of 469 ancient proteins were found after filtering, including proteins related to immunity, habits, oral health, or diet. Multiple oral species connected to dental pathology such as periodontitis were confirmed based on both DNA and proteins, such as *Porphyromonas gingivalis*, *Tanarella forsythia*, or *Treponema denticola*. High abundance of Clostridiales was confirmed by metagenomics.

Discussion and conclusion: Dental calculus was showed to preserve valuable ancient biomolecules offering a new insight into the life and death of Mendel. For example, results are consistent with the deteriorating dental health, especially severe periodontitis Mendel suffered from. Bacterial composition also confirms groups involved in mummification process, which agrees with the stage of preservation of the studied remains.

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SPEAKERS

S39 Johann Gregor Mendel – His family and origin

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Here I show the family tree, tell something about his birthplace in Heinzendorf, where he spent his childhood, about the people who helped him to go to Secondary School in Troppau and his further way to the St. Thomas Monastery in Brünn. In the end I mention his nephew Alois Schindler, who was an intimate friend and the witness of Mendel's death and autopsy in January 1884.

SPEAKERS

S40 Mendel's birth date

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The literature gives two dates for Mendel's birth, 20th or 22nd July 1822. In his paper for Folia Mendeliana (1972), P. W. van der Pas wrote that a solution to this problem will probably never be found. A. Matalová notes that in the surviving documents, the date of Mendel's birth is consistently given as 22nd July 1822. There are only two exceptions to this in numerous archival documents: the parish register of the village of Vražné, and a baptismal certificate derived from it twelve years later, in 1834. In both of these exceptions, the date 20th July 1822 is identically written as the day of Mendel's birth. No date of birth is included in any education certificate, passport or registration form of the University of Vienna. Some authors offer an explanation for the existence of two dates. This explanation has become very popular. Mendel's baptismal certificate, issued by the parish priest Johann Edmund Schreiber in 1834, was based on the original entry in the parish register. In the baptismal certificate, Schreiber confirms the date of birth in the parish register as being 20th July 1822. Schreiber was a conscientious priest and teacher and obeyed the imperial order to give the date of birth rather than the date of baptism. Therefore, Mendel's baptismal certificate is, in fact, his birth certificate with a date of birth of 20th July and not a baptismal date of 22nd July. However, neither Mendel himself nor his family respected this date and consistently gave the date of baptism instead of the date of birth. Therefore, we probably encounter two dates. However, Mendel himself always celebrated his birthday on 22nd July, the feast of St. Mary Magdalene. So, the question today is not when Mendel was actually born, but why did he always give a date other than the official date from the parish register? Apart from the answer that it was due to tradition in Mendel's family, another reason can be suggested: Mendel's special relationship to mathematics and symmetry.

SPEAKERS

S41 A New Reconstruction of Mendel's 1865-lectures and a Content Comparison with the 1866-paper

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Recently discovered historical sources allow us to make a new reconstruction of Mendel's two lectures at the Brünn Natural Sciences Society early in 1865 and to understand the relationship between the content of the lectures and his famous 1866 paper. In the lectures, Mendel announced the results of his pea-crossing experiments to the world. They are monumental scientific milestones in biology, comparable in significance to the papers by Darwin and Wallace read at the Linnean Society meeting in 1858.

We describe first the setting of the lectures and then the content as reported in newspaper articles a few days later. Based on these articles, we analyse the correspondence between the contents of the lectures and the 1866 article.

We find indications that Mendel added text to the discussion section of the manuscript, probably influenced by the reception of his lectures. Therefore we must be careful in interpreting the 1866 paper concerning the original motives of the experiments, which Mendel devised more than 12 years earlier.

SPEAKERS

S42 Mendel's Contemporaries: Convergence and Strategies in 19th Century Plant Breeding

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In the second half of the 19th century, empirical breeders made use of key plant breeding techniques such as hybridization and selection. In contrast to Gregor Mendel's manifold motivations for his research, they focused almost exclusively on the development of new varieties. Therefore, the aim of the present investigation is to highlight connections and common grounds in the state of knowledge among contemporary actors, as this might have influenced Mendel and other researchers in the field.

Methods: A review of literature and a comparative analysis of key findings published by 19th century plant breeders were carried out. Major findings by Charles Naudin, Wilhelm Rimpau, William J. Farrer, William J. Spillman, Pehr Bolin and Emanuel von Proskowetz were discussed comparatively.

Results: Experimental hybrid researchers and empirical plant breeders working independently of each other and in different countries described all the main phenomena which were similarly elaborated by Gregor Mendel. This includes the uniformity of the F1 generation or the segregation of parental characters in F2. In addition, a Moravian contemporary of Gregor Mendel, Emanuel von Proskowetz, for the first time outlined the importance of landraces in plant breeding (Haná pedigree barley).

Discussion and conclusion: Although 19th century empirical plant breeders were aware of various phenomena associated with progeny from crosses, they did not carry out quantitative analysis of segregating populations. Gregor Mendel might informally have been aware of these phenomena, but he carried out crosses based on the hypothesis of the existence of hereditary information in germ and pollen cells, which had large explanatory and predictive power. In contrast, Emanuel von Proskowetz – although working in the same geographic region as Mendel – seemed to be unaware of Mendel's results. However, his successful strategy of utilizing landraces led to "genetic resources" management in later decades.

SPEAKERS

S43 New insights from a new critically commented edition of Mendel's classic article on plant-hybridization and its role in the transformation of science and agriculture

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G. Mendel's article on plant-hybridization was first presented to the public in 1865. While initially mostly ignored, his work since then not only became the starting point of a new scientific discipline called Genetics, but also unfolded an unpredictable influence on the transformation of science in general. His work soon turned into one of the great standard texts of modern natural sciences. Thus, in the meantime almost every generation of scientist since the rediscovery of Mendel's work found their own way to read and interpret his work. Yet, especially in Agriculture the influence of this work is much harder to quantify. Shortly after the rediscovery lauded as the future for plant breeding, it is retrospectively surprising to note that the exact application in early 20th century breeding programs is often vague. Especially in the first decades of the 20th century Mendelian Genetics was promoted to a core discipline and plant breeding and agricultural stations around the globe were established. While those were partly successful and helped to further promote Genetics it became only the Green Revolution, the rapid increase of crop yields from new varieties and a new understanding of plant crossings that made hybridization and genetics a tool of choice to feed the world. Notably this was a development in which leading figures in Biology and Plant Breeding heralded Mendel's work again, yet then turning a stronger focus on additional effects such as heterosis. The new German edition of his article tries to highlight how there could have been so many different readings over more than a century. Furthermore, the edition also tries to clarify key aspects of his experiments including for example new insight on the early origins of his experiments, their historical background and the selection of plant material. It also provides a fresh view on the people that influenced his work and interest in other areas of science, e.g. Meteorology, Plant Physiology, and Apiology.

SPEAKERS

S44 The Role of the Cold War in Transforming a Statistical Puzzle about Mendel's Pea Data into a Scientific Scandal

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The history of interest in the question of whether, statistically considered, Mendel's data from his hybrid-pea experiments are "too good to be true" has an intriguing structure. When the British mathematician and evolutionary theorist Ronald Fisher published his classic analysis in 1936, knowledge that Mendel's data conformed improbably closely to the predictions of his theory was long familiar among specialists. Furthermore, for decades after Fisher published, the issue largely remained a matter for specialists to puzzle over. There was no "Mendel-Fisher controversy," and no public hand-wringing about Mendel's truthfulness. What turned this long-running minor concern into a major scientific scandal, I will suggest, was a particular 1960s/70s conjunction of historical developments, notably (i) the centennial celebrations of Mendel's 1865 lectures and 1866 paper; (ii) the changing cultural dynamics of the Cold War, on both sides of the Iron Curtain; and (iii) a new public mistrustfulness towards science, and scientists, in the West.

Acknowledgement: My talk will draw on research set out in my paper "Mendel the Fraud? A Social History Truth in Genetics," *Studies in History and Philosophy of Science* 93 (2022): 39-46.

SPEAKERS

S45 The fate of Mendel's grapevine

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Japanese people are keen to know what Gregor Mendel discovered from the beginning. Shortly after the rediscovery of Mendel's rule, the first lecture on Mendel's rule was given by Dr. Y. Hoshino of Sapporo College of Agriculture in 1902. In 1906, Dr. K. Toyama found the segregation of cocoon colors of silk worm followed the Mendel's rule. When the Mendel stature was founded at Brno by the initiative of Prof. E. Tschermak in 1910, Japanese botanists spent certain amounts of budget for this construction. When Prof. M. Miyoshi visited St. Thomas monastery at Brno in 1913 during his world trip, the Mendel's grapevine was offered to him as the expression of their thankfulness and transported to Tokyo via Siberian railway. Since then it is vigorously growing at the Botanical Garden of the University of Tokyo. However, during the Russian domination after the World War II in Czechoslovakia, the original grapevine was lost from the monastery. After the velvet revolution in Czechoslovakia, people at Brno knew that the Mendel's grapevine survived in Tokyo and wished eagerly to get back cuttings of the grapevine. However, as the first cutting did not survive, the second one was sent to Brno. People in Tokyo didn't know the fate of the grapevine which came back to home. When I was nominated to be an Associate Member of the EMBO in 1998 and was asked to speak of my studies at the Members' meeting held at Prague, my fellow colleagues at the Botanical Garden asked me to confirm the fate of the grapevine. After the meeting, I visited Brno and confirmed that the grapevine started to grow at the Botanical Garden of Mendel University. This visit aroused me to study the life and the science of Mendel and I have been in Brno several times. As I am involved in various activities of the Japan Mendel Society, I would like to tell you these including the current plan to organize the special exhibition to celebrate 200 years from the birth of Mendel in Japan.

SPEAKERS

S46 Development of medical genetics in the Czech Republic

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In 1950 Lysenkoism was introduced but had not been accepted in medical genetics. In 1962 prof. M. Hašek on a plenary session of the Czech Academy of Sciences refuted it as a non- scientific theory. Subsequently, in 1961 je supported the creation of 1st department of medical genetics at Faculty of Pediatrics, Charles University. Lysenkoism was officially abandoned in 1965.
In 1966 the Czech Ministry of Health through the 1st Conception of Medical Genetics legalized medical genetics as medical speciality, and fostered obligatory creation of departments of medical genetics at all medical schools and established postgraduate medical education. In 1962 the Section of Medical Cytogenetics of the Biological Society. In 1967 the Czech Society of Medical Genetics of the Czech Medical Society of J. E. Purkyne was founded, and by the end of 1969 six departments of medical genetics and 7 cytogenetic laboratories started their operation.
In 1971 the first successful prenatal cytogenetic diagnosis was performed, and since then prenatal prevention of chromosomal aneuploidies and metabolic disorders in close cooperation with Western European biochemical laboratories. Prior to 1979 all regional departments guaranteed complex genetic services, including prenatal diagnosis with ultrasound and obstetrical examination in pregnancies with a genetic risk.
In 1980 the new government Conception of Medical Genetics incorporated genetic services into national health care system and funded their further development. First trimester prenatal diagnosis was introduced, as well.
Between 1990-2022 there has been a substantial progress in early diagnosis, prenatal screening, prevention and treatment of genetic disorders, including development of private genetic centres. There has been extended international cooperation and support from national/European grants, broader molecular genetic examinations for individualized medical genetics diagnostics and care still fully reimbursed by the Czech health care system.

SPEAKERS

S47 An Analysis of Mendel's Two Hybridist Theories and of Their Relationships

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Based on a statistical analysis of his experiments, which was a novelty for the tradition of "horticulturalists" (or "plant breeders") as well as for the tradition of "hybridists", and seeking a "generally applicable law governing the formation and development of hybrids" (Mendel 1865: 3), Mendel states "the law of development/evolution found for Pisum" (Mendel 1865: 32). When he tries to provide the "foundation and explanation" (Mendel 1865: 32) of the law of formation and development of hybrids, he does it in terms of the production and behavior of egg cells and pollen cells, and, ultimately, in terms of the nature and behavior of what he calls "elements" (Mendel 1865, p. 41) or "cell elements" (Mendel 1865, p. 42). Besides, Mendel recognizes the existence not just of hybrids that behave like those of Pisum – i.e. of "variable hybrids" – but also of hybrids that "remain perfectly like the hybrid and continue constant in their offspring" (Mendel 1865: 38) and "acquire the status of new species" (Mendel 1865: 40) – i.e. of "constant hybrids" (Mendel 1869: 27-28, 31). The law that would govern the behavior of constant hybrids would also find its foundation and explanation in terms of the nature and behavior of elements (or cell elements). Mendel's hybridism consists of two theories: a theory that moves on a more "empirical" level, according to Schleiden's first "special guiding maxim" (Schleiden 1849: 141,142, 146), which can be called "Mendel's theory of the development/evolution of hybrids" (DEH), and a theory that moves on a more "theoretical" level, according to Schleiden's second "special guiding maxim" (Schleiden 1849: 146, 148), which can be called "Mendel's theory on the cellular foundation of the development/evolution of hybrids" (CFH).
The aim of this communication is to present an analysis of these two theories and of their intertheoretical relationships, carried out within the framework of the so-called Metatheoretical Structuralism.

S48 *SPEAKERS*

Genius Loci: Mendel in Context of Central and Peripheral Categories

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Mendel's isolation is often stressed as a chief factor for the delay of the acceptance of his ideas. Yet, we document an interesting historical feature how works on a periphery of science influenced some of the most conceptual aspects of radical scientific developments thus turning isolation to an advantage. Our examples range from various sciences.
Even in 19th century the information was good but the location mattered. And Mendel's position was unique in this context.
This is a joint work with Prof. Helena Nešetřilová. It is an elaboration of some ideas of the following paper:
Jaroslav Nešetřil, Helena Nešetřilová: Remarks on Mendel's mathematics, Folia Mendeliana 53/1-2, 5--22.

Acknowledgement: This research has been partially supported by ERC Synergy grant DYNASNET, grant No. 810115.

SPEAKERS

S49 On the Bicentennial of Mendel's Birth, Attempting to Recover Mendel's Inheritance Principles with Mendel's Eyes

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It was reported that at the second lecture in 1865 Mendel directly presented his discovery of inheritance as the principles regarding plant reproduction. Here, we recovered Mendel's mathematical expression representing the F1 hybrid reproduction by using the symbols of his own: $Aa \times Aa \rightarrow (A + a)(A + a) = A/A + a/a + A/a + a/A = A + 2Aa + a \rightarrow 3A + a$. Clearly, Mendel's pair of symbols, *A*, and *a*, had three implications on different occasions, gametes, factors, and traits, and the sequential equations could perfectly represent "the principles of gamete formation, of fertilization, and of seed development", quite consistent with his lecture content in Brno. Jointly, it is Mendel's Gamete Theory of Particulate Inheritance, all could be picked out between the lines of the 1866 paper.
Principles of gamete formation
m: Law of segregation:
Aa (♀)→ (*A*+*a*); *Aa* (♂)→ (*A*+*a*); *A*/*A* → (*A*+*A*); *a*/*a* → (*a*+*a*);
n: Law of free combination:
AaBb(♀) →(*A*+*a*)(*B*+*b*) = (*AB*+*Ab*+*aB*+*ab*);
AaBb(♂) →(*A*+*a*)(*B*+*b*) = (*AB*+*Ab*+*aB*+*ab*)
Principles of fertilization
o: Law of random fertilization: (*A*+*a*)(*A*+*a*)→*A*/*A* + *a*/*a* + *A*/*a* + *a*/*A*
p: One pollen factor uniting one egg factor: (*A*)(*A*)→*A*/*A*; (*A*)(*a*)→*a*/*A*; (*a*)(*A*)→*A*/*a*;
(*a*)(*a*)→*a*/*a q*: Equal contribution of parents: *A* (♀) = *A* (♂); *a* (♀) = *a* (♂); *A*/*a* = *a*/*A*
Principle of Seed Development
Law of qualitative characters inheritance
r: Gene and character; *A*→ A, *B*→ B, *C*→ C…;
Character = *r*(*Gene*): Character ∈ {A, B, C, ……}; *Gene* ∈ {*A*, *B*, *C*, ……}
Trait difference = *r*(*Allele difference*): Trait difference ∈ {A1-a, A2-a, A3-a, ……An-a}; *Allele difference* ∈ {*A1- a*, *A2- a*, *A*3- a, ……An- *a*}
s: One pair of alleles and one pair of contrasting traits: "*A*, *a*"→"A, a" *t*: Genotype and phenotype: *A*/*A* + *a*/*a* + *A*/*a* + *a*/*A* = A + 2Aa + a *Law of interallelic interaction*
u: complete dominance: *A*/*A* + *a*/*a* + *A*/*a* + *a*/*A*→ 3A +a;
v: incomplete dominance (co-dominance): *A*/*A* + *a*/*a* + *A*/*a* + *a*/*A*→ A + 2Aa + a
Law of quantitative characters inheritance
w: Polygeny, epistasis plus environment determining quantitative traits
(*A1* + 2*A1a* + *a*)(*A2* + 2*A2a* + *a*)(……) + Environment →

1.	A1A2	2A1aA2	1A2a
2.	A1A2a	4A1aA2a	2A2aa
3.	1 A1a	2A1aa	1aa

Acknowledgement: We are very grateful to the Organization Committee of Mendel Genetics Conferrence, for their hard work as well as for the admission of the abstract presentation.

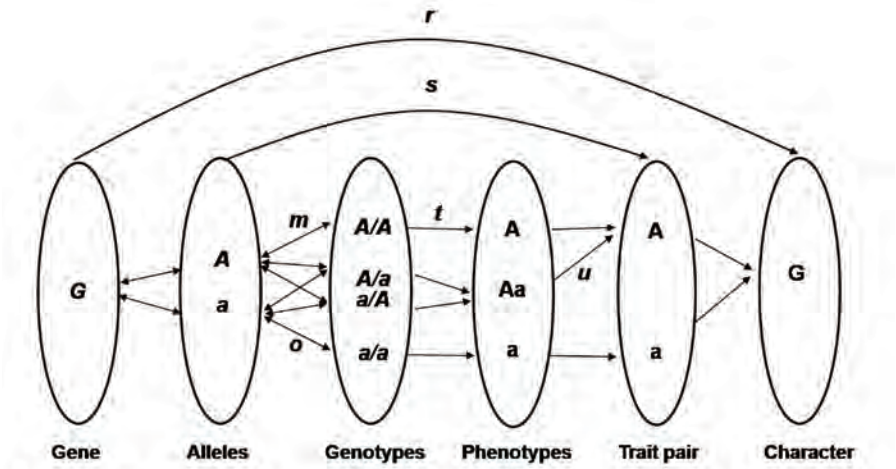

Figure 1 Mendel's mapping relationships between gene and character, alleles and traits, genotypes and phenotypes in language of modern genetics, but Mendel's gene and character are both italic.

Fig. 1: m: Law of segregation; o: Law of random fertilization; r: correspondence between Gene and character; s: correspondences between allele pair and trait pair; t: correspondence between genotype and phenotype; u: complete dominance of interallelic interaction

SPEAKERS

S50 Mendel's Genetic Algorithms Optimize Problems in Business and Economics

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Gregor Johan Mendel is the founder of genetics and is considered the discoverer of the basic laws of inheritance, which later became known as Mendel's laws of inheritance. His laws have affected many areas. In connection with the development of computers, the laws were used in the creation of so-called genetic algorithms. Especially, computer calculations are performed in binary form (0,1) and the fact of the successful development of mankind, was the inspiration for the application of the law of evolution in nature to the computer. Computer implementation of genetic algorithms began to appear in the 1970s and is associated with the names of J. Holland and D. E. Goldberg. Genetic terms were used: selection (selection of the strongest individual), crossover (creation of a new individual) and mutation (random change in an individual). This process forms one generation (iteration in the computer) and is repeated. A genetic algorithm is a heuristic procedure that seeks to apply the principles of evolutionary biology to find solutions to complex problems for which there is no applicable exact algorithm. The algorithm began to be used to optimize processes in the technical sciences, and its success spread to other fields, including economics and business. The reason is that optimizing the processes that the entrepreneur solves is maximizing profits and minimizing costs. Perhaps most famous problem is the Travel Salesman Problem. Various optimization tasks are solved e.g., minimization of material consumption, minimization of waste in cutting plans, minimization of distribution costs, waste collection costs, planning of optimal production etc. Various modifications of genetic algorithms have been created. But genetic algorithms based on Mendel's laws were among the first ones and still in use. Development has not stopped, new genetic algorithms are being applied to quantum computers, which significantly speed up calculations.

ABSTRACTS OF PARTICIPANTS

INHERITED DISEASES

P01 Application of Array CGH in Diagnostics of Mendelian Disorders

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Mendelian cytogenetics links structural chromosomal anomalies and monogenic diseases. Up to 15% of all mutations causing monogenic diseases are submicroscopic deletions and duplications, from 1kb to 10 Mb in size. Microarray based comparative genomic hybridization (array-CGH) represents a powerful method for mapping these genomic copy number alterations.
We will present several examples of monogenic diseases that have been diagnosed by array CGH at the Institute of Human Genetics, University of Belgrade.

Methods: The array-CGH procedure was performed using Agilent oligonucleotide microarrays in format 8x60K. Patients with pathogenic variants that include one gene responsible for a specific phenotype, regardless of whether other genes are involved, were selected for the research.

Results: We identified four patients with well known disorders that could be caused by mutations or deletions in a single gene: three had Phelan McDermid syndrome (MIM606232), with deletions ranging from 240 kb to 7.97 Mb, encompassing SHANK3 gene; one patient had Mowat Wilson syndrome (MIM235730) with deletion of 2,9 Mb including ZEB2 gene. Three patients had duplications, two of them with MECP2 duplication syndrome (MIM300260) and one with Charcot Marie Toothe type IA (MIM118220) caused by duplication of PMP22 gene. Other patients had rare variants: two siblings with intragenic deletion of ERBB4 gene and one patient of NRNX1 gene, and two unrelated patients with intragenic duplication of MYT1L. All patients had various neurodevelopmental disorders and/or congenital anomalies. One patient had specific phenotype with lymphedema caused by deletion of 5 Mb in 4q34.1-q34.3 region, including VEGFC gene.

Discussion and conclusion: Sophisticated DNA technology such as array-CGH significantly increases monogenic diseases diagnostic yield and accelerates novel gene discovery which will help many more patients with genetic conditions than previously thought possible.

INHERITED DISEASES

P02 Molecular Genetic Diagnostics of Neuromuscular Diseases

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Introduction: Neuromuscular disorders (NMDs) are a broad group of conditions that affect muscles, nerves, and neuromuscular junctions. NMDs are frequently inherited and extremely heterogeneous with more than 600 implicated genes. Due to the significant clinical and genetic heterogeneity, either for the many genes involved and the great variety of mutation types in a single gene, NMDs are often challenging to diagnose. Next-generation sequencing (NGS) techniques have helped greatly in diagnosing NMDs and underpinning the genetic pathways.

Methods: We present results of molecular genetic diagnostics of NMDs obtained by the application of NGS at the level of a selected list of genes that are known to be associated with NMDs and at the level of whole exome.

Results: Totally, 1500 patients with NMD suspicion were analysed. Genetic diagnosis was confirmed in 32% of these. In addition to single nucleotide variants and small insertions/deletions, we identified large gene rearrangements.

Discussion and Conclusion: Our study points to the need to process NGS data both for the identification of small scale variants, and for large gene rearrangements by analysis of copy number variations. Our study also shows that diagnostics NMD in diagnostic practise should be based on different methodological approaches, i.e. in addition to NGS on other techniques such as multiplex ligation-dependent probe amplification, repeat-primed PCR, Souther blot and hybridisation, and comparative genome hybridization.

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INHERITED DISEASES

P03 Monogenic Diabetes in Slovakia

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Monogenic diabetes is a disorder where the result of DNA diagnostics can influence the patient management, as some subtypes allow alternative treatment to insulin therapy. The aim of the study is to describe the mutational spectrum of monogenic diabetes in Slovakia and to perform functional studies of variants identified in one of the monogenic diabetes genes - HNF1A. Patients and methods: Since 2004, DNA samples from 637 patients with suspicion on monogenic diabetes and from 704 relatives were collected throughout Slovakia. The relevant genes were analysed using Sanger sequencing, MLPA or NGS approaches. Variants in the HNF1A gene encoding transcription factor HNF1α were functionally analysed using luciferase assay, EMSA, and immunofluorescence staining for nuclear localization. Results: We established molecular diagnosis in 198 out of 637 probands (31%). 125 had causal variant in GCK, 39 in HNF1A, 8 in HNF4A, 6 in mtDNA (m.3243A>G), 5 in HNF1B, 2 in ABCC8, 5 in KCNJ11, 4 in the INS gene, and single cases in RFX6, WFS1, TRIM37, and EIF2AK4 genes. Subsequent analysis of family members identified further 221 patients. To confirm the pathogenicity, we functionally tested 8 HNF1A variants that were newly described or classified as variants of unknown significance (VUS). The pathogenicity was proved in 5 tested variants, as they showed reduced transactivation activity (<40%) and/or decreased DNA-binding ability of HNF1α (<40% of the wild-type). Nuclear localization was not altered in any of the examined mutated proteins. As a result, we were able to reclassify 4 tested variants as likely pathogenic, and 2 variants as likely benign/benign. Conclusions: The main cause of monogenic diabetes in Slovakia are mutations in the GCK gene (63% of probands), followed by HNF1A (20%). Functional studies are important for correct classification of variants and lead to proper diagnosis and accurate treatment.

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INHERITED DISEASES

P04 Development of strategy for screening primary immunodeficiencies by measuring the number of copies of TRECs and KRECs by RT-PCR with further analysis of melt curves

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Primary immunodeficiency diseases (PID) are a clinically and molecularly heterogeneous group of inborn errors of immune system. It occurs in approximately 1-100 in 100 000 newborns in the world, but, unfortunately, many patients do not receive a timely diagnosis, which is crucial for an appropriate treatment. The quantification of T-cell receptor excision circles (TRECs) and κ -deleting recombination excision circles (KRECs) by qPCR has been used for screening most of PID in USA and several European countries. Unfortunately, there is no such screening program in Ukraine. We propose a modified method of screening for PID by using qPCR with further analysis of melt curve. The melt curve analysis allow us to eliminate fluorescence of primer dimers and other non-target PCR products.
Genomic DNA was extracted from dried blood spots (DBS) with AmpliSens® DNA-sorb-B nucleic acid extraction kit. In extracted DNA, the copy number of TREC and KREC were measured by qPCR reaction with 5xHOT FIREPol® EvaGreen® qPCR Mix (no ROX) and appropriate primers. The copy number of albumin gene were considered as an internal control. We used patients with known immune abnormalities as outer positive control. We tested 8 000 newborns from Ternopil region to develop a strategy for screening program for PID in Ukraine. Since we observed amplification even in NTC wells due to primer dimers, it was proposed to use evaluating melt curve profiles. A primer dimer or nonspecific type amplification will result in DNA products with a melt peak different from that of the correct qPCR product. In our case, melt peaks of TREC and KREC were 87.5°C and 87°C, respectively, while melt peaks of non-target product – 76°C or 83°C. Even considering 16.2% of patients required qPCR re-testing and 2.25% third retest from another DBS, the cost of screening for PID was 1.35 EUR per 1 patient, which is justifiable in terms of eliminating or decreasing adverse health consequences.

INHERITED DISEASES

P05 Association of rs634008 LRP5 Gene Polymorphism with Biochemical Markers and Densitometric Parameters in Slovak Postmenopausal Women with Osteoporosis

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Introduction and aims: The LRP5 gene has a significant effect on the WNT signaling pathway, which is closely related to osteoblast differentiation and regulation and bone density regulation. The aim of our study was to determine the possible association of the rs634008 LRP5 gene polymorphism with selected densitometric parameters (BMD, T-score) and biochemical markers (OC, CTx, Ca, P, Mg) in Slovak postmenopausal women with osteoporosis.
Methods: The research group consisted of 234 postmenopausal women, which we divided according to the T-score into two groups osteoporosis (OP = 114) and control group (CG = 120). Genomic DNA was isolated from peripheral blood using a commercial NucleoSpin® Blood Macherey-Nagel kit according to a standard protocol. Real-Time PCR and TaqMan® SNP Genotyping Assay according to the appropriate protocol were used for genotypic analysis. Biochemical marker analyzes were performed using Cobas Integra 400 plus analyzers (Switzerland) and Cobas e411 (Japan). Densitometric measurement of postmenopausal women was performed with a DXA Hologic body densitometer (DXA HologicDiscovery, Hologic Inc., Waltham, MA, USA).
Results: Based on Student's T-test, we found statistically significant differences ($p<0.001$) between the two groups of postmenopausal women between all monitored genotypes (CC, CT and TT) in ALP, Mg, BMD and T-scores in the area of femoral neck and lumbar spine. Using a Kruskal-Wallis nonparametric analysis of variance, we found statistically significant differences in the group of women with osteoporosis between the genotypes TT and CC ($p <0.025$) and CT and CC ($p <0.048$) in the biochemical parameter osteocalcin.
Discussion and conclusion: Our results provide initial information on the representation of genotypes and alleles of the rs634008 LRP5 gene polymorphism in the Slovak population of postmenopausal women. In the future, it would be beneficial to carry out further studies of the rs634008 LRP5 gene polymorphism and to expand the research population of postmenopausal women.

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INHERITED DISEASES

P06 Mendel's Laws of Inheritance in Preimplantation Genetic Testing

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More than 150 years have passed since Johann G. Mendel published revolutionary discoveries of the principles of inheritance, but even today we still use these basic genetic laws in methods of Preimplantation Genetic Testing of Monogenic diseases (PGT-M). In 1990, prof. Alan Handyside successfully tested embryos for X-linked disease for the first time and thus began a whole new era. Since then, PGT has experienced a dramatic evolution leading to today's methods that allow us to test embryos for any possible monogenic disease.

Material and methods: In our laboratory, we started performing PGT-M in 2007 using haplotype analysis on STR markers, but the real change came in 2014 when we adapted the SNP array-based Karyomapping platform. This approach uses up to 300,000 polymorphic SNPs spanning the entire human genome, allowing us to perform linkage haplotype analysis for all monogenic diseases together with aneuploidy and polyploidy detection. Using Karyomapping, we have already analyzed 3840 embryos of a total of 602 couples.

Results: During the time we have been using Karyomapping, we have analyzed embryos with 365 unique monogenic diseases. The most commonly affected genes (*HTT, CFTR, FMR1, HBB, PKD1, BRCA1*) cover 35% of all PGT-M cases, but the remaining are rare variants. The SNP array also allows us to detect microdeletions and microduplications, which are responsible for almost 9% of cases. One of the exceptional uses of this universal haplotype analysis is to determine the HLA match between a sick child with, for example, leukemia and embryos. In two cases we were able to find HLA compatible embryos.

Conclusion: PGT-M has undergone a great development in its relatively short existence. Currently, we are able to prevent the transmission of any monogenic disease with a known causal mutation to offspring. Together with the increasingly popular carrier panels and exome sequencing of monogenic diseases, PGT-M provides a very effective way to significantly reduce the risk of having an affected child.

INHERITED DISEASES

P07 Use of Genomic Analyses in Identification of Molecular Basis of Rare Paediatric-Onset Diseases.

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Genomic analysis is a very efficient diagnostic and gene discovery tool. It should ideally be applied in the diagnosis of all patients with rare diseases when the results of clinical, biochemical, metabolic, molecular and cytogenetic analyses do not lead to a specific diagnosis.

Methods: Over the past eight years, we investigated 374 carefully phenotyped children and their families with rare diseases of paediatric-onset and unknown etiology using a whole exome sequencing (WES). In 35 cases with negative results of WES, we used whole genome sequencing and/or RNA sequencing to search for molecular diagnosis.

Results: In 162 cases (43%) we were able to establish definitive genetic diagnosis using WES. Five cases (POLRMT, MADD, OVOL2, NDUFAF6, PLD1) represented novel gene-disease associations. In 86 cases novel disease causing mutations were identified in genes with established pathogenicity. In additional 20 cases (6%) we identified a potentially causal genetic variants and are in the process of their functional characterization. In eight cases, whole genome sequencing enabled the diagnosis to be closed and one novel gene-disease association was described (SAA1).

Discussion and conclusion:
Genomic analysis is not a single-time process; data should be revisited and reanalyzed repeatedly in the light of new scientific findings. In 31 cases (18%), the causality of the corresponding genes was established in less than 5 years ago. This suggests that there are still many answers hidden in the data. Furthermore, we are revealing more and more cases where the clinical condition can be explained better by digenic or oligogenic conditions.
In about 30% of the cases we identified mutations in genes which are not investigated in any of the gene panels currently used in clinical genetics laboratories. WES and/or genome sequencing thus should be considered a first-tier diagnostic test in cases with no specific diagnosis.

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INHERITED DISEASES

P08 Downstream Open Reading Frames in The Ptchd1-c Transcript may Mitigate Social Deficiencies in Ptchd1 Exon 2 Deletion Mice

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Patched domain-containing 1 (*PTCHD1*) is a risk factor gene for autism, and is predicted to produce a multi-pass protein encoded by three exons. Two isoforms exist in the brain: *PTCHD1-a* (exons 1-3; high expression), and *PTCHD1-c* (exons 1 and 3; low expression). To investigate the role of *Ptchd1* on behaviour and cognition, researchers have deleted exon 2 (*Ptchd1*$^{\Delta 2}$). This relegates expression to only the *Ptchd1-c* isoform; absence of the 661 -bp exon 2 creates a frameshift thatis predicted to truncate the C-terminus. *Ptchd1*$^{\Delta 2}$ mice recapitulate clinical symptoms of ASD, but do not exhibit social deficits. A downstream open reading frame (ORF) still exists in the final exon of the *Ptchd1-c* transcript, which we hypothesize produces an N-truncated Ptchd1 protein that retains some degree of wildtype function. Concomitantly, we have generated a mouse model with a mutation disrupting the downstream ORF (*Ptchd1*^{G387Vfs*2}) that displays social deficits. This study seeks to determine a molecular mechanism for the social phenotypic disparity between the *Ptchd1*$^{\Delta 2}$ and *Ptchd1*^{G387Vfs*2} mice.

Methods: CRISPR-Cas9 was first used to insert a 3xFlag epitope tag at the C-terminus of *Ptchd1* (*Ptchd1-3xFlag*) in the male mouse embryonal carcinoma cell line P19. CRISPR-Cas9 was next used to generate subclones analogous to the two mouse models, with either a deletion of exon 2 (*Ptchd1*$^{\Delta 2}$*-3xFlag*) or a disruption of the downstream ORF (*Ptchd1*^{G387Sfs*44}*-3xFlag*). Transgenic P19 lines were differentiated into neurons using retinoic acid, fractionated to enrich for synaptosome proteins, and analyzed by western blotting.

Results: *Ptchd1*$^{\Delta 2}$*-3xFlag* neurons express several putative N-truncated Ptchd1 proteins, which approximately correspond to computationally-predicted alternative start codons, all of which are absent in *Ptchd1*^{G387Sfs*44}*-3xFlag* neurons.

Conclusion: The downstream ORF in *Ptchd1-c* produces putative N-truncated Ptchd1 proteins that may mitigate social deficiencies in *Ptchd1*$^{\Delta 2}$ mice.

INHERITED DISEASES

P09 Analysis of Czech Genomes for Theranostics

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During the variant interpretation, choosing the right population as the reference dataset is one of the crucial steps. However, the current genomic data of Central European populations have been only poorly represented in the publicly available databases. This makes it very difficult for Czech geneticists and researchers to interpret variants found in the whole genome sequence of local individuals. Therefore, we aim to map genetic variants in the Czech Republic thoroughly.

Methods: Volunteers involved in the project fulfill several criteria: 1) they are proportionally selected from all the Czech regions, 2) their mother comes from the same region as father, 3) they do not suffer from any severe inherited disease, 4) their age ranges from 30 to 55 years old. The whole genomes libraries are prepared with a PCR-free protocol and paired-end sequenced by NovaSeq (2×150 bp). Sequence variants are detected by a Sarek workflow.

Results: So far, approximately 1,200 volunteers have donated their blood sample for the project. Almost 1,000 genomes have been already sequenced and the sequencing data have been gradually analyzed. First analyzed genomes have already aided e.g., in the research of ANKRD26-related thrombocytopenia – it was discovered, that a 5'UTR variant which was previously published as causal for the phenotype is present in 11.9% (21/176) of Czech healthy individuals.

Discussion and conclusion: The data produced in our project will be published as a publicly available database of variant frequencies at the individual positions in the genome. Our first findings suggest the major potential the research might have in the interpretation of variant causality. Potentially, the results can influence not only molecular diagnostics in the Czech Republic but also European genomics thanks to our involvement in the European "1+ Million Genomes" initiative.

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INHERITED DISEASES

P10 Unique cases of chromosome 11q inverted duplication deletion

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Inverted duplication deletion (inv dup del) is complex structural intrachromosomal rearrangement – a terminal deletion of one arm and an inverted duplication of intersticial segment localized at the end of the arm. Inv dup del of almost all chromosomes (p or q arms) have been described. Duplicated region is usually (not exclusively) larger than deletion. To our knowledge, nor postnatal neither prenatal case of inv dup del 11q has been published yet.
We present two unique cases of inv dup del 11q originated *de novo*. Proband 1 carries 15,2 Mb large duplication of region 11q23.3q25 and terminal deletion 11q25 of 1,4 Mb. Especially, pathogenic duplication is causal for patient phenotypic features. Clinical diagnosis of proband 2 is Jacobsen syndrome due to 10,3 Mb large terminal deletion 11q24.2q25, thatis accompanied by duplication 11q24.1q24.2 of 1,53 Mb.
Common phenotypic features of both probands are craniofacial dysmorphism, congenital heart defect, hypotonia and global developmental delay. However, sizes of duplicated and deleted regions are not identical and inv dup dels were probably formed by different mechanisms: U-type exchange (chromatids fusion) versus non-allelic homologous recombination (between segmental duplications or repetitions).

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INHERITED DISEASES

P11 Genetics of Adult-onset Focal Segmental Glomerulosclerosis (FSGS) in Czech Population, Multicentric Study of Kidney Disease

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Focal segmental glomerularsclerosis (FSGS) is a clinically and genetically heterogeneous entity that affects glomerular function of the kidney and manifests itself by proteinuria. It often leads to loss of kidney function, accounting for about 15 % of end-stage renal disease (ESRD). A portion of the FSGS cases is of a monogenic etiology and fulfills criteria for a rare disease. The mutations in FSGS genes cause damage in podocytes and glomerular basement membranes. The genetic FSGS was so far studied in pediatric populations. The prevalence of adult-onset genetic FSGS is considered largely underestimated and its clinical, genetic and histological features have not been clearly described.
Methods: Multicentric study, cohort inclusion criteria: adult-onset FSGS confirmed by kidney biopsy, FSGS family history or a sporadic primary FSGS resistant to steroids. The analyses were performed by whole exome sequencing, in some instances also by whole genome sequencing and various bioinformatic methods.
Results: The current cohort consists of 235 patients of average age 48 years. The DNA analysis revealed so far pathogenic or likely pathogenic variants in genes associated with FSGS at 10,6% unrelated patients. The most frequent mutations were variants in COL4A genes (45%), compound heterozygous mutations in NPHS2 gene (9,6%) and in FSGS families *INF2* gene variants (6,4%). By the statistical enrichment analysis we identified two Czech-specific founder mutations within *NHPS2* (c.G868A) and *COL4A4* (c.G1598A). The strong enrichment of *NPHS2* mutation within our cohort implies that there is another, not recognized, pathological variation in the second allele. The most significantly enriched gene with rare mutations within our cohort is *SMARCAL1*.
Conclusion: This is the first large-scale adult-onset genetic FSGS study in Czech population. It brings new insights into genetic and clinical features of the adult-onset FSGS and the results will improve personalized medical care.
Support: This work was supported by grant **NV19-06-00443** from **AZV ČR** (Czech Health Research Council), Ministry of Health of the Czech Republic

INHERITED DISEASES

P12 NPHS2 R229Q: the first variant with a mutation-dependent pathogenicity in human genetics

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NPHS2 is the most frequently affected gene is autosomal recessive steroid-resistant nephrotic syndrome. It encodes podocin, a key membrane-associated component of the glomerular slit diaphragm. The most frequent non-silent NPHS2 variant, c.686G>A, p.R229Q, rs61747728, (AF in Europe: 3.6%) was formerly considered pathogenic in-trans with NPHS2 mutations.
Based on its high allele frequency, we first hypothesized its incomplete penetrance, and found indeed 6/129 unaffected parents of children with NPHS2-associated nephrotic syndrome to carry R229Q in trans to a pathogenic variant. Next, we showed that the variants associated to R229Q in affected individuals cause amino acid substitutions in the C-terminal of podocin while these variants are rare among the NPHS2 mutations (p=1.2 x 10-35), indicating that R229Q is exclusively pathogenic when trans-associated to specific 3' mutations. We next proved that podocin oligomerizes exclusively through the C-terminal helical regions by three different approaches and showed by FRET measurement and structural modelling that the structure of pathogenic oligomers is altered. In cell culture experiments we also found that the pathogenic oligomers are not membrane localized.
We next developed a population-genetic algorithm to screen 17 other frequent autosomal recessive disorders for incompletely penetrant (IP) variants and found 22 novel frequent IP variants. With the exception of NPHS2 R229Q, no other was found to be subject of interallelic interaction.
In conclusion, NPHS2 R229Q variant is the first variant in human genetics with a trans-associated mutation-dependent pathogenicity. This phenomenon has important consequences in the clinical practice, since heterozygous carriers get frequently identified and the knowledge of pathogenic associations in necessary for the proper genetic counselling. Interallelic interactions may modify the pathogenicity of genetic variants in autosomal recessive disorders.

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INHERITED DISEASES

P13 Frequency of hereditary variants of factor V Leiden G1691A, prothrombin G20210A gene and locus 10976 G / A of factor VII gene among patients at high risk of thrombosis.

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Venous thromboembolism is a multifactorial disease that develops through the interaction between genetic, environmental and personal acquired factors. The frequency of alleles and genotypes of the 10976 G/A locus of factor VII gene and mutations in factor II and coagulation factor V were determined among patients at high risk of thrombosis.

Methods: The group of studies included 150 patients with thrombosis of various localizations and pulmonary embolism. The control group of studies consisted of 225 healthy individuals. Amplification of DNA sequences in vitro was performed by PCR followed by restriction analysis using specific endonucleases.

Results: Mutations were diagnosed in 46 patients, including 5 combined mutations. The frequency of mutant allele A of Leiden G1691A factor Vis 9.3% in the experimental group and 1.5% in the control group. The frequency of prothrombin G20210A gene alleles is 3.3% in the experimental group and 0.7% in the control group. The frequency of mutant allele A of the 10976 G/A locus of the factor VII gene in the experimental group is 12.5%, in the control group - 15.2%. No mutations in the homozygous state were reported, which may be due to the low frequency of homozygotes in populations. The mean age of thrombosis in the study group was 32 years.

Discussion and conclusion: Mutation of the 20210G/A locus of the FII coagulation gene increases the risk of thrombosis by 5 times compared to a healthy population, with hereditary factor V Leiden the risk of thrombosis increases by 6,5 times. Factor VII levels are strongly associated with the risk of thrombosis. However, the research did not show a statistically significant result. The presence of pathogenic allele A of the 10976 G/A locus of coagulation factor VII does not provide protection against acute thrombotic events and is not associated with increased development of thrombosis and pulmonary embolism.

INHERITED DISEASES

P14 Inherent Variability of Matrix Metalloproteinases and Their Inhibitors and Its Association with Hemodynamic Parameters in Patients with Ischemic Heart Disease

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Matrix metalloproteinase activity is tightly regulated by the endogenous tissue inhibitors (TIMPs), and dysregulated activity contributes to pathological extracellular matrix remodelling. MMP/TIMP balance is associated with atherosclerotic plaque progression and instability.
Aim of our work is to associate hemodynamic values of treated patients with ischemic heart disease with 19 polymorphisms in matrix metalloproteinases genes.
Methods: A total of 410 patients with ischemic heart disease (IHD) were enrolled in a prospective study. Invasive intra-aortic SBP and DBP were measured using a fluid-filled catheter in the ascending aorta. Peripheral blood pressures were measured on brachial artery non-invasively by standard method. The polymorphisms in MMPs and their inhibitors were detected by standard molecular biology methods.
Results: We proved significant difference in central aortic pressure (systolic, diastolic and especially in pulse pressure- P=0.0001) between men and women with IHD.
In women, we proved these significant associations with hemodynamic parameters: MMP7 rs11568819 with maximal end-diastolic pressure in left ventricle (P=0.03), TIMP2 rs8176329 with ejection fraction (P=0.04) and MMP2 rs243866 with peripheral diastolic blood pressure (P=0.005). In men, polymorphism MMP8-Sfcl is associated with pulse pressure in aorta (P=0.01). The MMP13 rs2252070 (P=0.02), MMP12 rs2276109 P=0.01) and MMP12 rs7123600 (P=0.02) were
associated with peripheral blood pressure values.
Discussion and conclusion: We proved significant influence of gene polymorphisms in MMPs and TIMPs on variability of hemodynamic parameters in patients with IHD. Further research is essential to elucidate more definitively the subtypes of MMPs and TIMPs as well as their genetic variants involved in the remodelling process which is needed for deeper understanding of ischemic heart disease pathophysiology. The ability of MMP regulation to confer additional protection over coronary reperfusion and current standard therapy also needs consideration.

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INHERITED DISEASES

P15 ADAM17 Gene Polymorphism and Chronic Venous Insufficiency

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Introduction and aims: Chronic venous disease (CVD) is a common disorder of lower extremities caused by venous hypertension , which is caused by venous valve reflux, venous blood flow obstruction or both. The study was scheduled to investigate the relationship between polymorphism in proinflammatory ADAM17 (3'TACE) and CVD risk. Genotype–phenotype study was calculated to test possible association between ADAM17 genotypes and phenotypes of CVD. Methods: Finally, 150 CVD patients as well as 227 control subjects were enrolled to the study. A double genotype of polymorphisms A+/-in position 2698 and G2712A in the 3'region of ADAM17 gene, which are in complete linkage disequilibrium, was detected by standard molecular biology method.

Results: A significant differences in allelic frequencies in ADAM17 gene polymorphism was found between CVD women and control ones (P=0.05)
From the clinical point of view, there are often significant doubts about real beneficial effect of genotype-phenotype study results for clinical practice. To confirm usefulness of our results, we tested significant clinical associations of severe CVD phenotype (ulceration) including ADAM17 genotype. Though sensitivity, specificity and power tests value are not as significant as in PAD and diabetes, the values for ADAM17 polymorphism are comparable with ischemic heart disease, venous inflammation and erysipelas occurrence in our CVD patients. When multiple regression analysis for associated phenotypes/ alleles with ulceration had been performed (P=0.02), the A allele of ADAM17 polymorphisms was significantly associated with ulceration (P=0.03) similarly as PAD (P=0.03) and diabetes (P=0,03). In the model, the highest significance with ulceration was observed for venous inflammation (P=0.00008).

Discussion and conclusion: Now it would be necessary to use the results of cross section study in further prospective study and revaluate genetic association with respect to continuing time dynamics of CVD in the same group of patients.

INHERITED DISEASES

P16 Loss of Heterozygosity in EIF3F Gene Leading to Intellectual Disability in Two Siblings

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Intellectual disability (ID) belongs to the group of neurodevelopmental disorders (NDD), which is characterized by significant limitations in intellectual functioning and adaptive behavioral skills. Genetic causes of ID are thought to be present in 25–50% of cases, although this number increases proportionally with severity. In particular, whole exome sequencing (WES) and whole genome sequencing help explain the underlying genetic cause of the disease in many cases.
Case Summary:
We report two affected male siblings with intellectual disability, epilepsy, developmental delays including delayed speech development, sensorineural hearing loss and facial dysmorphy. The Fragile X chromosome, Xp22.3 microdeletion and Kallman's syndrome were all excluded by standard genetic analyses in childhood. Recently, whole exome sequencing was performed in both siblings and four healthy family members. Homozygous extremely rare variant c.694T>G (p.Phe242Val) in the EIF3F gene was detected, segregating with the phenotype in both affected brothers. Moreover, array CGH analysis together with SNP genotyping detected extensive regions of homozygosity (ROH) – 50 Mb, which correspond to 2% of autosomal genome, including EIF3F gene. The observed size of the ROH indicating the inbreeding coefficient of 1/32 could point to a very distant parental consanguinity.
Conclusion:
The identified variant c.694T>G in EIF3F gene was recently associated with severe autosomal recessive NDD in patients from Germany and the UK, so our case supports the pathogenity of this rare variant. The determination of inherited genetic basis in both siblings was relevant to specify the diagnosis, prediction of possible problems related to NDD and identification of carrier status in family members.
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INHERITED DISEASES

P17 Two Siblings with Crisponi Syndrome Caused by a Homozygous CRLF1 in-frame Deletion-insertion Mutation

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We herein report the first two known cases of Crisponi Syndrome in the UK, an 8-year-old boy and his 19-year-old sister, and their genetic diagnosis.

Case summary: The boy and his sister are the third and first children of healthy consanguineous Pakistani parents (second cousins). Their middle child, another girl, is healthy and unaffected.
The boy suffered from developmental delay, plagiocephaly, severe scoliosis, overriding fingers and toes, bilateral finger contractures, frequent respiratory infections requiring antibiotics and admissions, otitis media with effusion, hyperhidrosis, hypersalivation, hypernatremia, hyperthermia, gastro-oesophageal reflux, and torticollis. A genetic diagnosis of Crisponi Syndrome was made at six months of age. Sanger sequencing of CRLF1 exon 4 and the flanking intronic regions (NM_004750.4) was performed. He is homozygous for the novel CRLF1 in-frame deletion-insertion mutation, c.566_631del (p.Thr189_Val211delinsMet), consistent with cold-induced sweating syndrome type 1 due to biallelic CRLF1 gene mutations. The CRLF1 gene has two consecutive fibronectin III-like domains (amino acid 134-229 and 234-334). The first domain has two conserved cysteine doublets (amino acid 143 -> 153 and 184 -> 195). This mutation deletes the second conserved cysteine doublet (184 -> 195) in the first fibronectin III-like domain.
His sister has a milder phenotype, with trismus, myopathic facies, camptodactyly, intellectual disability, severe progressive kyphoscoliosis and cold-induced sweating. Analysis of the coding region and conserved splice sites of 6110 genes by next-generation sequencing (Agilent Sure Select Focussed Exome v1/Illumina HiSeq) and Sanger sequencing described above were performed. Exome sequencing revealed a genetic diagnosis identical to that of her brother.

Conclusion: We presented the first two cases of Crisponi Syndrome in the UK. Dysmorphism and symptoms described should prompt consideration for genetic testing.

INHERITED DISEASES

P18 Proteinopathies and activation of endoplasmic reticulum stress as central pathogenetic mechanism in hereditary tubulointerstitial kidney disease

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Hereditary tubulointerstitial kidney disease (TKD) is characterized by tubular damage and interstitial fibrosis in the absence of glomerular lesions, with inescapable progression to end-stage renal disease. We contributed to genetic studies that in >1000 families world-wide have revealed causal mutations in at least four different genes, including UMOD, MUC1, REN, and SEC61A1 encoding for uromodulin, mucin-1, renin, and protein transport protein Sec61 (translocon) subunit alpha isoform 1, respectively.
Uromodulin, mucin-1 and renin are glycoproteins abundantly expressed in kidney and their biosynthesis depends on proper function of the translocon.
Seeking the pathogenetic mechanisms in affected tissues, cellular models and model organisms we found that mutations affect biogenesis and trafficking of the corresponding mutant proteins. Mutant proteins localize mostly to the endoplasmic reticulum (ER), the endoplasmic reticulum intermediate compartment (ERGIC) and TMED9 (transmembrane P24 Trafficking Protein 9) positive cargo receptor-containing vesicles, where the protein folding quality control system is present. Intracellular accumulation of mutant proteins activates endoplasmic reticulum (ER) stress and unfolded protein response (UPR). Renal epithelial tubular cells have high protein turnover, metabolic activity and limited regenaration capacities. Sustained ER stress and UPR therefore in a long term initiate processes leading to deterioration of kidney function and development of TKD. TKDs are thus toxic proteinopathies. Recent research identifying the BRD4780, a compound helping in removal of unfolded proteins from TMED9 transport vesicles into lysosomes for degradation, provides new opportunities to probe the pathologic and potentially therapeutic implications in TKD and other toxic proteinopathies beyond rare kidney diseases.

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HUMAN CANCER GENETICS

P19 Assessment of Genetic Instability in Interventional Radiologists Exposed to Chronic Low Dose Ionization Radiation

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High dose ionizing radiation (IR) is currently considered to be a generally accepted carcinogen. On the other hand effect of low dose (< 100 mSv) IR, which is typical for diagnostic radiology, is still under discussion. Interventional radiologists are one of the largest groups occupationally exposed to this type of IR for years. The aim of our work was to study DNA damage and genomic instability in peripheral blood lymphocytes of 12 radiologists and 14 control probands. Detection of double strand breaks was examined by quantifying fluorescently labeled residual 53BP1 (p53 binding protein 1) DNA repair foci. Analysis of chromosomal aberration (CA) and micronuclei (MN) were chosen for evaluation of genomic instability. The rearrangement in MLL gene (Histone-lysine N-methyltransferase 2A), which is one of the most frequently mutated genes in adult leukemias was investigated by fluorescence in situ hybridization using a break-apart probe. Our result did not show a significant difference in the production of 53BP1 foci between radiologists and controls. On the other hand, we found a significantly increased frequency of MN and CA (specifically: total CA, dicentrics, acentrics, ring chromosomes, chromatid gaps and fragments) in the radiology group compared to controls. We also detected that radiologists had a significantly higher amplification of the MLL gene segments, which were located between the breakpoint and the PHLDB1 gene (Pleckstrin Homology Like Domain Family B Member 1). These results suggest a possible effect of chronic low dose IR on the genetic instability of radiological personnel. Finally, our findings could contribute to the improvement of radiation protection and possibly to the regulation of safety limits in Slovak hospitals.

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HUMAN CANCER GENETICS

P20 Genomic profiles of primary and recurrent brain gliomas in patients with multiple tumor recurrences

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Introduction and aims: Diffuse gliomas are highly heterogenous tumors with occurrence of recurrent lesions in majority of patients. During disease progression gliomas undergo cellular and genomic evolution with newly acquired genetic properties. However, the mechanism of this complicated process associated with treatment failure is poorly understood. In this retrospective study, we performed genetic analyses of tumor cells of five patients with diffuse glioma who underwent surgical resections or biopsies of multiple recurrences.
Methods: The tumor samples were analyzed using combination of cytogenomic and genomic methods: I-FISH (Abbott, MetaSystems), aCGH/SNP (Agilent), MLPA, methylation-specific MLPA (MRC-Holland) and target NGS (ArcherDx).
Results: All five patients experienced recurrence with newly acquired genetic or epigenetic changes. As a primary event we observed mutation R132H in IDH1 gene. In addition, we detected methylation of the MGMT promoter, CDKN2A/B homozygous deletion, and RB1 deletion as later events that were probably associated with higher tumor grades. Besides the typical genomic changes, we detected aberrations with unknown or unclear prognostic relevance (e. g. inframe deletion in TP53, p.Met243_Asn247del). The progression to a higher grade of glioma occurred in four patients.
Discussion and conclusion: The evolutional patterns in glioma depend on clonal selection and/or the patient's treatment. Recurrence may arise from one major tumor clone or from one or more subclones within the primary tumor through. For more effective targeted treatment, it is necessary to examine all recurrent tissues. Because of high intratumor heterogeneity and limits of detection, it is advisable to perform a comprehensive range of molecular analyses.

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HUMAN CANCER GENETICS

P21 Comparison of DNA and RNA-based Approaches for the Genetic Characterization and Stratification of Patients with Diffuse Large B-cell Lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is an aggressive disease representing the most common type of Non-Hodgkin lymphomas worldwide. Significant heterogeneity in genetic features divides patients into several prognostic subtypes. The implementation of modern genetic tests is under extensive investigation as it can improve patient stratification and lead to personalized therapy. We aimed to compare RNA and DNA-based molecular methods for tumor characterization.
Methods: Nucleic acids were isolated from 9 lymph node DLBCL samples. RNA was analyzed by integrative next-generation sequencing (NGS) panel FusionPlex® Lymphoma Kit (Archer) analyzing 125 genes. DNA was analyzed by in-house comprehensive NGS panel LYNX and CGH+SNP array (Agilent).
Results: With the RNA-based FusionPlex panel, we gathered gene expression profiles (GEP), allowing us to distinguish ABC/GCB prognostic group in 6 patients. Furthermore, we were able to identify translocations in 4 patients and detect several gene variants in 7 patients. DNA-based LYNX panel detected a broader spectrum of gene variants in 8 patients, genome-wide chromosomal aberrations in 8 patients, translocations in 5 patients, and clonal IG rearrangements in all patients. As expected, the microarray analysis identified significantly more chromosomal changes due to the higher sensitivity and resolution of the assay.
Discussion and conclusion: The RNA-based method is appropriate for GEP but does not give satisfying results for variant detection as it can miss not expressed ones. Also, it does not provide accurate lymphoma translocation detection since they may not create fusion transcripts. DNA approaches are suitable for detecting all genomic alteration types (gene, copy number and structural variants), but the method selection depends on required sensitivity. We recommend combination of techniques or a comprehensive method for the appropriate characterization of DLBCL samples.

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Pt.	GEP (FusionP)	IGH transloc. (FusionP; LYNX)	Gene variants (FusionP; LYNX)	No. of affected chromosomes (LYNX; array)	Clonal IG rearrang. (LYNX)
1	uncl	BCL6; BCL6	0; 4	7; 10	yes
2	GCB	BCL6; BCL6	5; 15	0; 5	yes
3	GCB	no; no	3; 9	7; 13	yes
4	uncl	MYC; MYC	2; 3	10; 20	yes
5	GCB	no; BCL2	3; 3	5; 14	yes
6	GCB	no; no	4; 8	9; 12	yes
7	ABC	no; no	2; 4	9; 11	yes
8	uncl	no; no	0; 0	1; 12	yes
9	ABC	BCL6; BCL6	2; 5	8; 18	yes

Fig. 1: Results obtained by the RNA-based NGS panel FusionPlex (FusionP), DNA-based NGS panel LYNX and DNA microarray in nine DLBCL patients. GEP – gene expression profile; IGH – immunoglobulin heavy chain; uncl – unclassified.

HUMAN CANCER GENETICS

P22 Long-read Whole Genomes Sequencing Used to Detect Complex Chromosomal Rearrangements

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Highly complex karyotypes (CKs) are recognized as a feature with prognostic value associated with poor clinical outcome of cancer patients. They may be caused by chromothripsis, a genomic event leading to extensive chromosomal rearrangements. Traditional methods used to study CKs include classical cytogenetics and array-based methods, however sequencing techniques can provide much higher resolution. We aim to use the Oxford Nanopore platform to obtain long reads from the whole genome and analyze chronic lymphocytic leukemia (CLL) samples with known CKs resembling chromotripsis. We will then compare the traditional and sequencing methods.
Methods: Phenol-chloroform extraction was used to isolate high molecular weight genomic DNA from CLL samples with known somatic genomic rearrangements. The sequencing libraries were prepared with the Rapid Sequencing Kit and sequenced on the MinION sequencer. Data produced by the sequencer were aligned to the hg19 human genome reference with the Minimap2 aligner and structural variants were analyzed with the Sniffles variant caller.
Results: First, we compared the quality of sequencing results obtained with or without elimination of short DNA fragments from the libraries. Next, the MinION results were compared to the data obtained using classical cytogenetics, genomic arrays, and short-read sequencing on Illumina. When the target regions of known structural variants were covered, they were reliably detected by the split read method from long-read sequencing. Detailed analysis will be presented at the conference.
Discussion: Long-read sequencing represents a relatively fast and efficient alternative (or supplement) to traditional methods of analyzing complex genomic aberrations. We intend to perform a comprehensive comparison of different analytical methods and elucidate the benefits and drawbacks of the long-read sequencing methods for revealing the detailed structure of complex karyotypes in CLL.

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HUMAN CANCER GENETICS

P23 Long Read Targeted Sequencing Enables Genomic Analysis of Translocation Hotspots in Lymphoproliferative Diseases

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Structural variants (SV) are a major source of variability in the human genome and a cause of multiple hereditary and oncologic diseases. In addition, recurrent genetic translocations are a potent genetic marker for disease monitoring. SV identification is often hindered by their size and genetic context. Sequencing with the Oxford Nanopore Minion platform can overcome this problem with long reads (> 100 kb), effectively reading through the repetitive regions. Our assay utilizes the CRISPR-Cas9 enrichment method and Adaptive Sampling (AdS) to selectively target the recurrent translocation hotspots.

Methods : Genomic DNA was isolated from cell lines DOHH-2, MAVER-1, Ramos and JVM-2 using Wizard HMW kit (Promega). Sequencing library was constructed using kit LSK-110 (Oxford Nanopore) and custom gRNA probes (IDT). Alternatively, samples were processed using LSK-110 and enriched using AdS option in MinKNOW software. Libraries were sequenced using Minion R9.4.1 flowcell. For breakpoint detection, bioinformatics tools Minimap2, NanoSV and Sniffles were used.

Results: The sequencing run using the AdS generated 7.18 Gb of data and achieved the average on-target coverage of 13,7x. The sequencing of CRISPR-Cas9 enriched library yielded 2.86 Gb, with on-target coverage 18,8x. In both datasets, recurrent translocations were detected: t(8;14), t(11;14). Translocation t(14;18) was detected in AdS dataset, but not in CRISPR-Cas9 dataset. All these breakpoints are in accordance with the SV's attributed to the used cell lines.

Discussion and conclusion: The method has been validated on several well characterized lymphoma cell lines. We confirmed the potential for effective detection of SV's. Assay is well suited for detection of unknown translocation partners, because only a single directional probe is needed next to each hotspot. Main drawback of this approach at the moment is the lack of an indexing scheme for easy demultiplexing of results.

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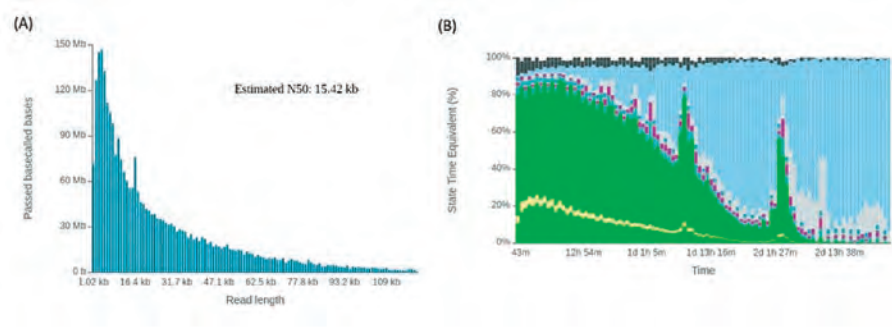

Fig. 1: A) Read length distribution and B) duty plot of the Cas9-enriched sequencing run

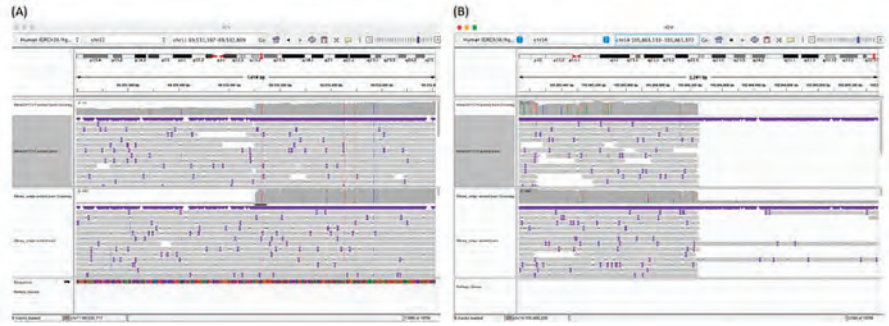

Fig. 2: A visualization of a translocation event t(11;14) detected using both Cas9 (top track) and AdS (bottom track) enrichment methods. A) detail on chromosome 11 breakpoint and B) detail on chromosome 14 breakpoint

HUMAN CANCER GENETICS

P24 Distinct p53 Phosphorylation Patterns in Chronic Lymphocytic Leukemia Patients are Reflected in Circumjacent Pathways' Activation upon DNA Damage

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Protein p53 has a central role in tumor suppression. p53 level in the basal state is kept low, but after DNA damage, it is stabilized and triggers transcription of its target genes. In chronic lymphocytic leukemia (CLL), aberrations in the *TP53* gene resulting in impaired function of the encoded p53 protein represent a marker of poor prognosis and chemorefractoriness. However, p53 function can also be disrupted by altered protein modifications, e. g. phosphorylations, even in the wild-type (wt) protein. We aimed to assess how p53 phosphorylations affect p53 function in CLL.

Methods: p53 phosphoprofiles induced by DNA damage were electrophoretically screened in *TP53*-wt primary CLL samples. RNA sequencing and qPCR were used to analyze the ability of p53 to trigger transcription. Gene aberrations were analyzed by a targeted NGS panel.

Results: DNA damage induced two phosphoprofiles. Profile I samples showed high p53 phosphorylation and standard response to DNA damage. Profile II samples showed low p53 phosphorylation and lower ability to activate the expression of p53 target genes upon DNA damage, which makes profile II similar to the control *TP53* mutated samples (*TP53*-muts). In the untreated state, profile II had significantly higher basal p53 levels than profile I cells. Also, untreated cells differed in the activity of the hypoxia pathway: the highest level was detected in *TP53*-muts, followed by profile II and profile I. Finally, ATM, a crucial part of the DNA damage-p53 axis, was more frequently mutated in profile II.

Discussion and conclusion: In CLL, *TP53*-wt cells with less phosphorylated p53 show *TP53* mutant-like behavior after DNA damage. p53 hypophosphorylation and the lower responsiveness to DNA damage are linked to *ATM* defects and the higher basal activity of the hypoxia pathway. It highlights the importance of phosphorylation in regulating p53 function in CLL.

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HUMAN CANCER GENETICS

P25 Genomic Risk Variants and HPV Infection Modulate Gene Expression at the Human Leukocyte Antigen Locus in Cervical Cancer

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Cervical cancer is the fourth most common gynaecological malignancy in women. Human papillomavirus (HPV) infection influences disease progression, and hereditary risk factors from genome-wide association studies (GWASs) have identified multiple genomic variants at the human leukocyte antigen (*HLA*) locus (6p21.32-33) containing genes with an essential role in modulating host immune response. We aim to validate these associations and investigate the functional relevance of variants by expression quantitative trait loci (eQTL) analysis in cervical tissues.

Methods: We genotyped four variants at the *HLA* locus from recent cervical cancer GWASs (rs17190106, rs535777, rs2763979, and rs3117027) in the German Cervigen cohort of 1099 invasive cervical cancers, 1345 dysplasias, and 1196 healthy controls. We tested these and five known GWAs variants (rs9272117, rs2844511, rs2856437, rs2299059, and rs28631719) to be eQTLs for 36 gene transcripts at this locus in 235 cervical tissues.

Results: In addition to previous findings from our cohort, rs17190106 associated with overall cervical disease (p=0.03, OR=0.82, 95%CI=0.68-0.98) and invasive cancer (p=0.001, OR=0.69, 95%CI=0.55-0.86), whereas rs535777 associated with adenocarcinomas (p=0.004, OR=1.63, 95%CI=1.17-2.27). We identified transcripts upregulated in HPV positive samples (*HLA-B* p=0.03, *NFKBIL1* p=0.02, *DDX39B* p=0.006, *LTB* p=0.04), and specifically upregulated (*MICA* p=0.01, *HCP5* p=0.02), and downregulated (*HLA-DPB2* p=0.04) in HPV16+ samples. We find strong eQTLs after correction for multiple testing, such as rs9272117 for *HLA-DRB6* (p=1.9x10E-5), rs28631719 for *HLA-DRB5* (p=1.0x10E-8) and *HLA-DRB1* (p=5.5x10E-5) in cervical tissues. We also identify rs9272117 as a master regulatory variant for coordinated *HLA* gene expression.

Conclusion: We corroborate recent GWAs signals at 6p21 in a hospital-based cohort. We identify genetic variants that modulate gene transcript levels together with HPV infection, indicating that highly controlled gene regulation underlies cervical cancer susceptibility at the *HLA* locus.

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HUMAN CANCER GENETICS

P26 Validation of DNA Damage Markers in Assessment of Radiosensitive Breast Cancer Patients

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Breast cancer (BC) is the most commonly diagnosed cancer worldwide. Although radiotherapy (RT) is one of the most effective treatments, it is impossible to target ionizing radiation only to tumors, which in radiosensitive (RS) patients causes serious side effects, e.g. bleeding, inflammation, or fibrosis. A major effort is currently being made to study potential biomarkers of individual RS allowing the identification of sensitive patients and the treatment personalization. This work is focused on the validation of DNA repair foci, chromosomal aberrations (CA), and micronuclei (MN) in the RS assessment. Our results showed a higher number of foci before RT in RS patients, but the receiver operating characteristic (ROC) analysis revealed this approach as moderately sensitive to distinguish between RS and non-RS patients. The number of DNA repair foci was also affected by chemotherapy (CHT) often administered to the patients before RT. In the CHT-treated patients, the amount of γH2AX/53BP1 focus was increased before RT, while during RT only the 53BP1 focus was elevated, and after RT, there was no difference. In vitro irradiation showed different DNA kinetics of foci between RS and non-RS patients. More 53BP1 and γH2AX/53BP1 foci were induced in RS patients than in non-RS patients, but during the subsequent loss of foci, the number of foci did not differ between patients. We also observed the accumulation of CA and MN during and after radiological treatment. The RS and non-RS patients did not differ in the number of aberrations, but during the whole therapy, a trend of a higher number of MN in RS patients was seen. For all biomarkers, we observed individual variability between patients, which precludes the individual use of these methods in RS assessment, but the combination of them could be a promising approach in the future.

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HUMAN CANCER GENETICS

P27 Exploring Different Aspects of Clonal Evolution in Chronic Lymphocytic Leukemia

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Introduction and aims: In chronic lymphocytic leukemia (CLL), an acquisition and clonal selection of genomic aberrations impact patients' clinical outcome. Frequent expansion of *TP53*-mutated subclones leads to disease course deterioration. We aimed to identify molecular genetic factors affecting the ability of *TP53*-mutated subclones to expand.

Methods: We focused on 53 CLL patients with a detailed clinical characterization and different scenarios of *TP53* mutation expansions. Using whole-exome sequencing, we analyzed the differences in mutation profiles and their changes over time in defined patient groups, as well as changes in clonal architecture in each patient. Furthermore, we extracted abnormal molecular pathways. To get a deeper insight, we performed bulk RNA-seq on a subset of cases and, moreover, implemented the single-cell RNA-seq in individual cases.

Results: We identified recurrent mutations in known CLL-associated genes but also novel unique mutations in other genes emerging or diminishing (i) under the treatment pressure and (ii) in relation to the clonal development of *TP53* mutations. We observed the mutual exclusivity and co-occurrence of specific gene mutations. In defined patient groups, we observed distinct pathways affected. In patients with early expanding *TP53* mutations, we detected decreased expression of the *ZYG11A* and *ZNF215* genes. The single-cell RNA-seq provided another level of information about the genomic complexity of the analyzed cases.

Discussion and conclusion: We explored the clonal composition of somatic mutations during the CLL disease course and assessed molecular pathways affected by gene mutations. We enriched these data with findings from RNA-based methods. Our results contribute to a better understanding of links between the CLL pathobiology and phenotype.

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GENETICS OF MICROORGANISMS

P28 Accumulation of PolyP under Nutrient Stress Conditions in vtc Mutants of Chlamydomonas reinhardtii

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Phosphorus (P) is one of the essential elements for cell growth and development. P, in the form of inorganic or orthophosphate, is integral to metabolic processes as a functional component of many biomolecules. Polyphosphates (polyP), another form of inorganic P, are ubiquitous, intracellularly found polymers. PolyP, occur in microalgae during the cell cycle and co-localize with cell nuclei. Both, Raman microscopy and conventional fluorescence microscopy detected groups of polyP granules on each side of the single nucleus, or later, on the outer side of newly formed nuclei in the synchronized culture of Desmodesmus quadricauda (Moudříková, 2021). PolyP production and consumption seem to be related to the progression of the cell cycle and nucleic acid synthesis. Moreover, it was proved, that the synthesis of polyP granules is crucial for the cell to cope with the energetic dynamics under stress conditions (Sanz-Luque, 2020). In most unicellular eukaryotes, the polyP synthesis is performed by the vacuolar transporter chaperone (VTC) complex. In Chlamydomonas reinhardtii, orthologues for Vtc1 and Vtc4, are essential for polyP accumulation. We hypothesize that polyP can serve as a short-term P source during the cell cycle as well as a long-term depot in adverse conditions. To elucidate the link between polyP formation and cell cycle progression, we followed the behavior of C. reinhardtii vtc1-1 mutant strain (vtc1), deficient in polyP synthesis, and vtc1-1 rescued with VTC1 (vtc1R), in the control medium, and under stress conditions of sulfur (S), or phosphorus (P) starvation. Both vtc1 and vtc1R strains were able to grow and divide up to 16 daughter cells (DC) in the control medium. Cells of vtc1 mutant were growing, but not dividing in S or P deprivation, in contrast to vtc1R strain able to divide to 4 DC. Cells of vtc1 mutant overproduced starch in -S and lipids in -P conditions. On the other hand, PolyP content was increased in vtc1R cells starved for S.

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GENETICS OF MICROORGANISMS

P29 Genetic Modification of the Diatom Phaeodactylum Tricornutum for Enhanced Production of the Carotenoid Fucoxanthin

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In this study, we focused on *Phaeodactylum tricornutum*, a marine diatom, which has been widely studied due to its potential for fucoxanthin production. Fucoxanthin is a carotenoid used as a dietary supplement for its high antioxidant, anti-inflammatory, antitumoral and antiobesity qualities. Due to its great biotechnological potential but relatively low content in algal biomass, improvement of the strain is needed to enhance production in algal biomass. This work aims to improve the fucoxanthin production in *P.tricornutum* by directed mutagenesis.
Methods: Three different approaches were used to construct strains with increased fucoxanthin content. 1. the Crispr/Cas9 modification system combined with a bacterial conjugation to knock out genes involved in chlorophyll c biosynthesis. 2. overproduction of selected genes involved in fucoxanthin biosynthesis. 3. Modifying the overproduction system by replacing promoters with new, stronger ones. The strength of these promoters was checked by the expression of a green fluorescence protein.
Results: The Crispr/Cas9 modification was successfully used, and the gene for F-DVR was knocked out.
The HPLC analysis showed no difference in the production of chlorophyll c.
Overproductive mutants were successfully constructed. Several mutants showed higher content of fucoxanthin when compared to the wild type. Experiments analyzed by scanning microscopy and flow cytometry showed much stronger GFP production when compared to the fcpA promoter.
Discussion and conclusion: Deleting the F-DVR divinyl reductase gene does not affect chlorophyll c production, presumably because another enzyme may replace it. Therefore, we assume it can be an N-DVR enzyme. Genetically modified strains with increased fucoxanthin content are valuable for biotechnology as they may decrease production costs which is among the most limiting factors of industrial production. In addition, a strong promoter with good properties has biotechnological benefits because it can be used, to create new vectors that will efficiently overexpress selected genes.

Acknowledgment: This work was supported by the Technology Agency of the Czech Republic programme National Centres of Competence: (project ID TN01000048).

GENETICS OF MICROORGANISMS

P30 Combination Therapy of Antibiotic with Metal Complexes in the Fight Against Staphylococcus aureus

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Antibiotic-resistant bacterial infections are a global threat. Nowadays, many previously manageable bacterial infections are becoming hard to treat. Furthermore, the difficulties of new drug development have resulted in few new antibiotics being approved in recent years. The development of novel compounds with lower costs and lower toxicity for the treatment of illnesses caused by multidrug–resistant (MDR) microbial pathogens should be a priority in current antimicrobial research. Transition metal complexes have important biological activities, including antibacterial, antifungal and anticancer. Modern medicine is trying to eliminate the overuse of antibiotics to avoid the development of bacterial resistance. One of possible approaches is the combination of conventional antibiotics with other compounds, e.g. metals to increase their effectivity. Increased susceptibility of gram-positive and gram-negative bacteria to antibiotics in the presence of metals has been reported. One of the mechanisms by which metals can potentiate the higher antimicrobial effect of antibiotics is their chemical properties, synergy effect and more effective penetration of antibiotics into the cell. Mixed antibiotics can also bypassing barriers that are anchored in the cell wall, such as efflux pumps. Recent reports of combined antibiotics with metals suggest a mechanism for disrupting efflux pumps to manage antibiotic resistance. Iron has proven to be a viable carrier for the transfer of specific antimicrobials due to its antimicrobial properties. Therefore, in our research we study the iron complex in combination with penicillin, which has been proposed as a potential antibacterial drug to manage bacterial resistance. Minimal inhibitory concentration, SEM microscopy, and qPCR were used to investigate the antimicrobial effect on Staphylococcus aureus. In comparison to the original medicines, the presence of metal compounds potentiated the increased antibacterial activity of S. aureus.

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GENETICS OF MICROORGANISMS

P31 Genetic drivers of chromosomal integrons stability

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The integron is a bacterial recombination system that allows acquisition, stockpiling and expression of promoterless genes embedded in cassettes. Some integrons, like the one we find in the second chromosome of Vibrio cholerae, can be particularly massive and contain dozens of non-expressed cassettes. It is unclear how such genetic structures can be stabilized in bacterial genomes. Here, we reveal that the orientation of integrons within bacterial chromosomes are essential to their stability. Indeed, we show that upon inversion of the chromosomal integron of Vibrio cholerae, its plasticity is dramatically increased. This correlates with a strong growth defect, associated with the excision of a particular type of cassettes that are toxin-antitoxin systems. Those observations provide a striking example of the relationship between genome organization and genome stability; and suggest that toxin-antitoxin systems can also act as "sensors" of genetic plasticity.

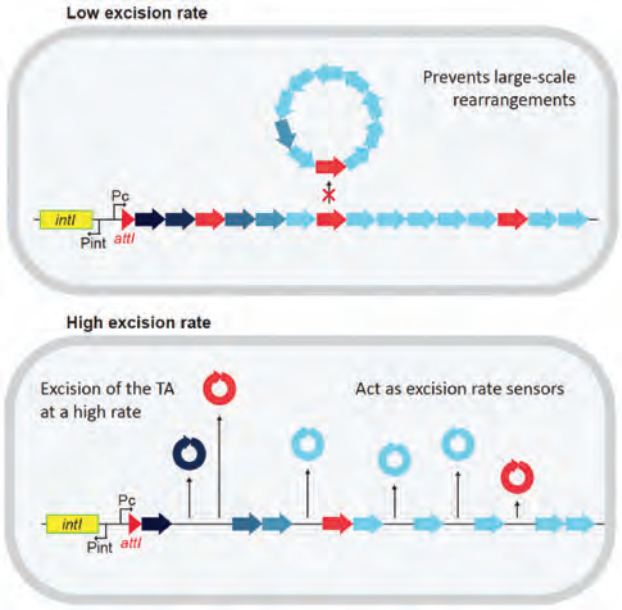

Fig. 1: Model of the double action of TA systems (red cassettes) within the SI to stabilize the array of cassettes. The classical activity preventing large scale rearrangements (up), and the proposed "sensor activity" driving low excision rates by killing the cell when the excision rate becomes too high.

GENETICS OF MICROORGANISMS

P32 Escherichia coli: Analysis of Zinc Involved Genes After Routinely Treatment with Agriculturally Used Zinc and Zinc Nanoparticles

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Introduction: Zinc oxide (ZnO) and zinc oxide nanoparticles (ZnO NPs) have been used as fertilizers and feed supplements in agriculture for many years. Many domestic animals are unable to absorb high concentrations of used zinc in feed, so it ends up in the environment in feces form, causing its contamination. For that reason, in the European Union (EU) from June 2022, zinc will be allowed as a feed additive only in quantities that meet daily animal requirements. Zinc application on bacteria can promote the development of resistance to it. Thus, using it for an extended period can result in the non-effectiveness of the material.

Methods: This poster is focused only on the biology of one model bacteria, Escherichia coli CCM 7929, exposed to a sub-inhibitory concentration of ZnO/ZnO NPs for the 40th time. The bacteria were divided into two groups: E. coli treated with sub-inhibitory concentrations of ZnO/ZnO NPs for the 40th time, and E. coli treated with sub-inhibitory concentrations of ZnO/ZnO NPs for the 20th time and then sub-cultured without them to obtain the 40th subcultures. Subcultural pellets were freezed in -80°C and DNA, RNA and proteins were isolated. Multi-omic approach was used to study effect of zinc treatment on zinc related genes from genomic, transcriptomic and proteomic point of view. DNA (cDNA) libraries were prepared from isolated DNA and RNA. Genome was sequenced by MiniSeq,Illumina and transcriptome was sequenced by Illumina and obtained data evaluated in Geneious program. were performed. Proteins were isolated and processed by LC-MS.

Results and Discussions: Bacteria developed resistance to zinc. At the same time some mutations in zinc related genes, as well as their different production.

Acknowledgement: The study was financially supported by IGRÁČEK MENDELU (No. CZ.02.2.69/0.0/0.0/19_073/0016670).

GENETICS OF MICROORGANISMS

P33 The Fim and FhaB adhesins play a crucial role in nasal cavity infection and Bordetella pertussis transmission in a novel mouse catarrhal infection model

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Pulmonary infections caused by *Bordetella pertussis* used to be the prime cause of infant mortality in the pre-vaccine era and mouse models of pertussis pneumonia served in characterization of *B. pertussis* virulence mechanisms. However, the biologically most relevant catarrhal disease stage and *B. pertussis* transmission has not been adequately reproduced in adult mice due to limited proliferation of the human-adapted pathogen on murine nasopharyngeal mucosa. We used immunodeficient C57BL/6J MyD88 KO mice to achieve B. pertussis proliferation to human-like high counts of 10^8 viable bacteria per nasal cavity to elicit rhinosinusitis accompanied by robust shedding and transmission of *B. pertussis* bacteria to adult co-housed MyD88 KO mice. Experiments with a comprehensive set of *B. pertussis* mutants revealed that pertussis toxin, adenylate cyclase toxin-hemolysin, the T3SS effector BteA/BopC and several other known virulence factors were dispensable for nasal cavity infection and *B. pertussis* transmission in the immunocompromised MyD88 KO mice. In contrast, mutants lacking the filamentous hemagglutinin (FhaB) or fimbriae (Fim) adhesins infected the nasal cavity poorly, shed at low levels and failed to productively infect co-housed MyD88 KO or C57BL/6J mice. FhaB and fimbriae thus appear to play a critical role in *B. pertussis* transmission. The here-described novel murine model of *B. pertussis*-induced nasal catarrh opens the way to genetic dissection of host mechanisms involved in *B. pertussis* shedding and to validation of key bacterial transmission factors that ought to be targeted by future pertussis vaccines.

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NOVEL EMERGING TECHNOLOGIES IN GENETICS

P34 Optogenetic Tools to Control Cell Death in the Zebrafish Skin

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A key response to the infection of cells is the initiation of specific inflammatory cell death pathways and a signaling response to warn and activate surrounding cells. One of the well-conserved systems for an innate immune response is the formation of the inflammasome. The inflammasome is a large multiprotein signaling platform formed for the activation of pro-inflammatory caspases and is activated by oligomerization of pattern-recognition receptors. The ability to control programmed cell death (PCD) in time and space at high resolution can specifically aid the understanding of cell and tissue responses to PCD in vivo. To study this, we have developed a genetically encoded tool for activating PCD (pyroptosis and/or apoptosis) in zebrafish skin.
For the optimal spatial and temporal control of cell death, we constructed Opto-ASC, a fusion of the light-responsive element of Cryptochrome 2 (Cry-2olig) and the inflammasome adaptor protein ASC (Apoptosis-Associated Speck-Like Protein Containing a CARD). We introduced a cassette of this construct under the control of heat shock element into zebrafish in which we can now induce ASC inflammasome (speck) formation in single cells of the skin.
We find that cell death resulting from ASC induced speck formation can differ in morphology and extrusion pattern. In the keratinocyte layer, some cells extrude apically and some basally. The apical extrusion in keratinocytes depends on Caspb and involves a strong Ca2+ signalling response in neighbouring cells.

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NOVEL EMERGING TECHNOLOGIES IN GENETICS

P35 Repair focus micro- and nano architecture in double strand break repair efficiency and pathway selection

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Ionizing radiation-induced foci (IRIFs) are a sensitive marker of DNA DSB induction/ repair. A development of microscopy techniques allows to study their internal composition and architecture at the nano-scale. The architecture of IRIFs could be expected to be dependent on both, local chromatin architecture and ongoing repair processes at individual DSB sites. It could be hypothesized that the multi-scale IRIF nano-architecture reflects various aspects of DSB repair or (co)determines repair processes. The IRIF nano-architecture may represent a new layer in DSB repair regulation and a carcinogenesis marker. We were interested if the IRIF architecture depends on the DSB repair stage, local chromatin architecture at damage sites and type of the incidental radiation. As the radiosensitivity of cancer cells often differ from normal cells, we also investigated if there exist some differences in IRIF architecture, that could be correlated to differences in chromatin architecture, repair pathway activation, persistence of unrepaired DSBs and the overall cell radiosensitivity. Combining techniques of particle accelerators, 3D confocal and Single-Molecule Localization Microscopy, we compared the micro- and nano-scale architecture of IRIFs in normal fibroblasts/ cancer cells by exposing to different doses of low/high-LET radiation. Using mathematical approaches based on Ripley's distance frequencies, cluster analyses, persistence homology, we found that the nano-architecture of IRIFs formed by gH2AX, 53BP1, RAD51 and MRE11 is protein-specific and follows the same principles in normal and cancer cells, with high mutual similarity of individual IRIFs. The topology was more similar for IRIFs associated with heterochromatin. This suggests that the internal architecture of IRIF has a functional role. The IRIF assembly, extent of topological similarity and protein composition differed for normal and tumor cells, which could be correlated to repair differences and/or radiosensitivity.

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NOVEL EMERGING TECHNOLOGIES IN GENETICS

P36 Automatic Analysis of Ionizing Radiation-Induced Foci (IRIFs) and Extraction of Advanced IRIF Parameters with our Newly-Developed Software Based on Artifcial Neural Networks and Deep Learning

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DNA double-strand breaks, marked by Ionizing Radiation-Induced (Repair) Foci (IRIF), are the most serious DNA lesions, dangerous to human health. IRIF quantification based on confocal microscopy represents the most sensitive and gold standard method in radiation biodosimetry and allows research of DSB induction and repair at the molecular and a single-cell level. Manual couniting of IRIFs is a very demanding and tedious process, whereas manual labeling of segmentation of individual IRIFs is almost impracticable. Our deep learning-based method for IRIF and nuclei segmentation analyzes those 3D images au- tomatically with a fidelity comparable to an experienced examiner, which is critical both for practical/clinical biodosimetry and radiobiological research. Moreover, as the software provides 3D segmentation of individual IRIFs and nuclei, it enables extraction of various micro-morphological IRIF parameters, such as the IRIF volume, solidity or volume of the nucleus, and other parameters, including the intensity of foci or the extent of mutual collocal- ization between IRIFs formed by different repair proteins. Importantly, the software can be trained for different IRIF and cell types. This is very advantageous when analyzing cancer cells, which often show diffuse, irregular and/or very heterogeneous IRIFs accompanied with an intensive background signal.

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NOVEL EMERGING TECHNOLOGIES IN GENETICS

P37 Comparison of Age Prediction from Blood by Pyrosequencing and Massively Parallel Sequencing

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Probably the most pursued and the most rapidly developing phenotyping tool all over the world is age prediction. Most of the published methods rely on Massively Parallel Sequencing (MPS) while one of the commercially available epigenetic typing kits, AgePlex from Qiagen, is based on pyrosequencing. In this project, we tried to develop an MPS age prediction model that would yield better performance characteristics than AgePlex.

We tested 59 CpGs in areas previously published as differentially methylated depending on age using methyl-specific qPCR in duplexes followed by next-generation sequencing on Illumina platform MiSeq and NovaSeq. The statistical model was developed using the 5 most informative CpGs in a testing set of 100 samples from healthy Czech blood donors and was validated using a set of another 100 samples. The same set of samples was tested by the AgePlex kit.

The prediction model, thatis being currently validated, consists of individual CpGs in genes *CCDC102B, ELOVL2, PDE4C, FHL2* and C1orf132. The successful rate of prediction in the range of 4 years was 89 % in the testing set, with a mean absolute error of 1.79 years and a mean square error of 3 years.

Here, we present more detailed results of prediction model validation and performance comparison for both our MSP based prediction method and commercially available pyrosequencing-based prediction method AgePlex.

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Acknowledgement: Results of the project described in this abstract will be presented also at ISFG 2022 conference in Washington, DC.

NOVEL EMERGING TECHNOLOGIES IN GENETICS

P38 Time-defined optogenetic control of bacteria adhesion

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Biofilms are bacterial communities that provide organisms within shield from external hazards and an environment to undertake complex metabolic tasks, as such, biofilms play significant roles in nature, medicine and industry. Biofilm formation is a process well characterized, but our ability to control it has been limited. Our strategy was to use optogenetic switches with high specificity and tunability to control biofilm formation by targeting its early stages with periodic light stimuli.

Methods: Synthetic adhesins *nMagHigh-eCPX* and *pMagHigh-eCPX* were expressed on bacterial membranes, allowing for the formation of multicellular clusters upon blue light illumination, with the adhesion reversing in darkness. A fluorescent reporter linked to the *lsr* promoter was used to measure quorum-sensing (QS) activation. Bacteria were exposed to darkness, constant blue light and nine different light-dark settings for 2h to achieve clustering and QS, and 48h for mature biofilms.

Results: Constant illumination resulted in bigger (~60%) and more compact bacterial clusters than darkness; however, tuning the dynamics of the photoswitches with consecutive pulses of light and darkness results in a variety of cluster sizes. Pulse illumination of 5-20 min light-dark resulted in the formation of even bigger (~400%) clusters and accelerated QS activation. Likewise, biofilms grown in constant illumination were ~20% thicker than those grown in darkness, but 5-20 pulse illumination was able to achieve a thicker (~90%), more massive (~500%) biofilm.

Discussion and conclusion: Optogenetic switches induced by periodic stimuli of light and darkness aided in the control of bacterial aggregation, quorum sensing and biofilm formation. From the tested pulse settings, 5-20 min light-dark maximized bacterial interactions and final biofilm mass. Pulse illumination provides a useful tool for controlling biofilm biology, and thus, has the potential to accelerate research in these areas.

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NOVEL EMERGING TECHNOLOGIES IN GENETICS

P39 LYNX: A web-based bioinformatic tool for targeted next-generation sequencing data analysis and visualization in lymphoid malignancies

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Next-generation sequencing (NGS) is one of the fastest-growing technologies that finds widespread application in medicine and related laboratory fields. The increasing availability and wide range of applications are pushing the use of NGS into routine diagnostics. However, NGS data processing requires bioinformatic expertise or specialized tools with a user interface (UI) for easy analysis and interpretation.
Methods: We have developed a comprehensive targeted NGS panel providing information about genetic markers in the most common lymphoid malignancies. Based on this NGS panel, we have designed a bioinformatic tool LYNX (LYmphoid NeXt-generation sequencing) with a UI providing easy access to data analysis and interactive visualizations of the results for smooth interpretation to clinicians in diagnostic settings.
Results: We have created a modular interactive bioinformatic tool for the analysis of targeted NGS data. Computational pipelines implemented in the tool provide information about mutations in selected genes, copy number variations across the genome, translocations, and rearrangements of antigen receptors in the most common lymphoid malignancies. All this information is presented via interactive tables and graphs in the UI. In addition, IGV software for the visual exploration of genomic data has been integrated.
Discussion and conclusion: The LYNX simplifies the process of targeted NGS panel data analysis and interpretation for laboratories without their own bioinformatic support. The UI enables users to execute the data analysis and then to explore the results and identify genetic markers for individual patients. Moreover, thanks to its modularity, LYNX can be adapted for other diagnostic panels, thus ensuring its transferability to other diagnostic applications.

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NOVEL EMERGING TECHNOLOGIES IN GENETICS

P40 Computational simulation of genomics, phenomics and environmental correlated variables using machine learning.

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We present a new computational system to simulate, in a modular manner, multi environmental trials in the form of an R package. The system allows for the simulation of environmental and genetic variables correlated. Phenomic characteristics are generated by machine learning models implemented in the package, which use genomic and environmental values simulated to create phenomic attributes. The package allows the simulation of any number of genotypes/genomic expressions across various environments. The system can be used to generate data to validate quantitative genetic models.

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NOVEL EMERGING TECHNOLOGIES IN GENETICS

P41 Current trends in performing large-scale multi-omics research projects

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Currently, different scientific fields generate large amounts of data that are no longer possible to store and analyze on the local computer. One of such examples could be genomics, transcriptomics and neuroimaging. With the rise of distributed computing, especially cloud computing services, we are not only able to analyze this data, but also combine those rich data sources to get novel insights by creating derived phenotypes. UK Biobank is a powerful biomedical database containing various data from half a million UK participants. List of the data includes omics (array data, whole exome and whole genome sequencing data), imaging (whole body MRI, retinal tomography, whole body bone density scan, etc.) and health records.
In this talk, I would like to present several studies that were made using UK Biobank data combining different data to get novel insights. Example of such a study is using accelerometer data to identify physical activity, performed by applying machine learning (ML) models and then using these derived phenotypes to perform genome wide association studies.
With greater adoption of distributed and cloud computing, providing storage for petabytes of data, enabling access to vast computing power tailored to specific use-cases such as ML, and allowing for easier collaboration, we can expect more novel approaches to data analysis leading to valuable insights.

NOVEL EMERGING TECHNOLOGIES IN GENETICS

P42 Deep Amplicon Sequencing of DNA Polymerase Epsilon for Effective Endometrial Tumor Diagnostics Using fastGEN Technology.

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Introduction and aims. Tumor DNA testing of ***POLE* gene**, which is component **DNA polymerase epsilon**, is a prerequisite for tumor risk-group assessment and personalized treatment in endometrial carcinoma. *POLE* mutated tumors are low risk and adjuvant chemotherapy should be deescalated. *POLE* mutations occur in 7 – 12% of endometrial cancers and have been associated with high tumor mutation burden, neoantigen load. Retrospective analysis showed that pathogenic *POLE* mutations are associated with clinical benefit to **immune checkpoint inhibitor therapy**, thus further thorough studies are warranted to validate *POLE* mutation as a predictive biomarker. Methods. **Deep amplicon sequencing** (DAS) has a potential to be suitable method for simultaneous detection somatic mutation within hotspot regions in exonuclease domain (exons 9 - 14) and with a defined detection limit down to 1% MAF. We have developed and validated a **unique fast method known as fastGEN** for genotyping of hotspot cancer mutations based on DAs using Illumina platform. Results. We have analyzed 33 endometrial tumors with 100% success rate. Pathogenic variants were found in 6 samples (2 with *POLE* p.P286R, 2 with p.V411L, 1 with p.M444K and one with p.S459F). Results of fastGEN were validated using larger somatic NGS panel (Nonacus Pancancer TMB/MSI; Qiagen QIAseq TMB Panel; Archer VariantPlex GyNcore and Illumina TSO 500). Using samples (n = 10) where results of both methods were available we observed perfect concordance with **100% specificity and sensitivity**. Variant detection using fastGEN *POLE* was highly reproducible (n = 4, *POLE* p.S459F, MAF = 29.8% ± 1.7% [mean ± SD]). Turn-around-time (sample to final report) was **less than 24 hours**.
Discussion and conclusion. Described method is routinely used in tumor diagnostics at our place and showed excellent performance; therefore it was licensed and thus could be used in other labs as a kit. The partner BioVendor Group is able to produce the kit in a high amount, perform rigorous QC, ensure the certification and distribute it worldwide.

NOVEL EMERGING TECHNOLOGIES IN GENETICS

P43 In Vitro Evolution of a Short Catalytic DNA Motif

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In Nature, we can find examples of catalytic RNA motifs called ribozymes. Short DNA motifs (deoxyribozymes) able to catalyze chemical reaction also exist but none such structure has so far been identified in the context of living organisms. In this work, we wanted to find unusually short artificial deoxyribozymes that are able to cleave RNA.

Methods: In vitro evolution experiments typically use libraries of ≈ 1015 sequences and require multiple rounds of selection to identify rare variants with desired activity. Based on the simple structures of some aptamers and nucleic acid enzymes, we hypothesized that functional motifs could be isolated from significantly smaller libraries in a single round of selection followed by high-throughput sequencing.

Results: Our selections yielded deoxyribozymes (and, in some cases, their sequence requirements) only 12 nucleotides long with activities only 8 to 30-fold lower than those previously isolated under similar conditions from libraries that were 7 orders of magnitude larger.

Discussion and conclusion: Here, we were able to identify short deoxyribozymes in a single step of an in vitro evolution. Our findings indicate that disadvantages of using less diverse pools and short catalytic motifs can be surprisingly small. Considering the functionality of known natural ribozymes, an RNA-cleaving deoxyribozyme would be the most probable motif to occur in Nature.

Acknowledgement: We thank Czech Science Foundation (GACR) [19-20989S] and 'Chemical biology for drugging undruggable targets (ChemBioDrug)' [CZ.02.1.01/0.0/0.0/16 019/0000729] from the OP RDE.

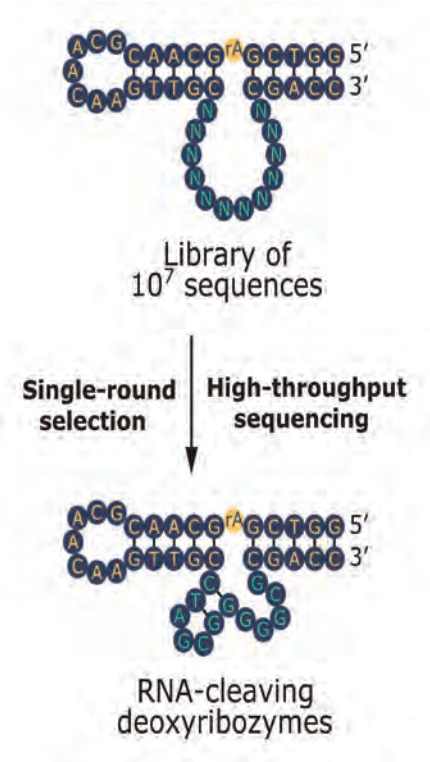

Fig. 1: From a DNA library with 12 randomized positions inserted into a stabilizing scaffold containing a single ribonucleotide as a substrate, we were able to select and characterize RNA-cleaving deoxyribozymes in a single round of in vitro selection.

NOVEL EMERGING TECHNOLOGIES IN GENETICS

P44 Long-read technologies identify hidden palindromic insertions in a family with X-linked congenital ataxia.

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Long-read techniques have complementary strengths to overcome the limitations of current standard genomic technologies, with a particular advantage in analyzing complex or repetitive genomic regions and identifying structural variants. By using Long-Read Whole Genome Sequencing (lrWGS), we aimed to identify the underlying pathogenic cause of a non-syndromic X-linked congenital ataxia with evidence of cerebellar atrophy at neuroimaging studies. In this large family, linkage to Xq25-q27.1 region was determined in 2008 (SCAX5 OMIM# 300703) but the underlying genomic defect remained elusive, despite all routine assessments including short-read genome sequencing (srWGS). Long-read sequencing analysis detected a complex genomic rearrangement including an inverted interstitial insertion of chromosomes 7p14.3 (99 kb) and 9q34.3 (160 kb) within the human-specific palindrome located in Xq27.1, which is known to be a hotspot for genomic rearrangements. Breakpoint junctions segregated with the X-linked inheritance in the family. To test the possible consequences of the disruption of topologically associating domains (TADs) combined with other positional or dosage effects, on the expression of cerebellar-specific genes located within the Xq27.1 palindromic region, we performed qPCR analysis on patient-derived lymphoblastoid cell lines and controls. Cerebellar degeneration protein 1 (*CDR1*), located downstream the palindromic rearrangement and Protein Phosphatase 1 Regulatory Subunit 17 (*PPP1R17*), present in the inserted 7p14.3 fragment, both specifically involved in cerebellar Purkinje cell development, were found to be upregulated in patients' cells. Our study highlights the added value of assembly-based lrWGS to detect new genomic disorders and provide insights into the pathogenesis of a previously undiagnosed rare condition.

Acknowledgement: We thank all family members for participation in this study. We are grateful to C. Bellcross and L. Boccuto. This study was initially sponsored by the Italian Telethon (grant n.492B to GZ)

GENETICS AND SOCIETY

P45 Health, Intelligence, Behaviour and Prosperity: a close look at the readings of our Genomes by the Environment

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Classical genetics officially starts with the publication of the seminal work of Gregor Mendel in 1866, which provides the first valid to this day basis of understanding of the rules of inheritance and in parallel with the book Hereditary Genius published by Francis Galton in 1869, a prequel of quantitative assessment of hereditary human traits. A century of follow up work led to the discovery of DNA as the hereditary material. Textbooks on Genetics now shuffle a fixed and given information. I argue that key questions about humans pertaining to classical genetics are still open, awaiting not only our modern molecular techniques, but also an intellectual framework to approach them:

1. Which methodological approach is more insightful in explaining human traits, the Mendelian view of humans as individuals or the Galtonian view of humans as populations?
2. How should we target cancer prevention, via gene forms and environments that are unique in each person or via a universal application of clinical screenings?
3. Was Cavalli-Sforza right in viewing peoples as being genetically comparable or Charles Murray in viewing peoples genetically apart?
4. Richard Lewontin and Edward O. Wilson, debated each other since 1975 till the end of their lives about the control of human behaviour by genes. Who was right?
5. Are there biological limits in achieving the humanitarian ideal of human equality?
6. Can we be partially or fully determined by our genes or environments and still responsible for our actions?
7. When are we more behaviorally free, when we follow our heart or when we plan for the future?
8. What does it mean to be human? Whatis more of an objective guide for our politics and ethics, our ideals or our universal behavioral attributes?

Providing better answers to these questions is to follow our environmental history and its instructions by our Books of Genesis in accordance with worldviews provided by humanities. The central theme of my work is that genome by environment interaction provides a basis of understanding humans in terms of wellbeing, sociality and ethics.

GENETICS AND SOCIETY

P46 Dopaminergic System Genetic Polymorphism and Physical Activity among Women

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While, there is a consensus regarding the health benefits of physical activity (PA), there is large variability in PA engagement, ranging from sedentary behavior to highly active. Overall, women do not meet the recommended weekly PA threshold compared to men. PA is a multifactorial trait, influenced by genetic and environmental factors, in which dopamine plays pivotal role. Dopaminergic system functional genetic polymorphisms cause differences in dopamine levels. The aim of the current study was to explore the association between Dopaminergic system functional genetic polymorphisms and PA engagement among healthy women. One hundred fifty-nine (159) healthy women, aged 25-60 years from similar demographic background participated in the study. PA engagement was assessed by the BAECKA questionnaire of habitual physical activity PA. Genomic DNA was extracted from buccal epithelial cells for Dopaminergic system related genetic polymorphisms analysis. Despite a similar demographic background of the participants, a large variance was found in all PA indexes. Significant association between catecholamine O-methyl transferase (COMT) A/G rs4680 polymorphisms and PA engagement was found. COMT rs4680 A-allele associated with low enzymatic activity and therefore higher dopamine levels was significantly more prevalent (83%) among highly active women compared to its prevalence among moderate (64%) and low (47%) active women. Overall, it seems that that A-allele might be associated with a relatively high rate of PA practitioners in general and running in particular

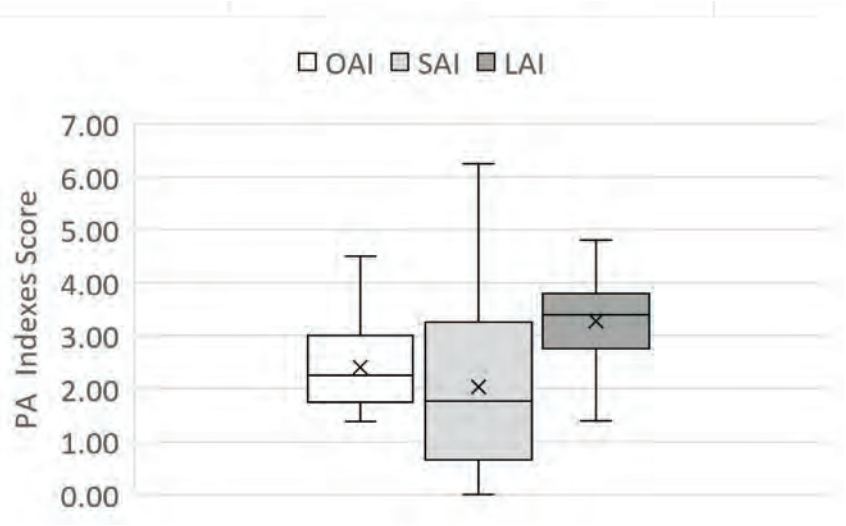

Fig. 1: PA Indexes distribution

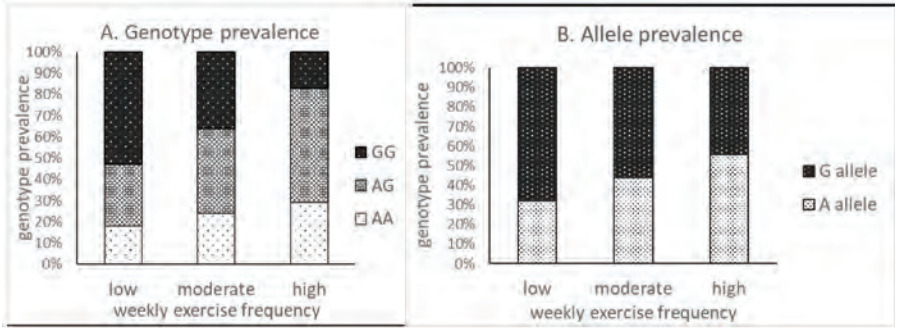

Fig. 2: 2 COMT A/G rs4680 genotype (A) and allele (B) prevalence among Israeli adult women divided to WEF

GENETICS AND SOCIETY

P47 Genomic techniques governance: what can we learn from EU-based reports

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Regulatory framework for the new breeding techniques (NBTs) in the European Union in the field of agriculture is subject to societal and political debates. The case study evaluates the findings of six Reports on the subject produced by the EU bodies between years 2017 and 2022. In addition, it is drawing lessons from ten research articles relevant to the subject. Key findings include (1) ongoing debate on NBTs between proponents' arguments, such as sustainability and productivity and opponents who are putting forward the safety and environmental concerns, (2) the EU regulatory framework for NBTs, including CRISPR, is stricter and is contrasting with the rest of the world, (3) current EU definition of GMO's is not fit for purpose and has negative impact on agriculture, especially in economic terms and (4) call for a revision of the current EU regulatory framework, such as the clarification of the GMO definitions, inclusion of new genomic techniques, product based approach and case-by-case risk assessment.

Acknowledgement: The opinions expressed in this abstract is the one of the author only and not of the European Committe of the Regions.

GENETICS AND SOCIETY

P48 Should Mendel Have Patented His Peas?

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The fundamental concept Gregor Mendel drew from his cross-hybridization experiments in the common pea plant was the notion of heritable traits transmitted to offspring as discrete "units" – what we now call genes. The tremendous impact of his discovery on biology and medicine need not be recounted here. But could the gentle friar ever have dreamed that these "units" would someday become the subject of fierce battles over ownership, application, and bodily autonomy, involving thousands of lawyers, millions of patients, and many billions of US dollars?

In the years 2008-2013, I served as Expert Witness in the famous U.S. Supreme Court case challenging the legitimacy of exclusive gene patents. Beginning in the 1980s, with the identification and cloning of genes of commercial importance, these Mendelian "units" began to be patented by the discoverers. The "claims" in these patents covered any and all uses of the described gene for diagnostic or therapeutic purposes – essentially granting monopolistic "ownership" of a disease. The patents challenged in the case were those covering the *BRCA1* and *BRCA2* genes associated with familial breast/ovarian cancer and "owned" by Myriad Genetics, Inc.

Since Mendel himself was a naturalist performing *in vivo* experiments, it is both poignant and gratifying that the argument we posed to the Court invoked a stipulation in U.S. Patent Law that "products and laws of nature are not patentable subject matter". We argued that genes, whether inside the body or reproduced *in vitro*, are natural products. In a momentous decision in 2013, the Supreme Court concurred, thus invalidating not only BRCA but all existing gene patents. After all, where would biomedicine be today if Watson and Crick had chosen to patent the double helix in 1953? Or if Mendel had patented his Laws of Inheritance, making them off-limits for genetic counseling and testing? Fortunately for all of humanity, Mendel was driven only by his selfless, innate curiosity.

GENETICS AND SOCIETY

P49 The Ethics of Human Genetic Enhancement

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With the development of biology and the discovery of CRISPR/Cas9, human genetic enhancement has moved from science fiction to the near future, raising ethical dilemmas and discussions that became particularly urgent when Chinese scientist He Jiankui announced the birth of the first genetically modified babies. The twins are supposed to have been made resistant to HIV. While the ethics of human genetic enhancement had interested bioethicists for a few decades, this precedent, which was a secretive, reckless, sloppy, and unnecessary procedure, caused a strong public outcry, resulted in Jiankui's imprisonment, strengthened the moratorium on the human germline genome editing, and raised even more acute debates.
Making humans genetically resistant to HIV, if successful, would be an etalon example of human genetic enhancement. There is also a plethora of genetic diseases that, though rare, all together affect almost 10% of the population. We could, in principle, get rid of them in the future. The idea of editing genomes to prevent diseases, although rather straightforwardly appealing, faces resistance in the general population. Changes that improve intelligence or moods, face a rather universal rejection.
First, the idea of eliminating genetic diseases and "bettering" the human genome raises an uncomfortable analogy with "eugenics" – the term that earned a terrible reputation in the 20th century. Concerns are also raised about the expensive gene editing procedures widening inequalities and, as sometimes frighteningly claimed, creating a separate race of superhumans. In this paper, I aim at investigating the ethical aspects of human genetic enhancement and analyzing the reasons for the scholars' and public's negative attitudes towards genetic enhancement. I evaluate which of the concerns are really connected to the matter of editing human genomes, which ones are rather addressing social or governance issues, and which ones are caused by the incomplete understanding of genome biology and genetic determinism.

GENETICS AND SOCIETY

P50 The Law and Ethics of Genome Editing in Europe

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We have come a long way since Gregor Mendel first described inherited traits in his sweet peas. However, scientific developments repeatedly draw our attention to the importance of addressing the challenges related to genetics. Science, which itself is a uniquely human endeavor, is challenging traditional ideas and values again and again. For example, in 2018 a Chinese researcher claimed that twin girls had been born via genome editing using a method called CRISPR/Cas9. So, the first designer babies were born. The era of routine somatic and germline gene editing is closer than ever. The pioneer is likely to be China. However, the possible consequences of that are difficult to predict for now. The risks of heritable, unpredictable off-target genetic mutations raise questions about the safety and permissibility. The paper presents the current legislation in the European Union (EU) and the ethical principles that are relevant in designing the regulation.

Methods: The paper is based on a review of the EU's legislation, policy, and relevant studies. A systematic literature review has been also conducted.

Results: Since the human cloning debate of the mid-1990s, countries have enacted their prohibitions on human gene editing. There are several legally non-binding and binding international regulations that regulate the topic, as well. One of the most important legislation in Europe is the Council of Europe's Oviedo Convention from 1997. However, not all the EU Member States have ratified this regulation, yet. It would be essential to do so, or, at a minimum, to introduce a ban on establishing a pregnancy with modified germ cells. The existing regulation is not free from contradictions. It is therefore doubtful that it is able to serve scientific progress while providing adequate protection against unethical or illegal human interventions into the human genome.

Conclusion: It is essential to develop up-to-date general legislation for the task of human genome editing which is based on common European values and ethical principles.

GENETICS AND SOCIETY

P51 Population fragmentation in spatially and temporally varying environments

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Loss of biodiversity associated with shrinking and fragmentation of habitat space is a core topic of current ecology and conservation. I show that such a fragmentation can arise dynamically, from a failure to adapt to continuously changing conditions. It leads to an abrupt loss of species' genetic diversity, and dramatically reduces the total population size. Therefore, range fragmentation can substantially increase the likelihood of a species' extinction. Moreover, the faster the conditions change through time, the more likely such a range fragmentation is. This can have profound consequences on biodiversity, which is facing escalating rates of environmental change.

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GENETICS AND SOCIETY

P52 Ancient DNA: Novel Insights into Human Past and Their Social Impact

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Ancient DNA (aDNA) is DNA isolated from specimens older than 100 years and its analysis has become a crucial part of multiple scientific disciplines, such as archaeology, anthropology, paleopathology and evolutionary biology. Archaeogenetics (aDNA research) has thus introduced radically novel perspectives on development of human societies.
Thanks to the latest advances in laboratory and sequencing technologies, aDNA can be used on an individual level to estimate personal characteristics such as sex and health status. On population-scale, the increasing sample size allows for inference of traits as migration, population size, inheritance rules, biological relatedness and relation of social stratification to genetic background. In our research, we are using archaeogenetics to study these patterns in particular in the Migration Period and Early Middle Ages of Central Europe. To fully describe populations in the highly turbulent period of the 1st millennium, it is important to capture a wide range of regional and social diversity. In our projects, this is achieved with an unprecedented amount of samples from more than 6,000 individuals, comprehensive sampling strategy and high success rate of aDNA retrieval from decomposed human remains using our cutting-edge protocols.
Discoveries achieved with help of aDNA have a large society-wide significance. This concerns in particular the impact of human settlements on the environment and vice versa (sedimentary aDNA, population constrictions), understanding past pandemics and their consequences (thanks to bloodborne pathogens preserved especially in teeth and immune-related markers in ancient human genomes) and comprehending social and biological processes regarding human migration and mobility. Currently, we are interested in changes in kinship systems (via IBD inference of distant relatives and large-scale genealogies) and a complex relationship of identity and ancestry widely discussed within identity politics today.

PLANT GENETICS

P53 Estimating Inbreeding Depression in a Long-Term Study of Snapdragons (Antirrhinum majus)

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Introduction and Aims: Self-incompatibility (SI) is an evolutionary mechanism of major importance that prevents inbreeding in flowering plants. However, it is theoretically predicted to be easily lost in a population with too low inbreeding depression (ID). Using a large dataset from a field population of the plant species Antirrhinum majus, we investigate whether ID is above the necessary threshold to maintain SI in this population.

Methods: We estimate inbreeding depression through heterozygosity-fitness correlations (HFCs). For 11 years, we measured trait data for six fitness proxies, and genotyped a panel of 91 SNPs in 22,353 individuals. We first confirm that there is significant identity disequilibrium by measuring the correlation in heterozygosity between markers. This implies that there is variation in inbreeding, providing the opportunity for inbreeding depression to cause HFCs. We then correlate fitness against heterozygosity in order to quantify inbreeding load for these traits.

Results: Despite significant variation in inbreeding, only one of six fitness proxies (number of flowering stems) increased significantly with heterozygosity, and has a high inbreeding load of 0.7. Discussion and Conclusion: Inbreeding depression is expected to be strong in SI species such as Antirrhinum majus. We found strong inbreeding depression for the number of flowering stems, but HFCs were not significant for the five other fitness proxies. Whilst identity disequilibrium is significant, it is still too low to allow detection of weak HFCs for these traits despite our exceptionally large sample size. This could be due to environmental and stochastic effects on traits related to size and fecundity, which swamp fitness effects due to heterozygosity.

Acknowledgement: We thank the volunteers who have contributed to data collection in the field, in particular those that managed field seasons. Part of this work was funded by Marie Curie COFUND Doctoral Fellowship.

PLANT GENETICS

P54 New Polyploid Hybrids of *Mercurialis perrenis* and *M. ovata* in North Bohemia

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Interspecific hybridization and polyploidization is believed to be important source of variability. Most flowering plants have undergone at least one polyploid or hybridization event. It is crucial driving force for evolution of new species. Hybridization and polyploidization within genus Mercurialis is well known, but mostly in annual species. Here we introduce new possible hybrids of *M. annua* and *M. perennis*, rhizomatous monoecious perennials in North Bohemia.
Methods: We used flow cytometry to establish genome size of our samples. Nuclei were isolated from fresh leaves and measured using propidium iodide staining according to their size. Flow cytometry data were compared to standard (*Pisum sativum*) and genome sizes of *M. perennis*, *M. ovata* and morphologically different individuals were calculated.
Results: Genome sizes for *M. ovata* are not available in the literature, genome sizes of *M. perennis* in databases vary between 3 and 7 pg per diploid genome. Samples of *M. perennis* from North Bohemia possess 6,70 ± 0,22 pg/2C. In *M. ovata*, 2,98 ± 0,12 pg/2C. Three plants that differed in morphology of leaves were identified as plausible hybrids with mean genome size of 4,89 ± 0,04 pg/2C.
There are only few notifications about hybrid of *M. perennis* and *M. ovata*. *Mercurialis x paxii* Grebn and *M. longistipes* (Borbás) Holub has been identified in central Europe, since there are no additional data, the situation is very confusing.
Discussion and conclusion: Since the habitat of both species does not collide in most cases, the probability of interspecific cross in this case is very low. Nevertheless, the analysis of morphology traits and mainly genome sizes suggests, that localities of common appearance of *M. perennis* and *M. ovata* in Czech republic might have the right conditions to form hybrids.

Unfortunately, no genome size data are available for *M. ovata* so no further comparison is possible. To confirm that these plants are hybrids of *M. perennis* and *M. ovata* requires further cytological and possibly molecular methods.

PLANT GENETICS

P55 Uncovering the Function of PHD-HD Proteins: a Lesson from HAT3.1

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HAT3.1 is a protein with a unique plant-specific domain architecture, comprising a homeodomain (HD) and a plant homeodomain (PHD)[1]. Even though the PHD was initially identified in the HAT3.1[1], our knowledge about the role of PHD-HD proteins in plants remains scarce. The aim of our project is to elucidate the HAT3.1 function, mainly in terms of its putative involvement in the developmental processes via epigenetic-mediated transcriptional regulation.
Methods: To analyze the *HAT3.1* expression pattern in plants, we created a *pHAT3.1::EGFP-GUS* transgenic line, in which the spatiotemporal specificity of the *HAT3.1* promoter activity was assayed using GUS staining and bright-field microscopy. Subcellular localization of the HAT3.1 protein was investigated via laser-scanning confocal microscopy using seedlings of *35S::HAT3.1-EGFP* transgenic line stained with DNA-specific Hoechst dye.
Results: During vegetative development, the strongest activity of the *HAT3.1* promoter was detected in the meristematic zone of the primary root tip, in the emerging lateral roots, and in the developing true leaves. In the generative developmental stage, the expression was restricted to the female reproductive organs and developing embryos. The analysis of the HAT3.1 subcellular localization revealed its specific targeting to the nucleus with a partially speckled distribution. Interestingly, the foci of HAT3.1 protein were shown to colocalize with chromocenters, corresponding to heterochromatic chromosome regions.
Discussion and conclusion: *HAT3.1* encodes a member of the PHD-HD protein family and is predominantly expressed in the developing organs, overlapping with regions of actively dividing cells. The observed intranuclear localization and partial heterochromatin targeting of HAT3.1 might be linked to its so far unknown transcriptionally repressive function.
References: [1] Schindler U., Beckmann H., Cashmore A. R. (1993) *Plant J.* 4(1): 137 -50.

Acknowledgement: This work was supported by European Regional Development Fund-Project „SINGING PLANT" (No. CZ.02.1.01/0.0/0.0/16_026/0008446).

PLANT GENETICS

P56 Study of Genetic Variability of Buckwheat Genotypes using Gene Specific Markers

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Buckwheat (*Fagopyrum*) is one of the most nutritionally interesting and important crops around the world. Buckwheat belongs to the pseudocereals whose grains are gluten-free so they are suitable for celiac people. Molecular markers are a suitable tool for the analysis of genetic variability. SCoT technique is a new one used for the detection of polymorphism in plant genes and the evaluation of genetic diversity. The aim of our work was to analyze 21 genotypes of *Fagopyrum esculentum* and 14 genotypes of *Fagopyrum tataricum* by using ten SCoT markers. The seedlings of buckwheat were used for DNA isolation and subsequent amplification of the DNA fragments by using SCoT primers was done. Percentage of polymorphism and polymorphic information content (PIC) values were calculated. A binary matrix from the data obtained was prepared and dendrogram based on hierarchical cluster analysis using UPGMA algorithm was constructed. Totally 162 polymorphic fragments were amplified with an average of 16.2. The percentage of polymorphism ranged from 58.33 % (ScoT 60) to 100 % (SCoT 12, SCoT 13, SCoT 29 and SCoT30). The PIC values ranged from 0.578 (ScoT 60) to 0.932 (ScoT 36). Genotypes of buckwheat were divided into two main clusters according to species. Two genotypes of common buckwheat (Siva, Špačinska I) from Slovenia and the Slovak Republic, respectively, were genetically the closest. According to our results, we can consider the SCoT technique appropriate for the differentiation of common and tartary buckwheat genotypes which demonstrates a high average percentage of polymorphism (90.29 %) and a high average PIC value (0.859).

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PLANT GENETICS

P57 Branching Shapes the Plants– Lessons from Pea

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Pea is a great model organism for study of the plant architecture. Plasticity of the plant shape is possible, among others, due to activity of the shoot apical meristem (SAM). From this single meristem originates the entire above-ground part of the plant mass.
The prerequisite for formation of a branch is initiation of axillary meristem. The regulatory mechanism, by which the SAM controls outgrowth of axillary meristems, is known as apical dominance. Removal of the SAM releases one or more axillary buds from their dormancy to replace the previously dominant apex. The most studied signal molecule originated in the SAM is auxin.
Aim of this work was to demonstrate that auxin canalisation and its polar transport mediated by PIN1 auxin transporters orchestrate the bud outgrowth control.

Methods: Pea plants were grown in perlite in a growth chamber at 20/18 °C, 16 h day/8 h night photoperiod. 7-Day-old plants, intact or decapitated 10 mm above the upper bud, were used for the experiments. Experiment treatment included 0.5% IAA, BA, Z, TIBA and GR24 application on different sites.

Results: The outgrowing buds in each experiments were measured and plant material was collected for PIN1 protein immunolocalization and confocal microscopy.

Discussion and conclusion: After decapitation the axillary buds establish directional auxin export by subcellular polarization of PIN1 proteins, while auxin application on the decapitated stem prevents this PIN1 polarization and canalization of laterally applied auxin. Moreover, axillary buds after decapitation are competing with each other for restoring the lost apex. This competition is based on auxin flow mediation. TIBA can interrupt auxin canalisation and auxin transport related processes. Direct application of BA or Z to axillary buds can promote their outgrowth, even in intact plants, while direct application of GR24 can inhibit their outgrowth. These hormones act as fine-tuners of auxin flow.

Acknowledgement: CEITEC 2020 (LQ1601) project with financial contribution of the Ministry of Education, Youth and Sports of the CR within special support paid from the National Program of Sustainability II funds,

PLANT GENETICS

P58 Pea breeding programmes in the Czech Republic

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The breeding of peas in Czech Republic (based on collaboration of several research and breeding institutions) has a clear conception and produces novel cultivars which are registerd and grown mostly in EU countries. Three companies are currently engaged in pea breeding, i.e. Selgen a.s., Semo a.s. and Agritec Ltd. Yield stability for producers and quality for end-users are the main aim of breeders. Seed quality of pea free from antinutritional factors and high nitrogen content (> 23 %) for feeding is demanded.
The breeding goals in green peas are: optimal green colour, size and shape of seeds, high content of resistance starch, high content of vitamins and carotenoids (luteins, β-carotene). Breeding pea is focused on biotic stresses, the major culprits of which are fungal and viral diseases. For both field peas and green peas resistances against fungal (Erysiphe pisi, Fusarium ssp., Ascochyta complex) and viral pathogens (PEMV, PSbMV) are important. Resistance of new lines was verified in standard biological inoculation tests. The results of cooperation between research and breeding companies are new resistant pea varieties. The varieties are resistant to powdery mildew (Erysiphe pisi f. sp. pisi; gene er-1) and fusarium wilt (Fusarium oxysporum f sp. pisi) races 1 (gene Fw). Up todate, 376 SNP markers were identified, some of them have shown high association with studied traits. SNP markers of these traits – e.g. downy mildew (Erysiphe pisi) resistance, resistance to Pea Enation Mosaic Virus (PEMV) and Pea Seed-borne Mosaic Virus (PSbMV) - were adopted to CAPS markers, routinely used in pea marker-assisted selection (MAS). The identified molecular markers may be immediately used for technological progress and genomic prediction of breeding value of novel pea cultivars in marketing programmes of the Czech enterprises.

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PLANT GENETICS

P59 Genetic Diversity of Maize Genetic Resources using Simple Sequence Repeats (SSRs)

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Maize is the major cereal growing all over the world and it is a considerable cereal ranked as the third in world cereal production after wheat and rice. The use of maize is broad-spectrum, especially in the food, feed, and biotechnology industries. Based on DNA polymorphism, it is possible to distinguish individual varieties and lines of maize and optimally direct the direction of their use. Detection of genetic diversity is also used in marker-assisted selection (MAS), which significantly shortens the breeding time and allows the selection of varieties suitable for the use. The aim of the work was to detect the genetic diversity of 25 Slovak maize lines from the company Zeainvent Trnava s.r.o. application of SSR markers. Five primer pairs designed by Elci and Hancer (2015) were used in PCR. The amplified DNA fragments were visualized in 10 % polyacrylamide gels due to their higher separation ability compared to agarose gels. The primer pairs amplified 24 types of polymorphic DNA bands, with the average number of alleles generated by one primer pair being 4.8. The average diversity index (DI) was 0.675, the probability index (PI) value averaged 0.118, and the average the polymorphism information content (PIC) value was calculated to be 0.646. Binary matrix from the data obtained was prepared and dendrogram using hierarchical cluster analysis using UPGMA algorithm was constructed. The dendrogram differentiated the lines into two main clusters, with 17 lines in the first cluster and 8 lines in the second cluster. Subsequently, the individual main clasters were divided into subcluster. Detection of genetic relationships of the analyzed sets of maize lines has potential use in MAs breeding in order to obtain varieties with improved characteristics for both the food industry and cultivation practice.

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PLANT GENETICS

P60 AHK5 Mediates ETR1-Initiated Multistep Phosphorelay and Controls Ethylene-Regulated Root Growth Responses in Arabidopsis

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Multistep phosphorelay (MSP) is emerging as a regulatory pathway allowing for the integration of multiple signal inputs in plants. MSP, previously thought mainly to mediate cytokinin signaling, was recently shown to be controlled by another phytohormone (ethylene), through the histidine kinase (HK) activity of ETHYLENE RESPONSE 1 (ETR1).
Methods: DNA constructs were prepared as reported earlier (1-3). Arabidopsis thaliana wild-type (Col-0, N1092) and the ahk5-1 mutant (N802391, SAIL_50_H11) were imaged as previously described (4).
Results: Here we show that although ETR1 is an active HK, its receiver domain (ETR1RD) is unable to accept the phosphate from the phosphorylated His in the ETR1HK to initiate a phosphorelay to ARABIDOPSIS HISTIDINE-CONTAINING PHOSPHOTRANSMITTERs (AHPs), the next downstream link in MSP signaling. Instead, ETR1 interacts with another HK ARABIDOPSIS HISTIDINE KINASE 5 (AHK5) and transfers the phosphate from ETR1HK through the AHK5 receiver domain (AHK5RD), and subsequently to AHP1, AHP2 and AHP3, independently of the HK activity of AHK5. We identify the orientation of the ETR1RD g-loop as critical for the inability of ETR1RD to be phosphorylated by ETR1HK and demonstrate that the determinants of functional and structural diversity are to be found outside this g-loop.
Discussion: We show that AHK5 mediates a majority of basal MSP signaling in the Arabidopsis root tip, and is necessary for ethylene-initiated, but not cytokinin-initiated, MSP signaling in planta. AHK5 acts as a negative regulator of root apical meristem (RAM) size, attenuating a number of ethylene-regulated root growth responses.

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PLANT GENETICS

P61 Transcriptome and proteome analysis of haploid Ginkgo biloba flavonoid metabolism for the first time

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Until 2016, it was thought that only diploids existed in ginkgo when the first natural haploid ginkgo material was discovered. The discovery of haploids is extremely valuable, especially for ginkgo, a gymnosperm. To investigate the effect of ploidy on ginkgo, we report the first combined transcriptomic and proteomic analysis of the differences in flavonoid content in haploid ginkgo. The latest Ginkgo reference genome and high-depth sequencing data were used in this study. While haploid ginkgo flavonoid content was significantly lower than that of diploids, 1324 genes with 397 proteins were upregulated, and 1161 genes and 289 proteins were downregulated. In the flavonoid metabolic pathway, the major regulatory genes, FLS, DFR, CYP75A, etc. were significantly downregulated in the haploid. In addition to flavonoid metabolism, we found that the weaker growth vigor and secondary metabolism accumulation in haploid Ginkgo were mainly caused by the reduced dosage of the corresponding regulatory genes. This study is the first to report the omics differences among different ploidy ginkgos, which will enrich the study of ginkgo ploidy and lay the foundation for the development and utilization of haploid ginkgos.

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PLANT GENETICS

P62 Study of Genetic Variability of Beans Genotypes using SCoT markers

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Beans are characterized by a high content of proteins, lipids, carbohydrates, vitamins, minerals and other health-promoting substances. The breeding of new bean varieties by the application of marker assisted selection is currently a new, highly effective trend in this area. DNA markers are widely used in plant genetics, where they are used to evaluate crop genetic diversity, to analyse population structure, and to create genetic maps. The main goal of the work was to detect the genetic diversity of 10 varieties of beans using 8 SCoT markers (SCoT 3, SCoT 19, SCoT 28, SCoT 29, SCoT 30, SCoT 34, SCoT 54, and SCoT 63). A total of 67 DNA fragments were detected, of which 48 were polymorphic and 19 fragments were monomorphic. The average percentage of polymorphism was 70,82 %. The size of the detected DNA fragments ranged from 190 - 2000 bp. Polymorphic information content (PIC) values ranged from 0,355 (SCoT 28) to 0,871 (SCoT 34), with a mean PIC of 0,705. Higher PIC values indicate a high degree of polymorphism in the analysed varieties. The diversity index ranged from 0,37 (SCoT 28) to 0,87 (SCoT 34) with an average value of 0,72. The probability value (PI) ranged from 0,002 (SCoT 34) to 0,072 (SCoT 54) and the mean value was 0.065. Through hierarchical cluster analysis using the UPGMA algorithm, a dendrogram of the genetic relatedness of the analysed varieties was constructed, which divided the individual varieties into two main clusters, which were further divided into subclusters. The dendrogram shows that the genetically closest varieties are Olga (Germany) and Pesak (Bulgaria), while the genetically furthest from the others was the Golden Dream (Denmark). The results show that SCoT markers are a useful tool for analysing genetic diversity and determining the degree of DNA bean polymorphism.

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PLANT GENETICS

P63 Comparative FISH Mapping of Carrot-derived Centromeric Repeats in Daucus (Apiaceae)

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The genus *Daucus* comprises about 40 wild species and the cultivated carrot, an economically and nutritionally important crop. Wild *Daucus* species exhibit great morphological and genetic diversity, and despite extensive research, the taxonomic and phylogenetic relationships between them have still not been fully resolved. To better understand these relationships, the application of cytogenetic data can be of great usefulness. Therefore, the aim of this study was to explore the chromosomal distribution of carrot-derived centromeric repeats in selected *Daucus* taxa and related species by fluorescence *in situ* hybridization (FISH).

Methods: In this study, 34 accessions (28 accessions of *Daucus*, 6 accessions of closely related non-*Daucus*) belonging to 22 taxa (species or subspecies) were used. Chromosome preparations were made by an enzymatic maceration of root meristems derived from greenhouse-grown plants. For comparative FISH analysis, we used a 36-nucleotide probe corresponding to a carrot centromeric repeat (CentDc), directly labeled with cyanine-5.

Results: The CentDc repeats were present in 26 accessions of *Daucus* (belonging to *Daucus* subclades I and II) and one accession of closely related non-*Daucus* species. Of these, in 20 *Daucus* accessions (representing 11 taxa), the CentDc repeats were detected in the centromeric regions of all chromosomes, whereas in the remaining ones, the number of chromosome pairs with CentDc signals varied, depending on the species; however, their centromeric localization was conserved.

Discussion and conclusion: The presence of the CentDc repeats in the genomes of taxa belonging to both *Daucus* subclades and one closely related non-*Daucus* species indicated the ancestral status of this repeat. Our observations provide useful information for further evolutionary, cytotaxonomic, and phylogenetic studies on *Daucus* and may contribute to a better understanding of the dynamic evolution of centromeric satellites in plants.

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PLANT GENETICS

P64 Mating System within the Hybrid Zone between Pinus sylvestris L. and P. mugo Turra in Slovakia

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Natural hybrid zones represent an excellent opportunity for studying evolutionary mechanisms of hybrid speciation and divergence of hybridizing species. It may provide great insight into the factors that contribute to the development of reproductive barriers, an issue thatis critical to understanding the mechanisms of speciation. Hybrid zone populations between Scots pine (Pinus sylvestris L.) and mountain dwarf pine (P. mugo Turra) have been found in several locations in central Europe, including northern Slovakia. To understand their evolution and phylogeny, various morphological, anatomical, and molecular traits including DNA markers were used, providing solid evidence for their hybrid nature. However, little is known about their mating system and selection patterns, for example, whether the presence of hybrids is a result of disassortative mating or heterozygote advantage in new habitats. Here, we analyzed the mating system within the hybrid zone in Zuberec using four reference populations of P. sylvestris and five populations of P. mugo. Based on five SSR (Simple Sequence Repeats) marker loci, we found that the hybrid zone generally represents a random mating population, as evidenced by an absence of significant positive values of Fis. Only one marker locus had significantly low heterozygosity in all populations tested, including the hybrid zone. Because no null homozygotes were observed within the locus, the heterozygosity deficit may indicate linkage with selectively important genes, such as mate choice loci that promote assortative mating. Still, the presence of hybrid individuals in the locality is indisputable. Despite this fact, our study shows that assortative mating may be common even within highly variable hybrid swarm populations with a unimodal distribution of genotypes. Heterozygote advantage as a mechanism of ecological selection should therefore be considered much more important in determining the genetic structure of natural hybrid zones.

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PLANT GENETICS

P65 Expression of Avena sativa L. Metallothioneins Under Soil Drought

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Oat (*Avena sativa* L.) is susceptible to soil drought which causes yield reduction. Some proteins e.g. metallothioneins (MTs) can protect plants during water-deficit stress. MTs are responsible for maintaining homeostasis of metals and may also take a part in response to various stresses (Mierek-Adamska et al. 2018). The aim of the study was to estimate the role of *Avena sativa* L. metallothioneins (*As*MTs) in drought response. Soil drought (25% field water capacity, FWC) has begun in the seedling phase and lasted 14 days. The control plants were watered to 70% FWC. At the 14th day of drought plant material was collected. Biochemical parameters were estimated spectrophotometrically, and real-time PCR was used for gene expression analysis. Leaves relative water content was 29% lower in drought treated plants compared to control. Also, the increase of activity peroxidase (0.8-fold) as well as the content of soluble sugars (2.0-fold) and abscisic acid (28.0-fold) in plants under drought was observed. While superoxide dismutase activity and carotenoids concentration were 1.4 and 3.3-fold lower, respectively. For each *AsMT* gene a 1500-bp long fragment including promoter and 5'UTR of genomic DNA was retrieved from PanOat database. All four *AsMT*s had multiple putative cis-regulatory elements involved in drought response. In promoter sequence of *AsMT1* 12% of all found responsive elements were associated with drought. For other MTs this number was lower – 8%, 4% and 2% for *AsMT4*, *AsMT2* and *AsMT3*, respectively. Expression of *AsMT2* and *AsMT4* increased 10 times in drought stressed plants but expression of *AsMT1 and AsMT3* was lower. According to the obtained data *As*MTs could play an important role in oat tolerance to soil drought.

Mierek-Adamska A, Znajewska Z, Goc A, Dąbrowska GB 2018. Molecular cloning and characterization of *Ipomoea nil* metallothioneins. Turk J Bot 42(3): 247–256. https://doi.org/10.3906/bot-1707-26

PLANT GENETICS

P66 Plant Microbe Interaction to Ameliorate Health and Agricultural Sectors

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Myself first used to pay homage to Mendel by way of explaining "Algebraic Interpretation of Mendelism" to graduate students that Mendel's mathematical skill of $(a+b)^2 = a^2+ab+ab+b^2$ yielded 9:3:3:1 on assuming a=3 & b=1, but it failed to convince them. So in order to quench their inquisitiveness, I took an unscientific & orthodox story of rebirth that I was first born in Czech Republic which gave me the opportunity to serve Mendel as his assistant where I realized his mathematical skill more perfect than biological knowledge in deriving Laws of Inheritance.
The present investigation actually highlights process of biotization to boost health and agricultural sectors. The ethanol extracts of in vivo and in vitro generated plantlets of spp. of Vernonia, Tinospora, Mucuna, etc., were assessed for their efficacy against cancer cell lines. High (>90%) cytotoxicity in *in vivo* and low (55%) in *in vitro* viscous samples against EAC, SiHa and HepG2 cell lines as per Dose and Time dependant as well as single peak and multiple peaks in these respective samples during spectrophotometric scan, all these discrepancies invited the in vitro plants to grow under stress of a fungus, Piriformospora indica. Biotization of micro propagated plantlets not only enhanced the survival rate and growth of the regenerated plants but it also increased the anticancer potentiality by restoring the level of inhibition from 55 to 85% against cell lines. Presence of hyphae and pear shaped chlamydospores in the cortical cells of stained roots in histological studies confirmed Plant-Microbes Interaction. The IC50 value of *in vivo* and *in vitro* biotized plants (100 and 95.5µg/ml, respectively) was significant as compared with *in vitro* non-biotized plant extract (660.6µg/ml). Apoptotic fragmentation of DNA Laddering at IC50 against HepG2 too appreciated cytotoxicity of ethanol extracts of investigating plants. Similarly biotization in Litchi plant with an actinomycetes, isolated in our lab Nocardiopsis umakantae SK75[L] (NCBI no. KF280241), was effective in plant's nutritional enrichment.

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PLANT GENETICS

P67 Creation of Low Phytate Pea Lines Using CRISPR/Cas9-assisted Transformation

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This work is focused on the development of pea (*Pisum sativum* L.) breeding lines with low phytic acid (lpa) content. Phytic acid is an antinutritional factor causing decreased bioavailability of mineral elements, inefficient feed utilization and increased phosphate content in excrement.
Three different constructs suitable for CRISPR/Cas9 genome editing technology were created, each of them targeting a different region of myo-inositol-3-phosphate synthase (*mips*) gene. These constructs have been used for pea agrobacterial transformation with high virulent EHA105 and GV3101 strains of *Agrobacterium tumefaciens*. Screening of putative transformants has been performed by GUS staining during four selection cycles based on *nptII* gene (resistance to kanamycin). DNA samples isolated from GUS-positive transformants have been analysed by PCR amplification and subsequent restriction.
According to observed transient expression of *uidA* reporter gene in transformed explants of tested pea varieties (Eso, Protecta and Trendy), Eso variety and pVO108 construct were selected for further transformations. In total 16 transformations using either individual constructs or a mixture of 2–3 constructs were carried out up to now. From over 7000 transformed node explants almost 400 were GUS positive, approximately 200 explants were tested by PCR and several selected samples were sequenced. By optimizing the individual steps, a protocol for successful pea agrobacterial transformation of the Eso variety was obtained. Our goal is to create transgenic lpa lines, which, however depend on the successful selection of a non-chimeric shoot in *in vitro* culture.

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PLANT GENETICS

P68 Mutagenesis as an Acceleration of Natural Selection?

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During the 20th century, the population grew by about 6 billion to the current 7.7 billion. It was necessary to achieve an extreme increase in crop yields in order to ensure a sufficient amount of not only food but also feed. This has been achieved through more efficient mechanization and the use of fertilizers and pesticides, but mainly through the breeding of higher yielding varieties and / or more resistant to abiotic and biotic stressors. The cornerstone in this process was laid in the years 1856–1863 by Johann Gregor Mendel, who devoted himself to crossing peas and monitoring his offspring. In 1866, based on analyzes of genetic crosses between bred pea (Pisum sativum L.) strains, differing in a well-defined trait such as seed shape, flower color ect., formulated the basic laws of inheritance. He laid the foundations of genetics and thus enabled the development of this branch of biology.
Mutagenesis has become the next logical step in obtaining crops with the desired properties. Thanks to her, great progress has been made. It is especially important in the experimental acquisition of crops with added value for humanity. Mutagens are substances that are structurally related to nucleotide bases and are incorporated into DNA during replication. Variations in their structure then cause mismatched bases and, as a result, mutations. By using a suitable selection marker, plants with the desired properties can be obtained in a very short time (compared to natural selection). However, new methods of mutagenesis are currently facing legislative problems on the part of the EU. Genetically modified organisms (GMOs) are organisms created by the transmission of hereditary information between unrelated species - ie transgenesis. From a legislative point of view, classical, non-targeted mutagenesis is not a GMO method and can therefore be used effectively.

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PLANT GENETICS

P69 Wheat as a Potential Source of Coloured Antioxidants – Cumulation of Genes for Increased Content of Anthocyanins and Carotenoids

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There is a growing interest in the production of crops and varieties rich in anthocyanins and carotenoids, which are widely known antioxidants with health benefits. Donors of these substances also exist in wheat, and it is possible to create wheat varieties (Triticum aestivum L.) with significantly increased contents of these substances in the grain. These are wheats with purple pericarp (Pp genes), blue aleurone (Ba genes) and yellow endosperm (Psy genes for the enzyme phytoene synthase). Genes for the biosynthesis of coloured substances are on different chromosomes. Therefore, it is possible to combine them with each other and obtain breeding material with remarkably dark grain. The purple colour is caused by the complementary influence of the genes Pp1 (on chromosome 7B) and Pp3a or Pp3b (on 2A). The Ba1, Ba2 and Ba3 genes are located on chromosomes 4B, 4A and 4D. Because the aleurone is a part of the triploid endosperm, it should be possible to obtain a set of nine alleles Ba1Ba1Ba1Ba2Ba2Ba2Ba3Ba3Ba3 by gene pyramiding. The homoeo-logic loci Psy-A1, Psy-B1, Psy-D1 are located on chromosomes 7A, 7B and 7D in wheat and Psy-A1 and Psy-D1 appear to be of the greatest importance for biosynthesis of carotenoids. In 2021, the first European variety with "black grain" AF Zora with a combination of the Ba2 gene and genes for purple pericarp was registered in the Czech Republic. This variety is characterized by an extremely high content of anthocyanins in its grain. Recently we have created breeding material with a combination of the Ba1 and Ba2 genes which resulted in darker blue grain. High-value pigmented wheat grain can be used as a functional food. These coloured substances can also easily be extracted to replace synthetic dyes currently used in food, drug and cosmetics. It is to be expected that coloured substances in wheat grain will expand its usability in the food industry.

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PLANT GENETICS

P70 Unusual Wheat Morphotype – Multirow Spike

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Development of bread wheat varieties (Triticum aestivum L.) for intensive cultivation technologies in Central European conditions is moving towards increased spike grain weight with same or lower stem length (Lejšová-Svobodová et al., 2020) and number of spikes per unit of area. In the context of this development, the possibility of using a multirow spike (MRS), which is mutation characterized by a higher number of spikelets growing from one spike rachis node, is considered. MRS has potential to achieve higher reproductive capacity of the spike (number of spikelets and grain per spike). In MRS, extra spikelets can grow directly from the primary spike rachis node, or form short secondary rachides, which is called spike branching. The spike branching is determined by several genes which are orthologous to FRIZZY PANICLE genes, which control inflorescence branching in many other plant species. In common wheat, these are the WFZP (WHEAt FRIZZY PANICLE) genes, specifically WFZP-A, WFZP-B and WFZP-D on short arms of second group of homoelogous chromosomes. Two recessive alleles for MRS expression were found in the WFZP-D locus – wfzp-D.1 and wfzp-D.2 (Dobrovolskaya et al., 2015). Two breeding lines with MRS were grown at high intensity of cultivation. Their yields reached 11.29 t/ha and 11.85 t/ha, which was 11 % and 7 % less than the average of a set of common varieties with normal spikes (12.67 t/ha). Lines with MRS achieved lower amount of spikes per square meter. The yield of the near-isogenic line ANBW 6A with MRS was 15.40 % lower than in recipient check variety Novosibirskaya 67 with normal spikes. Although it can be assumed that this yield reduction was due to the presence of MRS, this morphotype should not be marginalised, because it's higher spike productivity and reproduction capacity does not contradict the variety breeding trend. MRS is also very unusual interesting feature. The genes with great effect that determine this trait can easily be used in breeding program.

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PLANT GENETICS

P71 ATG8 Genes Expression in Wheat During Drought

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Introduction and aims. Drought is one of the major negative limiting factors for crops productivity. The study of ATG8 genes expression in wheat could provide with useful tools for further drought tolerance wheat varieties breeding. Methods. Ukrainian wheat variety Oksamyt Myronivskyy was used for comparative study of ATG8 genes expression levels during water deficiency (modulated by 10% of PEG 6000 addition to the Hoagland solution). Plantlets and roots of 6-, 9- and 12-days old seedlings were used for RNA extraction and cDNA obtainment. Genes TaATG8a,b,c,d,e,f,g,h,i were used for the expression levels estimation via quantitative PCR (ΔΔCt method). Results. A significant decrease of ATG8b,d,f,g genes expression was observed in roots on the 9th day of cultivation under drought conditions compared to the ATG8c,h genes that demonstrated only slight expression increase. After the significant decrease in expression on the 9th day ATG8b,d,f,i genes appeared upregulated on the 12th day, while other two genes – ATG8a,c – were gradually downregulated starting from the 6th and till the 12th day. In plantlets, three genes, ATG8a,b,e, reacted on the drought stress in a similar way – gradually downregulated from 6th till 9th day of drought and upregulated on the 12th day. ATG8c,g,i genes were different – their expression from 6th till 9th day was almost at the same level with Ubi, while by the 12th day it sharply decreased. The expression levels of other two genes – ATG8d,h – ranged within the endogenous control level. It should be noted that the expression of ATG8f gene was gradually decreasing from 6th till 12th day of drought stress. So, the amplitude of fluctuations of the ATG8 genes expression levels in plantlets, as well as in roots, was moderate (except ATG8a,i) and didn't exceed 2-4 times increase/decrease comparing to the endogenous control. Conclusion. Therefore, probably other highly specific ATG8 genes family regulate drought response, which needs further research.

PLANT GENETICS

P72 Bayesian Additive Regression Trees for Genotype by Environment Interaction Studies

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We propose a new class of models for the estimation of genotype by environment(GxE) interactions in plant-based genetics. Our approach, named AMBARTI uses semi-parametric Bayesian additive regression trees to accurately capture marginal genotypic and environment effects along with their interaction in a fully Bayesian model. We demonstrate that our approach is competitive or superior to similar models widely used in the literature via both simulation and a real-world data set. Furthermore, we introduce new types of visualization to properly assess both the marginal and interactive predictions from the model. An R package that implements our approach is available at https://github.com/ebprado/ambarti.

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PLANT GENETICS

P73 The Origin of Mendel's Pea: Pea Crop Domestication

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The origin of the agriculture was one of key points in human history, and a central part of this was the evolution of new plant forms, domesticated crops. There have been effort to identify selection signatures at the genome level. This knowledge will help geneticists to better understand the evolution of organisms, and at the same time, help breeders to implement successful breeding strategies. Timing of seed germination is one of the key steps in plant life. It determines when plants enter natural or agricultural ecosystems and is the basis for crop production and has been along with seed dispersal altered during the domestication. Case Summary: Using genome wide analysis of wild P. elatius, P. fulvum and domesticated (P. sativum landraces and P. abyssinicum) accessions we revealed genetic diversity in relation to the place of the origin and associated environment. In order to identify biological and genetic background of two key domestication traits (seed dormancy and pod dehiscence) we carried out comparative anatomical, transcriptomic, proteomic and metabolomic study of wild and domesticated pea seeds and pods. Conclusions: We demonstrated that domesticated P. sativum and the Ethiopian pea (P. abyssinicum), were derived from different P. elatius genepool; therefore pea has at least two domestication events. Genetic mapping identified 2 to 3 loci involved in seed dormancy and single locus governing pod dehiscence. We identified differentially expressed genes and altered biochemical pathways. One of the pronounced changes were found in phenylpropanoid pathway leading to complex phenolic compounds including lignin. Proanthocyanidin oligomeration showed correlation with seed dormancy. Non-functional polyphenol oxidase gene has been selected during pea domestication, related to the oxidation and phenolics polymerization in the seed coat. These findings will be discussed in context of various legume crops.

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PLANT GENETICS

P74 Changes in the Expression of Metallothioneins and the Content of Metal Ion, Water, and Total RNA During Canola Seed Development

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Industrialization and agricultural activities have been increasing heavy metal contamination for many years, and thus today it is widespread around the world. The presence of heavy metals in soil or nutrient solution alters plant nutrient absorption and results in growth retardation that might lead to the death of the organism. In plants, ion homeostasis is essential for plant growth, development, and in case of crops it is essential for high yield production. Metallothioneins (MTs) are highly conserved, small (6-7 kDa), cysteine-rich, metal-binding proteins that are important for copper (Cu) and zinc (Zn) homeostasis, and protection against heavy metal toxicity (mostly cadmium), oxidative stress, and DNA damage. In plants, MTs have four main isoforms, MT1-MT4. Here, we have studied the expression of canola (*Brassica napus*) metallothionein genes belonging to four types (*BnMT1-4*), and the content of metal ions, water, and total RNA in developing canola seeds (35, 56, 63, 70, and 80 days after flowering). Examining *BnMT* expression by sqRT-PCR in developing seeds demonstrated that *BnMT1-4* are expressed in each developmental stage except at 35 days after flowering. Interestingly, all *BnMT*s have their own unique pattern of expression during seed development. Furthermore, SEM-EDX analysis revealed that Cu and Zn levels slightly decreased while bioanalyzer results showed that total RNA content increased during seed development. The results suggest that each type of MT might have a unique role in the process of seed maturation. A better understanding of the mechanism of heavy metal homeostasis during seed maturation will be of crucial importance for increasing quantity and quality of seeds and thus for food security.

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PLANT GENETICS

P75 The Parthenogenesis Gene in Dandelions and Mendel's Hawkweeds

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After completing his pea crossing experiments, Gregor Mendel experimented for eight years with hawkweeds (genus Hieracium, now Pilosella). Pea hybrids produced variable offspring, whereas Hieracium hybrids produced constant offspring. We now know that hawkweeds reproduce by apomixis (clonal seeds). Parthenogenesis, the development of the embryo without fertilization of the egg cell, is an element of apomixis. We identified the parthenogenesis (PAR) locus in dandelion (Taraxacum officinale) by genetic and deletion mapping of Loss of Apomixis phenotypes. The dominant PAR gene was singled out by CRISPR-Cas9 targeted deletion of genes within the ~1 Mb locus. The PAR gene is a small 513 bp gene with a specific zinc finger domain and a repressor binding EAR motif. Complementation of the Taraxacum PAR locus deletion line resulted in the restoration of apomixis. When the dandelion PAR or par gene, driven by an egg cell-specific Arabidopsis promoter, is introduced into sexual lettuce, the egg cells produce embryo-like structures without fertilization. This is functional proof that the PAR gene causes parthenogenesis. In comparison to the sexual allele, the dominant PAR allele contains a large MITE transposon (1335 bp) in its promoter, 110 bp upstream of the start codon of the CDS. Remarkably, a similar MITE insertion was found in the PAR gene of apomictic hawkweed Pilosella piloselloides. We hypothesize that the MITE insertion causes parthenogenesis. The insertion site is 27 bp upstream of the insertion site in Taraxacum, suggesting independent parallel evolution of apomixis in these two genera. Segregation of the PpPAR gene in pollen meiosis likely explains Mendel's crossing results in hawkweeds.

PLANT GENETICS

P76 Association between Allelic Variability at the Phytoene Synthase 1 Locus and Yellow Pigment Content in Wheat and Tritordeum

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Endosperm carotenoid content in the cereals is a primary determinant of flour colour and this affects both the nutritional value of the grain and its utility for different applications. Utilising wheat rice synteny two genes, epsilon-cyclase (epsilon-LCY) and phytoene synthase (Psy-1), were identified as candidate genes for two of the QTL affecting carotenoid content in endosperm. Phytoene synthase (Psy-1) is a key gene that plays a main role in the biosynthetic pathway of carotenoids and affects their accumulation in grains. The aim of the study was to verify this role in genotypes with different grain colours for breeding. Grains of 14 genotypes of hexaploid wheat (standard wheat varieties with red grain: Bohemia, Julie, Matchball; with blue aleurone: Skorpion, AF Oxana, V1-80-17; with purple pericarp: Rosso, AF Jumiko, PS Karkulka; with yellow endosperm: Bona Vita, V2-49-17, Citrus; with black grain: V1-63-17, AF Zora) and 3 genotypes of tritordeum (HT 135, HTC 1380, and JB1) were analysed. The grains were from the harvest of the year 2018, 2019 and 2020. Spectrophotometrically measured values (Bulda et al., 2008) of total carotenoid content ranged from 0.71 to 14.82 µg/g depending on the genotype and the year of harvest. The total carotenoid content in grains of tritordeum genotypes was higher than of wheat. Molecular markers YP7A, YP7A-2, YP7B-1, YP7B2, YP7B4, YP7D-2 (Stepanenko et al., 2017), Psy1-A1_STS (Singh et al., 2009), PSY7A-1, PSY7A5, and PSY7A-7 (Crawford et al., 2011) have shown useful for analysis of variability of Psy-1 locus. 8 alleles of loci Psy-A1 (alleles a, b, c, l, and o), Psy-B1a or b, and Psy-D1a were detected in the total. There was statistically significant correlation ($p<0.05$) between carotenoids content and alleles combination in the analysed samples. The importance of studied gene on the content of carotenoids in grain and for the breeding program was verified.

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PLANT GENETICS

P77 Obtaining of Transgenic Nicotiana Benthamiana Plants with Heterologous ZRNase II Gene to Produce a Model for Study of Influence of Extracellular Ribonucleases on Plant Resistance to Virus Movement

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Ribonucleases (RNases) are supposed to be engaged to antiviral response in plants, so heterologous *RNase* gene expression can be a tool for production of cultivars with multiple resistance to viruses and viroids. *Nicotiana benthamiana* plants are susceptible to the large number of diverse plant viruses that can successfully infect it. The species is known to be a model used in plant virology as experimental host in the studies of plant –virus interactions.
The aim of our research was the production transgenic *Nicotiana benthamiana* plants expressing heterologous *ZRNase II* gene as a future model to determinate the susceptability of obtained plants to a number of plant viruses and their systemic movement in plants.
We have obtained the transgenic *N. benthamiana* plants with *ZRNase* gene *via Agrobacterium*-mediated genetic transformation of leaf disks. Resistant shoots were regenerated and rooted under the selective pressure of 100 mg/l of kanamycin in the cultural medium. The stable integration of *ZRNase II* gene was confirmed by PCR-analysis. Thus the transgenic plant material was created for future investigation of extracellular ribonuclease influence on plant resistance to virus movement.

ANIMAL GENETICS

P78 Salaria fluviatilis: Patterns of Distribution in Mediterranean Countries, an Insight into Sardinia and North Italy Freshwaters Genetic Variability.

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The freshwater blennies are a group of small benthic species that inhabit freshwaters in Mediterranean countries. Three species have been formally described to date: *Salaria economidisi*, endemic to Lake Trichonis in Greece, *Salaria atlantica*, endemic to the Seboui river basin in Morocco and the widespread *Salaria fluviatilis*. A total of 102 individuals of *S. fluviatilis* were collected from 4 different Italian regions (Sardinia, Liguria, Piedmont and Lombardy) and analysed using a portion of the control region as molecular marker. Phylogenetic and phylogeographic analyses were carried out on a dataset including the sequences from this study along with all conspecific available on GenBank. The aims of the present study were: (i) to depict the phylogeographic patterns of *S. fluviatilis* populations within its whole range of distribution in Mediterranean countries and, (ii) to clarify the genetic relationships occurring between samples from Sardinia and those from northern Italy.
Results showed the presence of two main genetic clusters: the first cluster was representative of northern Italy (except for Liguria) and the eastern part of Europe and, the second cluster spread in Sardinia, Liguria and Iberian Peninsula. A further third genetic cluster was found being represented by sequences from Middle East.
This scenario could be the consequence of an ancestral dispersion center located in the Iberian Peninsula by which individuals of *S. fluviatilis* could have migrated arriving in Liguria, Corsica and Sardinia following a step-in stone migration model. The presence in the Ligurian territory of geographical barriers, likely prevented the migration of individuals from Spain toward the northernmost Italian regions, where the original populations arrived from Balkans.
Further studies, involving a greater number of specimens from Balkan areas, Spain, and other Italian regions are needed to check the occurrence of these incipient division between east and west Mediterranean countries.

ANIMAL GENETICS

P79 Altered Active Demethylation Process in Zygotes Lacking the Maternal Genome

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Upon fertilisation, two highly specialized parental genomes meet to form a totipotent zygote. The post-fertilisation period is under the influence of maternal factors. However, little is known what these factors are and whether there is a functional crosstalk between parental genomes. To investigate this, we have performed a series of micromanipulations and removed the maternal genome at different stages of oogenesis. These manipulated eggs were fertilised. Interestingly, the maternal nuclear components are mostly redundant for the new pronucleus formation, but the active demethylation of specific paternal sequences seems to be absent or exhibits markedly altered kinetics.
To investigate this phenomenon closer, we asked whether this is caused by an altered ratio of a putative inhibitory factor to the male genome. To this end, we prepared androgenetic embryos using 1-5 male genomes. We also asked whether the demethylation activity is linked specifically to maternal chromatin. To investigate this, we constructed embryos comprising the paternal genome and a mammalian or an insect somatic cell. While the first experiment showed that the lack of pre-replication demethylation cannot be rescued by diluting the putative inhibitory factor, the latter experiment showed that the demethylation activity is not specifically associated with the maternal genome. It is generally accepted that the maternal genome is protected from demethylation by the maternal factor Stella. It is possible that in the absence of other genomic substrate, it ectopically binds to the paternal genome blocking the demethylation of specific sequences. However, the demethylation was blocked irrespectively of Stella's localization as shown by the fractionation of micromanipulated zygotes. At least in our system, we conclude that although the original notion of a maternal factor binding certain sequences to protect them from active demethylation is feasible, Stella is likely not the factor responsible.

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ANIMAL GENETICS

P80 CRISPR/CAS9-MEDIATED GENE KNOCK-OUT AND GENE EDITING AT A BIOTECHNOLOGICAL APPROACH TO CHICKEN RESISTANCE TO VIRAL DISEASES

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Avian sarcoma and leukosis virus (ASLV) diversified into seven subgroups (A, B, C, D, E, J, and K) present as either exogenous or endogenous viruses in domestic chicken. These subgroups are classified by the subgroup-specific receptor usage. ALV subgroups enter the cell through Tva, a member of the family of low-density lipoprotein receptors, Tvb, a tumor necrosis factor receptor-related protein, Tvc, a butyrophilin family protein with two immunoglobulin-like domains, or Tvj identified as the chicken Na^+/H^+ exchanger type 1 (chNHE1) with twelve transmembrane segments and prominent extracellular loop 1. For all ASLV receptors, virus-resistant alleles exist, mostly due to the frame shift mutations or amino-acid substitutions. For example, single W38 deletion or substitution makes the NHE1 receptor molecule resistant to virus entry. Some of ASLV-resistant receptor alleles segregate in domestic chicken and can be used for breeding the ASLV-resistant lines. Resistant alleles for NHE1, however, have not been found in chicken.
Using the CRISPR/Cas9 gene editing tools, we introduced frame shift indel mutations into the endogenous copies of *tva*, *tvc*, and *tvj* receptor genes. These mutations abrogated the receptor functions and conferred the resistance to the respective virus subgroup in cell culture.
Ultimately, we prepared a chicken line with W38 deletion within the endogenous NHE1 and chicken line with frame-shift knock out of *tva* gene using the CRISPR/Cas9 and demonstrated that theses chicken lines are resistant to ALV-J/ALV-A infection in vivo. The new techniques of gene knock-out and gene editing in chicken employed in this work will be described. Our original methods of orthotopic transplantation of primordial germinal cells into adult recipients improved efficiency of gene modification and enabled to skip the chimeric G0 stage. This approach might become the state-of-art for biotechnological use of chicken in virology, immunology and developmental science.

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ANIMAL GENETICS

P81 ACADM Frameshift Variant in a Cavalier King Charles Spaniel with Medium-Chain Acyl-CoA Dehydrogenase Deficiency

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Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common fatty acid oxidation disorder in humans and is caused by variants in the *ACADM* gene. The aim of this study was to investigate a Cavalier King Charles Spaniel (CKCS) that presented with a history of seizure like episodes including weakness and ataxia, resembling human MCAD deficiency.

Methods: We sequenced the genome of the affected dog and compared the data to 565 control genomes of different breeds. *ACADM* was considered the top functional candidate gene. Targeted genotyping by Sanger sequencing of PCR amplicons was performed in 162 additional CKCS dogs. Acylcarnitine measurements were performed in five dogs of each genotype.

Results: Variant filtering of the whole genome sequence data revealed a single homozygous private protein-changing variant in *ACADM* in the affected dog. The identified variant, XM_038541645.1:c.444_445delinsGTTAATTCTCAATATTGTCTAAGAATTATG, introduces a premature stop codon and is predicted to result in truncation of ~63% of the wild type protein, XP_038397573.1:p.(Thr150Ilefs*6). Targeted genotyping of 162 CKCS dogs revealed a variant allele frequency of 23.5% and twelve additional homozygous mutant dogs. The acylcarnitine C8/C12 ratio, specific for MCAD deficiency, was elevated ~65 fold in homozygous mutant dogs as compared to homozygous wild type dogs.

Discussion and conclusion: Based on the clinical and biochemical data together with current knowledge in humans, we propose the *ACADM* frameshift variant as candidate causative variant for MCAD deficiency and neurological phenotype in the investigated dog. Unexpectedly, the mutant allele was very common in the tested population. Further prospective studies are warranted to assess the clinical consequences of this enzyme defect in dogs. Testing the CKCS breeding population for the identified *ACADM* variant is recommended to prevent the unintentional breeding of dogs with MCAD deficiency.

ANIMAL GENETICS

P82 A de novo variant in the keratin 1 gene (KRT1) in a Shar Pei dog with severe non-epidermolytic ichthyosis

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Ichthyoses are a heterogeneous group of hereditary cornification disorders. They are characterized by generalized dry skin, scaling and/or hyperkeratosis. Several genetically distinct forms have been identified in a variety of dog breeds.

Methods: We investigated a Shar Pei with clinical signs of ichthyosis. A clinical evaluation was performed and biopsies were taken for histopathological and immunohistochemical examination. Additionally, we obtained whole genome sequencing data of the affected dog and both parents. A trio analysis was carried out, comparing the sequencing data to 793 canine control genomes.

Results: The 3-months old Shar Pei puppy showed generalized scaling, alopecia and footpad lesions. Histopathological examinations demonstrated a non-epidermolytic severe hyperkeratosis. The parents and siblings of the affected puppy did not show any skin lesions. A trio whole genome sequencing analysis identified a heterozygous *de novo* 3 bp deletion in the *KRT1* gene in the affected dog. The variant is predicted to delete a single asparagine from the conserved coil 1A motif within the rod domain of KRT1, NP_001003392.1:p.(Asn190del). Epidermal and follicular KRT1 expression, evaluated by immunohistochemistry, was visually comparable to that of healthy Shar Peis.

Discussion and Conclusion: The finding of a *de novo* variant in an excellent functional candidate gene strongly suggests that KRT1:p.Asn190del caused the ichthyosis phenotype in the affected Shar Pei puppy. The single amino acid deletion might interfere with keratin dimerization or another function of KRT1. Histologically, the dog showed a non-epidermolytic ichthyosis with retained KRT1 expression. Missense variants affecting the homologous asparagine residue of the human KRT1 cause epidermolytic hyperkeratosis. To the best of our knowledge, this is the first description of a *KRT1*-related ichthyosis in domestic animals.

ANIMAL GENETICS

P83 The Genetic Analysis of European Wisents in Lithuania

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By the beginning of the 20th century *Bison bonasus* specie had a dramatic bottleneck effect. None of the free-living individuals were left and the reintroduction of the European wisent was started based on a few captive individuals of 11 European (*Bison bonasus bonasus*) (LB - Lowland Bialowieza line) and only one Caucasian (*Bison bonasus caucasicus*) (LC - Lowland Caucasian line). This research was based on PCR microsatellite analysis of 14 (BOVIRBP, BTJAB1, BM6438, BM2830, TGLA122, ETH10, BM1225, BM1818, BM723, ETH121, TGLA53, TGLA227,
CSSM66, HEL9) markers of 29 individuals forming 3 subpopulations in Lithuania. Also, one individual from Belarus was included in this research. The goal of this analysis was to determine whether analyzed individuals are more familiar with LC or LB genetically to separate them for further specie restoration. The structure analysis showed that analyzed individuals are linked to contain two genetic clusters containing 11 and 18 individuals.

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ANIMAL GENETICS

P84 Genetics of Acral Mutilation Syndrome in Several Dog Breeds Equivalent to Human Sensory Neuropathies

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Canine acral mutilation syndrome (AMS) has been documented for decades as part of inherited sensory neuropathies. Clinical signs include ataxia and decrease/loss of tactile and pain sensation, leading to skin ulceration and severe auto-amputation of digits. Although the disorder was reported in several dog breeds, causal variants in only two genes were identified to date. In humans, the disease corresponds to hereditary sensory autonomic neuropathies (HSAN), characterized by insensitivity to pain, sometimes combined with self-mutilation. To date, 16 genes have been associated with HSAn but do not explain the disease origin of all patients. Our aim is to characterize the underlying genetic etiology leading to AMS in two dog breeds, as natural models for HSAN.
Samples from AMS-affected dogs (2 Fox terriers (FT), 4 Miniature pinschers (MP)) as well as unaffected dogs in each breed were collected. Whole-genome next-generation sequencing of cases and controls was carried out to search for causal genetic variants using the reference genome assembly UU_Cfam_GSD_1.0. The variant datasets were compared to two public datasets (~3000 dog genomes), which provide a powerful resource to identify breed-specific pathogenic variants.
Although all cases presented the same clinical features, they likely represent separate forms of sensory neuropathy. Preliminary analyses identified 199,405 homozygous small variants in related FT and 450,156 in a MP case, as well as no evidence for large chromosomal aberrations. After filtering with minor allele frequency of 1%, 35 in FT and 60 in MP candidate protein-changing variants in functionally important genes are under investigation.
The spontaneous dog models will help identify further HSAN-associated genes, which may be included in human neuropathy diagnosis, as previously shown in hunting dogs with AMS. In dogs, genetic tests will be developed to support precise diagnosis and breeding plans to select against the fatal disease. Finally, this research provides a double benefit for veterinary and human medicine.

Acknowledgement: We are grateful to all owners, breeders, and veterinarians for providing information and samples of their dogs, as well as the Cani-DNA Biological Resource Center for sample collection and storage.

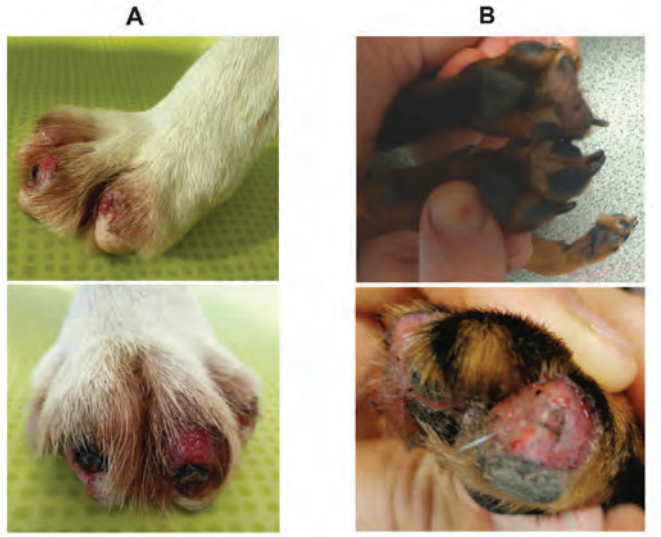

Fig. 1: Representative clinical phenotype of (A) Fox terrier and (B) Miniature pinscher cases with an age of onset of clinical signs less than 1 year. Fox terrier photos from Correard et al., 2019.

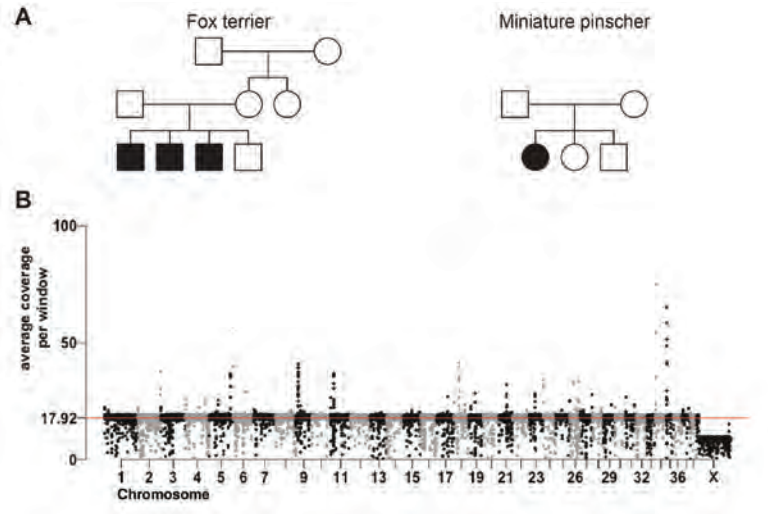

Fig. 2: (A) Analysis of available pedigree information supported an autosomal recessive mode of inheritance. Black and white symbols represent AMS-affected and unaffected individuals, respectively. (B) A representative plot of average coverage over 100kb genomic windows. This detected no evidence of larger chromosomal structural rearrangements.

ANIMAL GENETICS

P85 The benefit of crossbreeding beef cows with Wagyu bulls in the Czech Republic

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Wagyu is a Japanese beef breed (so-called Japanese black cattle) thatis expanding and becoming popular in the Czech Republic. Wagyu cattle meatis demanded because of its tenderness, marbling score, and nutritional qualities. The required quality is achieved primarily by the ability of intramuscular fat deposition and a high ratio of the valuable monounsaturated fatty acids (MUFA). Breeding of this cattle has specific aspects concerning nutrition or length of fattening period, which should be taken into account for obtaining the demanded performance. The wagyu breed has a genetic predisposition towards marbling. The production of marbling in muscles begins from the 12th month of age and progresses the most intensively around the 24th month of age. This is the reason why the animals of the wagyu breed and its crossbreeds should be slaughtered at an age higher than two years. The concentrated feed ratio with high-energy feedstuffs is used in the last fattening period. To obtain a full blood animal could be very difficult and expensive. Thus, the crossbreeding with other beef breeds has proceeded. Since 2011 the crossbreeding of beef cows with the wagyu bulls has been provided in Agrovýzkum Rapotín, Ltd. The goal is to assess the contribution of the genetic potential of wagyu not only to the meat quality but also to the other performance traits. Currently, 30 wagyu crossbred cows are raised in the mountain area. The growth ability, particularly the carcass quality of bulls, steers, and heifers is evaluated. Up to now, 28 wagyu-sired bulls and five heifers were raised and subsequently slaughtered at the age of 24 to 30 months. The effect of age was not significant on most carcass quality traits. Satisfactory results were achieved in all observed traits of growth ability and carcass quality. Concerning marbling, as the most important meat quality trait, the heifers surpassed the bulls significantly. Besides the carcass quality of the wagyu crossbred animals, the calving ease ability, good pregnancy rate, and docile temperament are appreciated.

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ANIMAL GENETICS

P86 A Mendel Legacy for Animals

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Within two years of the rediscovery of Mendel's results, Mendelian inheritance was reported for five traits in chickens and one in cattle (by Bateson and Saunders), and one in mice (by Cuénot). The next year, Castle reported Mendelian inheritance in guinea-pigs, rabbits and trout. Two years later, Hurst reported Mendelian inheritance for two more chicken traits, and Bateson and Saunders reported a classic 9:3:3:1 ratio for the joint segregation of two of the chicken traits. These same chicken data provided the first report, in any species, of interaction between genes, a phenomenon that Bateson later called epistasis. As the years passed, many animal traits/diseases were shown to follow Mendelian inheritance.

Methods: In 1980 one of us (FWN) commenced a computerized compendium of all reported Mendelian traits/diseases in non-human and non-model vertebrates. Being modeled on the human compendium called Mendelian Inheritance in Man (MIM) that had been commenced by Dr Victor McKusick in the early 1960s, the animal compendium was named Mendelian Inheritance in Animals (MIA). In 1995, MIA went online, becoming Online MIA (OMIA https://omia.org).

Results: Two hundred years after Mendel's birth, OMIA is the freely-available global resource for information on more than 2200 traits/diseases in more than 300 non-model animal species. More than 1400 likely causal mutations (variants) are tabled in a standardized format.

Discussion and conclusion: OMIA details the wide range of types of variants that can give rise to Mendelian inheritance. Likely causal variants have been reported for around 60% of Mendelian traits/diseases in animals, including for almost all of those traits first reported after the rediscovery. Mendel would be very pleased to know that by applying exactly the same inheritance principles that he reported in 1865, animal breeders in 2022 can make use of the information in OMIA to avoid the occurrence of many inherited diseases.

ANIMAL GENETICS

P87 Genetic Investigations of Amelogenesis Imperfecta in Doberman Pinscher Dogs

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Amelogenesis imperfecta is an enamel defect reported in humans and dogs of different breeds, such as Samoyeds and Italian Greyhounds. It is characterized by a dark, rough surface of the deciduous and permanent teeth.

Methods: We obtained samples from two unaffected parents, three unaffected and one affected puppy of a Doberman Pinscher family. Another affected and four unaffected puppies were not available for sampling. We additionally received a sample from a distantly related affected Doberman Pinscher. Genomic DNA from these seven dogs was isolated and genotyped with the illumina canine_HD SNV array. The genotypes of the six animals from the family were used for a parametric linkage analysis with the Merlin software and an autosomal recessive inheritance model. Homozygosity mapping was performed in the two available cases with the PLINK software.

Results: The obtained pedigree data were compatible with a monogenic autosomal recessive mode of inheritance. Linkage analysis in the sampled family members showed 56 linked intervals on 35 chromosomes comprising 1121 Mb with a maximum LOD score of 0.25. Homozygosity mapping identified 133 homozygous segments with shared haplotypes spanning roughly 520 Mb. A total of 68 genomic segments on 26 chromosomes showed homozygosity and linkage. This intersection comprised 271 Mb, representing approximately 9.2% of the dog genome.

Discussion and Conclusion: The limited number of available samples did not allow an unambiguous mapping of a single disease locus. With a single litter available, it is unlikely to reach a significant LOD score ≥3 in the linkage analysis. However, the obtained data are consistent with a hypothetical monogenic autosomal recessive mode of inheritance. Whole genome sequencing of an affected dog is ongoing. The sequencing data will be compared to 800 control genomes in a search for private variants that may cause the amelogenesis imperfecta.

ANIMAL GENETICS

P88 Brachygnathia inferior in Brown Swiss Cattle: A Simple Mendelian Trait?

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Shortening of the lower jaw (brachygnathia inferior) is a congenital malformation in domestic animals including cattle. Brachygnathia inferior often leads to reduced growth and poorer health and welfare of the animals, which affects productivity. As it is already described in sheep and buffalo, a complex inheritance is to be assumed. The aim was to decipher the mode of inheritance and to find associated genomic regions. We have observed several cases with a frequency of about 0.1% in the Brown Swiss cattle population of Switzerland. A simple monogenic dominant model of inheritance can be excluded, as none of the 46 specifically examined offspring of 147 affected cows showed the trait. Therefore, we have suspected that either a simple monogenic-recessive or an oligo- or polygenic inheritance is underlying. We conducted a genome-wide association study with 147 cases and 509 normal controls. The animals were genotyped with different routinely available array chips and imputation was done in a two-step approach. Using the final high-density 700k dataset, we applied single SNP regression analysis to identify a single major quantitative trait locus for this trait on chromosome 5 between 29 and 33 Mb. Numerous genes and loci are annotated in this 4 Mb interval, including genes of signalling pathways such as Wnt (Wingless-related integration site), which are important for the complex regulation of the development of craniofacial structures. The association signal in this genome region was supported by the results of a subsequent run of homozygosity analysis with the same data set. Currently, whole-genome sequencing from several cases is being performed to achieve both fine mapping and subsequent discovery of associated, potentially causal variants. Our work represents the first comprehensive study of the genetics of this undesirable trait in dairy cattle and points to a potentially simple Mendelian trait.

ANIMAL GENETICS

P89 PI3-Kinase Signalling Drives Cancer Initiation, Progression and Response to Targeted Treatment in Genetically Engineered Mouse Models of Intestinal Cancer

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Although frequently mutated, the role of phosphatidylinositol 3-Kinase (PI3-K) signalling in colorectal cancer (CRC) is incompletely understood. We aimed to address this issue by developing PI3-K hyperactive genetically engineered mouse models of intestinal cancer. Impact on tumour initiation and progression, its interplay with other signalling pathways, and role in treatment response were investigated.
To hyperactivate PI3-K signalling, we used a tamoxifen-inducible Cre recombinase to introduce a kinase-activating mutation in the endogenous locus of the *Pik3ca* gene, and genetically ablated its negative regulator *Pten*. Additionally, we crossed these mice to *Apc*-loss predisposed, and *Kras*^{G12D} mutant mice to investigate interactions with wnt- and MAPK signalling. To dissect dependence on pathway components, we genetically ablated *Rictor* (mTORC2) and utilized small molecule inhibitors of PI3-Kinase α and β, mTOR, Akt, Mek, and combinations thereof. Tissue samples were characterized by immunohistochemistry and to an extent by RNA-seq.
PI3-Kinase hyperactivation resulted in an allele-dose dependent increase in tumour initiation and progression to invasive adenocarcinoma in *Apc*-loss predisposed mice. We found marked synergy to drive intestinal cancer within the PI3-K pathway, but also with concomitant *Kras*^{G12D} mutation. Targeted inhibition and genetic ablation of pathway components revealed that PI3-Kα, in concert with MAPK signalling, sustains tumour formation and growth via mTORC1, likely in an mTORC2 independent manner. Kras mutation caused excessive rewiring of this signalling network and conferred resistance to PI3-K inhibition.
We show that PI3-K signalling is not a binary on/off switch in intestinal cancer initiation and progression to advanced cancer. Clinically, PI3-Kinase inhibitors have not yet shown efficacy in CRC and co-activation of PI3-K and MAPK may be a reason for this. The newly generated mouse models will be used to further trial clinically relevant treatment regimes.

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HISTORY OF GENETICS

P90 Building a Better Hop: How Mendelian Hop Breeding Launched the Craft Beer Phenomenon

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Introduction and Aims: Hops (humulus lupus) have been a known plant variety for millennia; mostly used in making beer. By 1900, hops were a flavoring agent in beer. Hops are dioecious; annual vines grow from perennial rhizomes. The female produces a hop cone; the male—a flower. Cones contain resin that provides flavor. In the mid-20th century, the female was viewed as the contributor to a cultivar's characteristics (a male's sole value—germinating a cone). This was contrary to Mendel's theories of plant breeding. In 1961, U.S. Department of Agriculture (USDA) geneticist Dr. Stanley N. Brooks proved the male contributed half of the characteristics of a cone. Further work by Brooks and USDA's hops research team resulted in a new hop—Cascade. In 1972, USDA registered it as the first American hop cultivar. In 1975, Anchor, a small brewery, was the first to use Cascade. Apparently, Anchor chose Cascade for an "all-American" beer for the nation's bicentennial. In 1980, Sierra Nevada used Cascade in its first beer. Its marketing highlighted Cascade's flavor. These first steps birthed the craft beer industry. Today, craft beer marketing often includes listing hop cultivars. But the journey began with Mendel and a research scientist who understood Mendelian inheritance—to build a better hop.

Methods: Historical; academic and USDA literature reviewed.

Results: Information from the literature indicates a connection from Gregor J. Mendel to Stanley N. Brooks.

Conclusions: American hop breeding did not employ Mendelian techniques until the 1950s. Brooks's mid-century research proved the male plant contributed to the flavor acids found in the female cone. Further research by Brooks and colleagues resulted in the Cascade hop, the first American hop cultivar. Cascade's flavors were vital to smaller brewers seeking to make beers with a variety of taste attributes. Today, the craft beer industry is an international phenomenon, with Mendel having a transformational influence.

Acknowledgement: Daniel Fairbanks, Ph.D., Utah Valley University

HISTORY OF GENETICS

P91 Mendel's research on plant hybridisation after 1865

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Gregor Mendel is best known for his famous 1866 article on plant hybridisation in the garden pea (*Pisum sativum*) and the common bean (*Phaseolus spp.*), which is the foundation of the science of genetics. Less known is the extent of his research on plant hybridisation in other species, most of it conducted during the years 1866–73. A common myth is the notion that Mendel focused his research during this period almost entirely on hawkweed (*Hieracium*) and that this research was complete failure due to apomixis. In fact, Mendel conducted hybridisation research on at least twenty plant genera, and his efforts with *Hieracium* were extensive, successful, and informative. In this classic 1866 article, he classified plant hybrids into two categories: those with variable progeny (including pea and bean), and those with constant progeny that retain the characters of the hybrid parent through subsequent generations of self-fertilisation. In an attempt to develop a more comprehensive theory of inheritance, he intentionally selected plant genera that he knew beforehand were likely to exemplify variable types and constant types. He wrote that hybrids of *Matthiola*, *Zea*, and *Mirabilis* "behave exactly like those of *Pisum,*" and others, especially *Geum* and *Hieracium* hybrids, produce constant progeny. He also confirmed in *Mirabilis* that one pollen grainis sufficient for fertilisation, confirming his theory of parental equality and contradicting claims by Naudin and Darwin of parental inequality. He published one article on *Hieracium* in 1870 but unfortunately left his abundant post-1865 hybridisation research unpublished, evidence of it mostly confined to his letters to Carl von Nägeli.

HISTORY OF GENETICS

P92 Mendel as a student at the University of Vienna

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Gregor Johann Mendel was sent to Vienna by the abbott Cyrill Napp in 1851 to enroll as a student at the University of Vienna to get a formal education in the natural sciences. This talk focuses on the skills, which Mendel acquired during his university studies, and how his University education inspired him to conduct his famous hybridization experiments on peas a few years later.

Methods: historical records, primary literature

Results: Thanks to preserved university records, we know which subjects Mendel studied as well as the names of his teachers, among them the physicists Doppler and Ettingshausen and the botanist Unger.

Discussion and conclusion: The scientific atmosphere at the University of Vienna in the middle of the 19th century fostered Mendel's growth as a scientist. Mendel got a rigorous education in the natural sciences and acquired quantitative research skills as applied by the physicists at the time. He also learnt about evolutionary ideas, which one of his teachers, Franz Unger, adhered to and published on.

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HISTORY OF GENETICS

P93 Integrating Mendelian genetics within an inclusive inheritance framework for science engagement activities

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Many have argued that it is misleading to start and end with Mendelian traits and rules of inheritance in K-12 education, as it fails to address the full complexity of genetic inheritance, leaves out the reality of extra-genetic inheritance, and ignores the developmental and ecological contexts of inheritance, which can lead to or reinforce fallacious genetic deterministic and essentialist reasoning (Dougherty 2009; Dougherty et al. 2011; Jamieson and Radick 2013, 2017;
Donovan 2014; Radick 2016; Stern and Kampourakis 2017; Kampourakis 2021; Aivelo and Uitto 2021). In this talk, I present a project carried out at the "Deck50," an interactive science center at the Natural History Museum of Vienna. Led by Barbara Fischer and Lynn Chiu, four programs on Mendel and his scientific work were developed under the frameworks of inclusive inheritance frameworks (Danchin et al. 2011; Jablonka and Lamb 2014, 2020; Bonduriansky and Day 2018) and the concept of the "reactive genome" (Gilbert 2003). Mendel's work is presented in an interactive theater format ("Meet a Scientist, Gregor Mendel") to showcase the human side of the scientist-priest (motivation, success, failure, etc), highlighting the decisions involved in choosing simplified model organisms. An art-science activity (a mission-based activity to create cardboard "peasts" from a variety of genetic and environmental developmental factors and pass these factors on as "instructional recipes"), a wet-lab activity (the staining and examining of onion chromosomes through a microscope, with the understanding that they are activated and reactive to external stimuli), and a science quiz activity (the investigation of a wide variety of inheritance mechanisms and complex traits) were developed to situate Mendel's life and science within the 21st century science of inheritance. With these examples, we report a case study that draws from philosophy of science, history of science, and science communication principles to responsibly honor Mendel's bicentennial through science engagement activities.

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HISTORY OF GENETICS

P94 In Their Ninth Decade, the First Visit of C C Li and C C Tan: Founder of Human Population Genetics Meets the Guardian of Chinese Mendelism

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In the mid-1990s at the US University of Pittsburgh, two senior China-born geneticists, Li Ching Chun and Tan Jiazhen, first met. Much alike, they had never met: Li born in 1912, in Tianjin near Beijing; Tan in 1909, in Ningbo near Shanghai. Both got bachelor degrees, Li at Nanking in 1936, Tan at Soochow in 1930. Both earned PhDs at famous US genetics departments: Li, at Cornell University, Ithaca, New York, in plant breeding and genetics; Tan at California Institute of Technology, Pasadena, California, where he was Dobzhansky's first PhD student, describing the salivary gland chromosomes and genetic maps of Drosophila pseudoobscura, with Morgan and Sturtevant. Both did post-doctoral work at Columbia University in New York before returning to China. The 1948 Communist Revolution bifrucated their lives. Having done a book on human population genetics and, at age 34 years, become a chair in Beijing, Li was told to stop all work on Mendelism and adopt the truth of Lyzenkoism from Russia. He escaped China via Hong Kong and got a fellowship in Pittsburgh in 1951, aided by Muller. Li was critical of Tan, who had survived academically by switching from animal genetics per se to evolution and paleontology. Politicians considered Li's training in agriculture as more crucial for the Chinese economy than Tan's obscure insect work. I first met Tan in Shanghai in the late 1970s, after the Cultural Revolution. He recounted his meeting with Mao Zedong, who allowed him to teach the alternate genetics of Mendel, but only at Fudan University. From our contact, Tan agreed to visit Pittsburgh. Despite premonitions of tension between these genetic giants (both hard of hearing), they happily yelled at each other for hours the Li's office adjacent to mine and at dinner. It is a life-long joy to witness the founder of human population genetics and the sole guardian of Mendelism in China join hands near the end of their lives. Li died in 2003 at age 90, and Tan in 2008 at age 99 years.

HISTORY OF GENETICS

P95 G. Mendel Memorial Symposium 1965 – celebrating Mendel's legacy and international cooperation

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The International *G. Mendel Memorial Symposium* was organised in Czechoslovakia in August 1965 to commemorate the 100th anniversary of the public presentation of Mendel's experiments on heredity. The Symposium, divided into the celebrational gathering in Brno and the scientific meeting focused on mutation processes in Prague, was attended by 925 scientists from both sides of the Iron Curtain.

Case Summary: In the early 1950s, classical genetics was suppressed in favour of Lysenkoism in Soviet block countries. However, the situation was slowly changing with destalinization, and the Czechoslovak Academy of Science (ČSAV) started to seek international collaboration and became more open to West science. In 1958, ČSAV suggested to the International Union of Biological Sciences that the event marking the 100th anniversary of Mendel's lectures on heredity should be held in Czechoslovakia. The four days long commemorative part of *G. Mendel Memorial Symposium* took place in Brno and focused on the history of genetics and its contemporary discoveries and applications. The programme also included visits to the sites connected to Mendel as his native house in Hynčice or the newly opened Mendel Memorial Hall with the exposition about Mendel. The meeting continued in Prague with *Symposium on the Mutational Process* with four scientific sessions dealing with the research topics close to the recently established biological institutes of ČSAV and the Slovak Academy of Sciences.

Conclusion: *G. Mendel Memorial Symposium* represents the culmination of the efforts of ČSAV for international cooperation, the presentation of their research on the global level and an opportunity to show the international scientific audience that Mendel and his discoveries are acknowledged and commemorated in Czechoslovakia. Therefore, Symposium was perceived among scientists both from the East and West as the symbolic confirmation of the end of the Lysenkoism era.

HISTORY OF GENETICS

P96 Jaroslav Kříženecký – propagator of Mendel's legacy

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Jaroslav Kříženecký was not only a Czech zootechnician, geneticist and university teacher but is considered one of the biggest propagators and experts on Gregor Mendel's life. He was the founder of Gregor Mendel Genetics Department (now known as Mendelianum) in the Moravian Museum in Brno and the author and editor of numerous publications on Mendel.

Case Summary: Jaroslav Kříženecký, throughout his professional life, aimed to establish organisations commemorating Mendel's life and work. Between the 1930s and 1950s, such institutions were missing in Brno. However, they were frequently sought by visitors of Brno, who were keen to see Mendel's memorabilia. This led to the first attempt by Kříženecký to found the genetics department together with Mendel museum and library in 1932, but the plan was cancelled due to the financial crisis. In 1946, it was offered to Kříženecký by representatives of Masaryk University to be the head of the Genetics Department at the Faculty of Science. During the next two years, Kříženecký prepared this project, which included experimental, bibliographical sections and the museum. Kříženecký started the propagation campaign, international cooperation and gathered literature for the future department. Nevertheless, the Communist coup happened in 1948, and soon genetics became suppressed in Czechoslovakia. It took Kříženecký another fourteen years until the idea of the Mendel institution came finally to life through Gregor Mendel Genetics Department at Moravian Museum.

Conclusion: Jaroslav Kříženecký was the main leader of efforts to establish institutions focused on Mendel's legacy in Brno. The culmination of his endeavours represents the opening of Mendel Memorial Hall at the Augustinian Abbey during G. Mendel Memorial Symposium in 1965. Unfortunately, Kříženecký could not attend this celebrational event, as he died in December 1964.

HISTORY OF GENETICS

P97 Mendel's Experimental Data of Backcrosses in Pisum Backs up His Presentation in 1865 Lectures (the final version)

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No doubt, Mendel's 1865 speech in Brno merits more attention for its value rarely recorded in the local newspapers. According to the report summary of the two lectures, Mendel likely aimed to compare the developmental features of characters in successive generations of hybrid self-crosses and of backcrosses with parents. As a result, he not only confirmed Köelreuter's belief that parental pollen were more effective than hybrid pollen to speed up the reversion tendency, but also discovered the law of inheritance regarding cross-generation transmission of hereditary factors[1]. However, as we known, the hybridization system in *Pisum* only embraces prior self-crosses of hybrid and subsequent testcross designed to verify the hypothesis of segregation, where no position for the so-called backcross.

In theory, the mate of F1 hybrid in testcross is a recessive individual but in backcross it could be a recessive and/or a dominant individual of parents. Here we report the hybridization data of backcrosses could be easily picked out in his later (testcross) experiments for checking gametes (Table 1), substantially backing up his experimental framework in the lecture. Finally, considering Mendel was too intelligent to use a dominant individual as a tester when designing his testcross, we speculate that to testify certain evolutionary opinion, Mendel parallel initiated "self-crosses + backcrosses" with seed characters of shape and color of F1 hybrids; then the ratio 3:1 and the two ratios of 1:1 collectively inspired him to model the perfect square formula to express the behaviors of bisexual gametes in process of reproduction; at last, the real "testcross" was designed to validate it (Figure 1). Sure, such a successful multistage research project made Mendel free to choose different narrative styles in his oral and writing presentation one after another.

1. Zhang H, etc. Mendelism: New Insights from Gregor Mendel's Lectures in Brno. Genetics.2017; 207: 1-8.

Acknowledgement: We are very grateful to the Organization Committee of Mendel Genetics Conferrence, for their hard work.

Table 1 The cross data regarding testimony of reproductive cells in the 1866 paper

Traits	Backcross combinations[#]		Cross types[#]	Characters of the progeny	Results Tallied	Ratio[†]
A: round seed a: wrinkled seed B: yellow albumen seed b: green albumen seed	1	*AaBb*×*ab*	BC_I/ Testcross	*AaBb*: *Aab*: *aBb*: *ab*	31: 26: 27: 26	1:1:1:1
	2	*ab*×*AaBb*	Reciprocal BC_I/ Testcross	*AaBb*: *Aabb*: *aaBb*:*ab*	24: 25: 22: 27	1:1:1:1
	3	*AaBb*×*AB*	BC_{II}	*AB*: *ABb*: *AaB*: *AaBb*[‡]	20: 23: 25: 22	1:1:1:1
	4	*AB*×*AaBb*	Reciprocal BC_{II}	*AB*: *ABb*: *AaB*: *AaBb*[‡]	25: 19: 22: 21	1:1:1:1
A:violet-red flowers a: white flowers B: long axis b: short axis	5	*Aab*×*aBb*	Testcross	*AaBb*: *aBb*: *Aab*: *ab*	47: 40: 38: 41	1:1:1:1
				Aa: a: Bb: b[*]	85: 81: 87: 79	1:1:1:1

Notes: ¤, the order of experiments is renumbered for the sake of visualization; #, the types are determined by the definitions mentioned above; †, the ratios are calculated here; ‡, the seeds of the progeny are all dominant, but the character combinations are deduced from the data of the next generation, see Mendel's text; *, each trait was counted independently.

Fig. 1: no

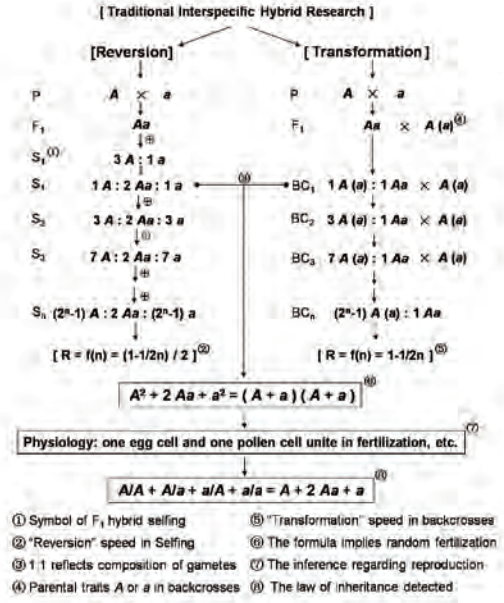

Fig. 2: Figure 1 Putative "T shape diagram" of the experimental framework of Mendel's discovery of the law of inheritance using the perfect square formula

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Jaroslav Doležel
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ANIMAL GENETICS
Michael Lampson
University of Pennsylvania, US
Petr Hořín
Masaryk University, CZ

HISTORY OF GENETICS
Daniel Fairbanks
Utah Valley University, US
Uwe Hoßfel
Friedrich-Schiller Universität Jena, GE

ROUNDTABLE: ETHICAL QUESTIONS IN GENETICS
Věra Franková
Charles University, CZ
Milan Macek
Charles University, CZ

NOTES

PEAS FOR MENDEL

„A statue for Mendel, who was ahead of his time in exceptionalism, is a kind of thanks and a natural moral obligation for us. Not only were the results of Mendel's discoveries not appreciated during his lifetime, but he also did not receive any scientific acknowledgment until many years after his death. Mendel's work is unique and far-reaching. For example, his actions express an idea that does not exclude the possibility that belief in God and science can stand peacefully side by side."

Jan Emil Biernat, OSA

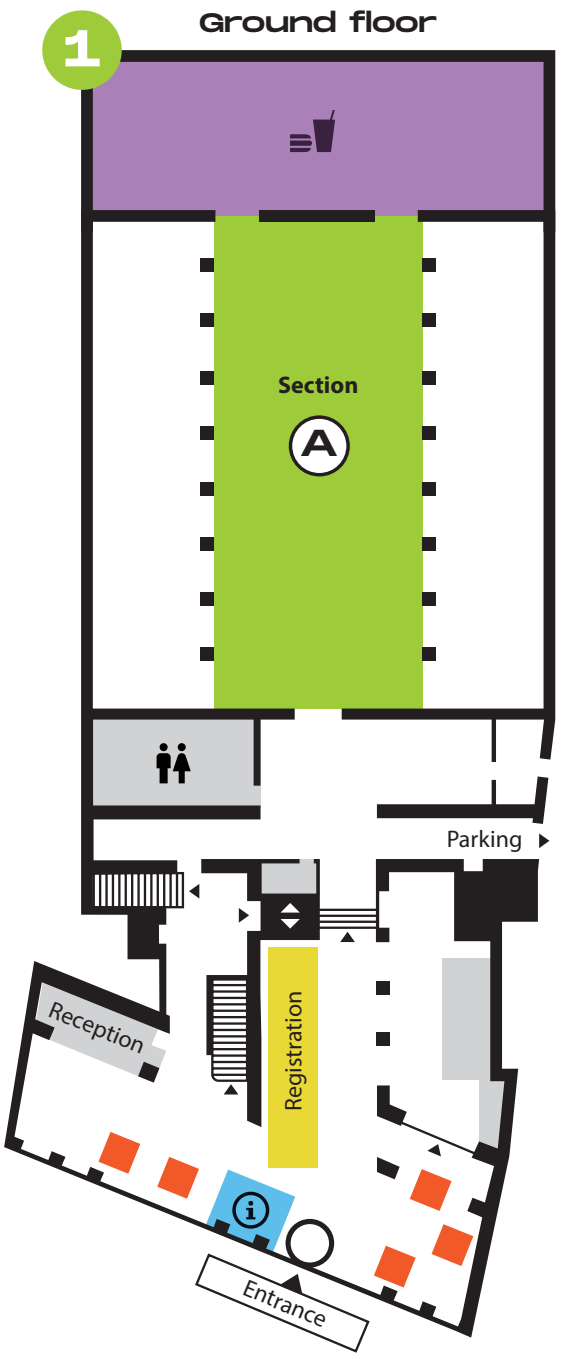
The monument of Hrachovina, which refers directly to Mendel's pea plant experiments, will form a new landmark of Mendel Square in Old Brno, it should remind present and future generations of the personality and groundbreaking discovery of G. J. Mendel.

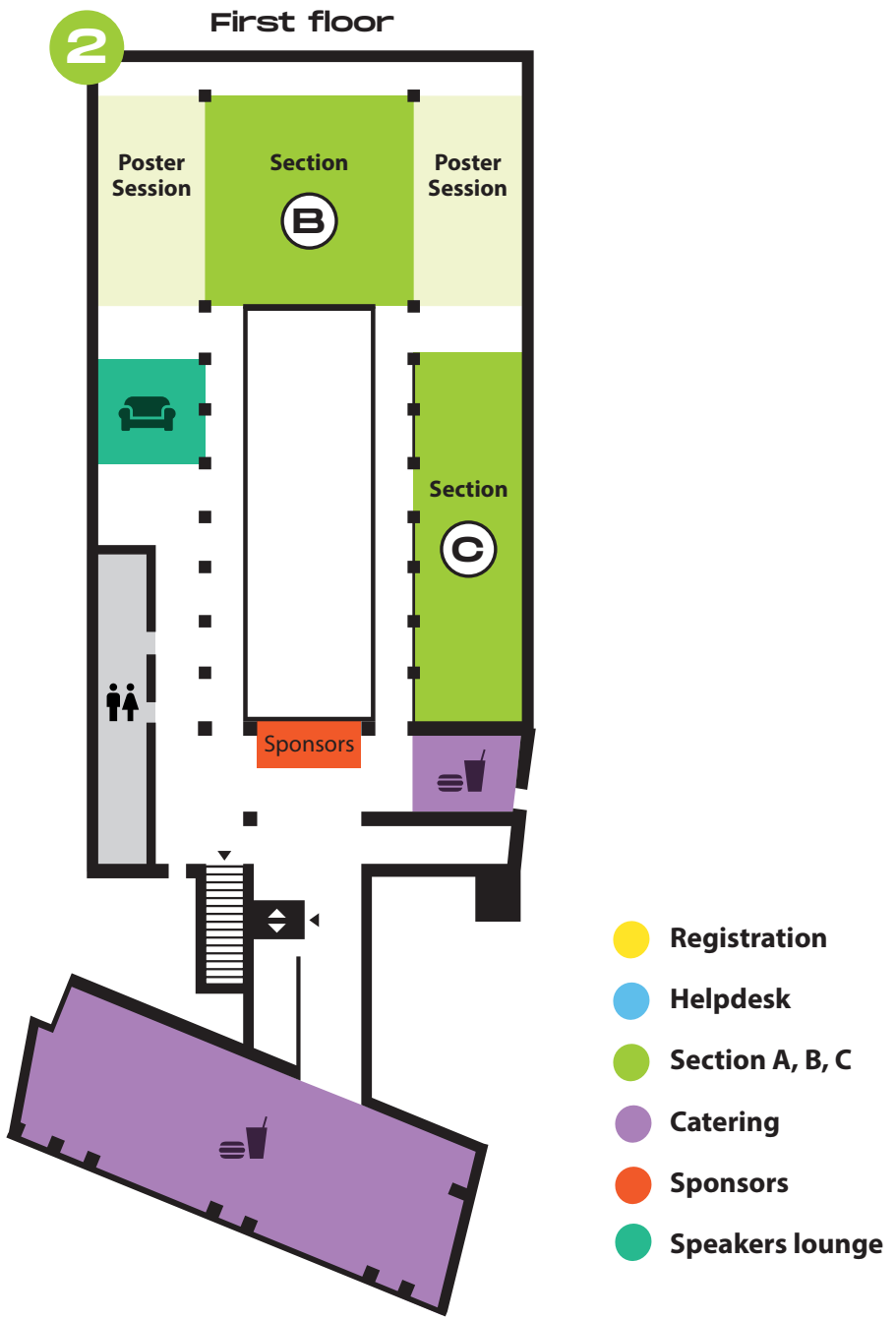
Donate to the realization of the artwork and become part of Mendel's legacy!

We would be grateful to receive a donation of any amount to our transparent account:
IBAN: CZ07 0800 0000 0061 7514 0379, BIC (SWIFT) code: GIBACZPX

PEAS
author J. Gargulák

HOTEL PASSAGE FLOOR PLAN





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See you later...