2012. LXXXVII. évfolyam, 2. különszám

A GRADUÁLIS ÉS POSZTGRADUÁLIS KÉPZÉS folyóirata
Alapítva 1911-ben

2012.
LXXXVII.
évfolyam,
2. különszám

ORVOS-KÉPZÉS

ORVOSKÉPZÉS

SEMMELWEIS SYMPOSIUM 2012

Principal Questions of the Genomic Medicine: Prediction, Prevention and Personalized Treatment

Semmelweis Kiadó

Az ORVOSKÉPZÉS megjelenik negyedévente. Megrendelhető a Kiadótól.

ORVOSKÉPZÉS
A graduális és posztgraduális képzés folyóirata
Alapítva 1911-ben
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2012; LXXXVII. évfolyam,
S2:285-336.
SEMMELWEIS SYMPOSIUM 2012

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Nyomdai előállítás:
Avaloni Kft.

ISSN 0030-6037

Semmelweis Kiadó
www.semmelweiskiado.hu

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XXI. INTERNATIONAL SEMMELWEIS SYMPOSIUM 2012

Principal Questions of Genomic Medicine: Prediction, Prevention and Personalized Treatment

November 9-10, 2012.

Semmelweis University, Budapest, Hungary
Basic Medical Science Center

PRESIDENT OF THE SCIENTIFIC COMMITTEE
Maria Judit Molnar

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VENUE
Semmelweis University Basic Medical Science Center
H-1083 Tűzoltó Street 34-37. Budapest, Hungary

REGISTRATION FEE: 100 €
The registration is free for workers and students of Semmelweis University
The Semmelweis Symposium is accredited by the OFTEX and provides 44 credit points for medical specialists. This symposium has been approved by the School of Doctoral Studies of Semmelweis University and provides 1 credit point for PhD students.

REGISTRATION OFFICE IS OPEN
09. 11. 2012. Friday 08:00-19:00
10. 11. 2012. Saturday 08:00-14:00

REGISTRATION FEE CONTAINS
Participation of scientific programs
The program and the abstract book
Lunch and coffee breaks
Social programs

BADGES
All participants are kindly requested to wear their name badge when attending the congress or social events.

LUNCH
Lunch area is located in the entrance hall.
Complimentary coffee, tea and snacks will be served to registered delegates during coffee breaks.

VENUE OF SOCIAL PROGRAM
Semmelweis University Basic Medical Science Center
H-1083 Tűzoltó Street 34-37. Budapest, Hungary

SOCIAL PROGRAM

Exhibition:
Zsuzsa Pannonhalmi (Ferenczy Noémi-prize holder, ceramic artist)

Concert: 09. 11. 2012.
The pearls of chamber music from Bach to Bartók, Gyorgy Geiger (Liszt and Kossuth prize holder, trumpet artist) and Eva Maros (harp artist)

Banquet dinner: 19:40 09. 11. 2012.

General information
English is the offical language of the Congress
Website: http://www.semmelweissymposium2012.hu

POSTER INSTRUCTION
Posters must be mounted on November 9, before the morning session and remain on display until the end of the Symposium. Size of poster boards: 1 m (width) x 1.3 m (height). Stickers will be provided.
Authors are requested to be present at their posters during the poster session. At the session, poster chairpersons will walk around and discuss posters. There are 3 minutes for the authors to present their work and 2 minutes for discussion.

ORVOSKÉPZÉS folyóirat szerzői útmutatója

A folyóirat célja: Az 1911-óta megjelenő Orvosképzés legfontosabb célja a hazai orvoskollégák folyamatos graduális és posztgraduális képzésének támogatása. A lap elsősorban olyan munkák közlését tartja feladatának, amelyek az orvostudomány egy-egy ágának újabb és leszűrt eredményeit foglalják össze magas színvonalon úgy, hogy azok a gyakorló orvoshoz, szakorvoshoz, klinikushoz és elméleti orvoshoz egyaránt szóljanak. Emellett lehetőség van eredeti közlemények és esetismertetések benyújtására, és az újság a Semmelweis Egyetem szakmai kötelező szinten tartó tanfolyamok előadási összefoglalóinak is teret ad. Az eredeti közlemények a rendszeres lapszámokban, vagy a témához kapcsolódó tematikus lapszámokban kapnak helyet. Fontos feladatunknak tartjuk, hogy rezidens kollégák tollából származó esetismertetéseket is közöljünk, melyeket mentori ajánlással kérünk benyújtani. A beadott dolgozatokat a szerkesztőbizottság előzetes bírálatra adja ki, és a kézirat közlésére a bírálat eredményének függvényében kerül sor. Tudományos dolgozat benyújtására az alábbiak szerint van lehetőség:

- Esetismertetés (case report)
- Fiatal doktorok (PhD) tudományos beszámolója, új eredményeinek összefoglalása (nem tézisek vagy doktori értekezések!)
- Klasszikus összefoglaló közlemény az elméleti és klinikai orvostudomány bármely területéről, a legújabb irodalmi eredmények felhasználásával
- „Update" jellegű közlemény, azaz nem egy téma kidolgozása, hanem adott szakterület legújabb tudományos eredményeinek összefoglalása
- Előadási összefoglaló (a tanfolyamszervezők felkérése alapján)

A kézirat: A tudományos közleményeket elektronikusan, Word dokumentum formátumban kérjük eljuttatni a szerkesztőségbe. Az illusztrációkat, ábrákat és táblázatokat külön file-ként kérjük elküldeni. Az ábrák címeit és az ábramagyarázatokat a Word dokumentumban külön oldalon kell feltüntetni, az ábra/táblázat számának egyértelmű megjelölésével. A digitális képeket minimum 300 dpi felbontásban kérjük, elfogadunk tif, eps, illetve cdr kiterjesztésű file-okat. A kézirat elfogadása esetén az ábrákat a szerkesztőség nyomtatott formában is kéri elküldeni. Az orvosi szavak helyesírásában az Akadémia állásfoglalásának megfelelően, a latinos írásmód következetes alkalmazását tekintjük elfogadottnak. Magyarosan kérjük írni a tudományágak és szakterületek, a technikai eljárások, műszerek, a kémiai vegyületek neveit. A szerkesztők fenntartják maguknak a stiláris javítás jogát. A mértékegységeket SI mértékrendszerben kérjük megadni.

A kézirat felépítése a következő: (1) címoldal, (2) magyar összefoglalás, kulcsszavakkal, (3) angol összefoglalás (angol címmel), angol kulcsszavakkal, (sorrendben): magyar cím, angol cím, (4) rövidítések jegyzéke (ha van), (5) szöveg, (6) irodalomjegyzék, (7) ábrajegyzék, (8) táblázatok, (9) ábrák. Az oldalszámozást a címoldaltól kezdve kell megadni és az egyes felsorolt tételeket külön lapon kell kezdeni.
(1) A *címoldalon* sorrendben a következők szerepeljenek: a kézirat címe, a szerzők neve, valamint a szerzők munkahelye, a kapcsolattartó szerző pontos elektronikus és postai címének megjelölésével. (2–3) Az *összefoglalást* magyar és angol nyelven kell beküldeni, külön oldalakon, a következő szerkezet szerint: „Bevezetés" („Introduction"), „Célkitűzés" („Aim"), „Módszer" („Methods"), „Eredmények" („Results") és „Következtetések" („Conclusions") lényegre törő megfogalmazása történjék. A magyar és az angol összefoglalások terjedelme – külön-külön – ne haladja meg a 200 szót (kulcsszavak nélkül). A témához kapcsolódó, maximum 5 kulcsszót az összefoglalók oldalán, azokat követően kérjük feltüntetni magyar és angol nyelven. (4) A kéziratban előforduló, nem általánosan elfogadott *rövidítésekről* külön jegyzéket kell készíteni abc-sorrendben. (5) A szövegtörzs szerkezete világos és az olvasó számára átlátható legyen. Eredeti közlemények esetén a „Bevezető"-ben röviden meg kell jelölni a problémafelvetést, és az irodalmi hivatkozásokat a legújabb eredeti közleményekre és összefoglalókra kell szűkíteni. A „Módszer" részben világosan és pontosan kell leírni azokat a módszereket, amelyek alapján a közölt eredmények születtek. Korábban közölt módszereket esetén csak a metodika alapelveit kell megjelölni, megfelelő irodalmi hivatkozással. Klinikai vizsgálatoknál a kézirathoz csatolni kell az illetékes etikai bizottság állásfoglalását. Állatkísérletek esetén a Magyar Tudományos Akadémia – Egészségügyi Tudományos Tanács – állatkísérletekre vonatkozó etikai kódexe érvényes, melyre a metodikai részben utalni kell. A statisztikai módszereket és azok irodalmát is meg kell adni. Az „Eredmények" és a „Megbeszélés" részeket világosan kell megszerkeszteni. *Referáló közlemények* benyújtása esetén a szövegtörzs altémákra osztható, melyeket alcímek vezessenek be. *Összefoglaló referátumoknál* a szövegtörzs terjedelme ne haladja meg a 30 000 karaktert (szóközzel), *eredeti közleménynél* (klinikai, vagy kísérletes) ne haladja meg a 20 000 karaktert (szóközzel), *esetismertetésnél* ne haladja meg a 10.000 karaktert (szóközzel), *előadási összefoglaló* esetén pedig ne haladja meg a 8000 karaktert (szóközzel).
Irodalom: a hivatkozásokat (maximum 50, előadási összefoglalónál maximum 10) a szövegben való megjelenés sorrendjében tüntessék fel. A szövegben a hivatkozást a sorszáma jelöli.
Hivatkozás cikkre: sorrendben: szerzők neve (6 szerző felett et al./és mtsai), cikk címe, folyóirat neve (Index Medicus szerint rövidítve), év; kötetszám:első-utolsó oldal. Példa: 1. Kelly PJ, Eisman JA, Sambrook PN. Interaction of genetic and environmental influences on peak bone density. Osteoporosis Int 1990; 1:56-60. *Hivatkozás könyvfejezetre,* sorrendben: a fejezet szerzői. A fejezet címe. In: szerkesztők (editors). A könyv címe. A kiadás helye, kiadó, megjelenés éve; fejezet első-utolsó oldala. Példa: 2. Delange FM, Ermans AM. Iodide deficiency. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid. 7th ed. Philadelphia, Lipincott-Raven, 1996; 296 316.
Ábrajegyzék: a megjelenés sorrendjében, arab számmal sorszámozva egymás alatt tartalmazza az ábra címét és alatta rövid és lényegre törő ábramagyarázatot
Táblázatok: külön-külön lapokon kérjük, címmel ellátva és arab számmal sorszámozva. Törekedjenek arra, hogy a táblázat könnyen áttekinthető legyen, ne tartalmazzon zavaróan sok adatot.
Ábrák: külön-külön lapokon kérjük. Csak reprodukálható minőségű ábrákat, fényképek küldését kérjük (min. 300 dpi felbontásban), a korábban megjelölt file formátumokban. A kézirat elfogadása esetén a nyomtatott ábrát kérjük beküldeni a szerkesztőségbe és az ábra hátoldalán puha ceruzával kérjük jelölni a szerző nevét, arab számmal az ábra sorszámát és a vertikális irányát.

A formai hiányossággal beküldött kéziratokat nem tudjuk elfogadni. A gyors lektori és korrektúrafordulók érdekében kérjük a legbiztosabb levelezési, illetve e-mail címet, telefon- és faxszámot megadni. Elfogadás esetén külön levélben kérjük jelezni, hogy a szerzők a közleménnyel egyetértenek (és ezt aláírásukkal igazolják), valamint lemondanak a folyóirat javára a kiadási jogról. Írásbeli engedélyt kérünk mellékelni a már közölt adat/ábra felhasználása, felismerhető személy ábrázolása, szerzőnek nem minősülő személy nevének említése/feltüntetése esetén. A szerkesztőség az általa felkért szakértők személyét titkossággal kezeli. A kézirat tulajdonjoga a megjelenésig a szerzőt illeti meg, a megjelenés napján tulajdonjoga a kiadóra száll. A megjelent kéziratok megőrzésére szerkesztőségünk nem tud vállalkozni.

A kéziratok benyújtását a következő címre várjuk:

Dr. Szelid Zsolt szerkesztőségi titkár
Semmelweis Egyetem, Kardiológiai Központ
1122 Budapest, Városmajor u. 68
Tel: (06-1) 458-6810
E-mail: orvoskepzes@kardio.sote.hu

DR. MOLNÁR MÁRIA JUDIT
Vice-Rector
President of the Scientific Committee

Dear Collegues,

It is my pleasure to announce the XXI. **Semmelweis Symposium,** one of the the most important scientific event of the Semmelweis University.

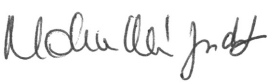
Semmelweis University is an internationally recognized biomedical university with state-of-art medical, teaching and research activity. Its most prestigious annual scientific event is Semmelweis Symposium and the main topic this year is **„Principal Questions of Genomic Medicine: Prediction, Prevention and Personalized Treatment"** reflecting modern scientific trends.

Personalized medicine is a paradigm shift that paves the way for a superior health care system. Our rapidly expanding knowledge of genetics and molecular biology is creating a stronger platform for us to move ahead into a future in which care is more closely tailored than ever before. New diagnostics for identifying disorders and new therapeutic possibilities are most likely to obtain their benefits in healthcare. Semmelweis University acts openly in the interest of prevention beside the diagnostic and therapeutic approaches. Thus, 2012 has been declared the Year of Prevention at Semmelweis University.

Semmelweis Symposium 2012 is organized by the Prevention Module of the Research Project „Modern Medical Scientific Technologies at Semmelweis University". Our University keeps up with international scientific trends and embraces groundbreaking research in genomics, proteomics, immunomics, cardiology, neurology, neurogenomics and pharmacogenomics. It was thus important for us to bring these new trends to the platform of our prestigious international conference. We aim to open attendees a wide window on the field of personalized medicine with leading invited scientists and with 12 session (7 oral and 5 poster session) representing and demonstrating us the entire spectrum of it at **Semmelweis Symposium 2012**.

This interdisciplinar meeting will appeal to researchers, PhD students, clinicians, residents, drug/diagnostics developers, and policy regulators. On behalf of the Organizing Committee, I would like to cordially invite you to the XXI. Semmelweis Symposium (Budapest, Hungary, the 9-10th of November, 2012). I look forward to welcoming you to a very successful Congress.

Budapest, 2012. November



Maria Judit Molnar
Vice-Rector
President of the Scientific Committee

Tisztelt Kollégák!

Nagy megtiszteltetés számomra, hogy a Semmelweis Egyetem egyik legjelentősebb tudományos rendezvényét, a **XXI. Semmelweis Symposiumot** ajánlhatom Önöknek.

A Semmelweis Egyetem egy nemzetközileg elismert biomedicinális egyetem, mely kitűnő oktatási, kutatási és betegellátó tevékenysége alapján elnyerte a megtisztelő Kutató Egyetemi címet. Egyetemünk évente megrendezi tudományos konferenciáját, a Semmelweis Symposiumot, mely idei témája **„A Genomikai Medicina Fő Kérdései: Predikció, Prevenció és Személyre Szabott Terápia”** a tudományos világ legújabb kutatási trendjére fókuszál.

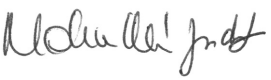
A személyre szabott medicina az egészségipar paradigmaváltását alapozza meg. A robbanásszerű technológiai fejlődés, a rendelkezésünkre álló, gyorsan bövülő genetikai és molekuláris biológiai ismeretanyag olyan jövő reményét kínálja, melyben az egészségügyi ellátás sokkal individualizáltabb, mint bármikor korábban. Új diagnosztikák segítenek a betegségek azonosításában, a betegségek stratifikációjában és új kezelési lehetőségek teremtik meg az egészségügyi ellátás alapját. A Semmelweis Egyetemen a betegségek diagnosztikája és kezelése mellett egyre nagyobb hangsúlyt kap a betegségek megelőzése. Így 2012-t Egyetemünk a Prevenció Évének deklarálta.

A Semmelweis Symposiumot 2012-ben a „Modern Orvostudományi Technológiák a Semmelweis Egyetemen” című Kutató Egyetemi Pályázat Prevenciós Moduljának témavezetői szervezik. Ezen kutatócsoportok tudományos munkájának fókusza a nemzetközi trendekhez illeszkedik a genomika, proteomika, immunomika, kardiológia, idegtudományok és a farmakogenomika területén. Fontosnak tartottuk, hogy ezeket az új trendeket egy olyan konferencián mutassuk be Önöknek, ahol számos nemzetközileg elismert külföldi előadót is vendégül láthatunk. A program összeállítása során legfontosabb célunk a személyre szabott orvoslás területének széleskörű bemutatása, ezért a Semmelweis Symposium 2012 programjában 12 szekció (7 oralis és 5 poszterszekció) igyekszik a teljesség igénye nélkül a széles spektrumra rávilágítani. Az interdiszciplinális symposiumot ajánljuk alapkutatók, PhD-hallgatók, klinikusok, rezidensek, gyógyszeripari, diagnosztikai cégek és a hatósági szereplők figyelmébe.

A Szervező Bizottság nevében engedjék meg, hogy tisztelettel meghívjam Önöket a XXI. Semmelweis Symposiumra 2012 november 9-10-én a Semmelweis Egyetem Elméleti Oktatási Központjába.

Szeretettel várjuk Önöket konferenciánkon.

Budapest, 2012. november



Molnár Mária Judit
tudományos rektorhelyettes
a Symposium Tudományos Bizottságának elnöke

SEMMELWEIS SYMPOSIUM 2012 / Program / Contens

9. NOVEMBER, 2012 (FRIDAY)

09.00-09.25	***OPENING CEREMONY*** Welcome *Széll, Ágoston* (Rector of the Semmelweis University) – 10 minutes Semmelweis University facing the new era of medicine: Personalized Healthcare *Molnár, Mária Judit* (Chair of the Symposium) – 15 minutes		
09.25-11.15	**IMPLEMENTING A PERSONALIZED MEDICINE STRATEGY – IS THERE LIGHT AT THE END OF THE TUNNEL? – CHAIRPERSONS: LÁSZLÓ HUNYADY AND GYÖRGY NÉMETH**		
	Golubnitschaja, Olga (General Secretary of the European Personalized Medicine Association) – 25 minutes	Predictive diagnostics, targeted preventive measures and personalized treatment:new philosophy in health care	p. 294
	Németh, György (President of the Hungarian Society of Personalized Medicine, Budapest, Hungary) – 20 minutes	Personalized medicine: from theory to the clinical practice	p. 294
	Nagy, László (University of Debrecen, Institute of Personalized Medicine, Debrecen) – 20 minutes	The triad of success in personalized medicine: pharmacogenomics, biotechnology and regulatory issues from a Central European perspective	
	Ádám, Veronika (Semmelweis University, Budapest, Hungary) – 20 minutes	Key dehydrogenases in the mitochondrial generation of reactive oxygen species	
	Discussion – 20 minutes		
11.15-11.40	**BREAK**		
11.40-13.00	**IMMUNOMICS – CHAIRPERSONS: ANDRÁS FALUS AND EDIT BÚZÁS**		
	Brusic, Vladimir (Harvard Medical School, Boston, USA) – 25 minutes	High-throughput Immunomics: data mining of vaccine targets in viral pathogens	p. 295
	Szalai, Csaba (Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary) – 20 minutes	Genomic investigations in asthma	p. 295
	Falus, András (Semmelweis University, Budapest, Hungary) – 20 minutes	Systems biology approach in tumor genomics and epigenetics	p. 296
	Discussion – 15 minutes		
13.00-13.45	**LUNCH BREAK**		

13.45-15.45 METABOLOMICS – CHAIRPERSONS: ZOLTÁN TAKÁTS AND LÁSZLÓ SZŐNYI

	Kertesz, Vilmos (Oak Ridge National Laboratories, USA) – 20 minutes	The modern methods of metabolomics and proteomics	p. 296
	Szőnyi, László (Semmelweis University, Pediatric Clinics 1 Budapest, Hungary) – 20 minutes	Population level newborn screening for inherited metabolic disorders and other genetic risk factors	p. 297
	Römpp, Andreas (Justus Liebig University) – 20 minutes	Imaging mass spectrometry in predictive tumor diagnostics	
	Szatmári, Ildikó (Semmelweis University, Cell Screen Research Center, Budapest, Hungary) – 20 minutes	Mass spectrometry methods as confirmation analyses in the diagnosis of inherited metabolic disorders	p. 297
	Takáts, Zoltán (Semmelweis University, Cell Screen Research Center, Budapest, Hungary / Imperial College London, Department of Surgery and Cancer, London, UK) – 20 minutes	Mass spectrometric profiling of biological tissues – a comprehensive tool for personalized medicine	p. 298
	Discussion – 20 minutes		
15.45-16.00	**BREAK**		

16.00-17.40 PERSONALIZED CARDIOLOGY THE WAY TO THE OPTIMAL PREVENTION AND THERAPY – CHAIR PERSONS: BÉLA MERKELY AND ZOLTÁN NAGY

	Staessen, Jan A. (University of Leuren, Belgium) – 25 minutes	Reduction of salt intake in the general population: recommendations to be taken cum grano salis	
	Szelid, Zsolt (Semmelweis University, Budapest, Hungary) – 20 minutes.	Primary prevention population cohort: Budakalász epidemiology study	p. 298
	Soós, Pál (Semmelweis University, Budapest, Hungary) – 20 minutes	Heart failure epidemiology in Hungary	p. 299
	Maurovich-Horváth, Pál (Semmelweis University, Budapest, Hungary) – 20 minutes	Cardiac-CT in population screening	p. 299
	Discussion – 15 minutes		
17.40-18.45	**POSTER SESSION**		
	PREDICTION OF TREATMENT EFFECTIVITY AND SIDE EFFECTS – CHAIRPERSONS: KOVÁCS, GÁBOR AND SZALAI, CSABA		
	PREDICTION AND PREVENTION OF CARDIOVASCULAR AND CEREBROVASCULAR DISORDERS – CHAIRPERSONS: SOÓS, PÁL AND VASTAGH, ILDIKÓ		
	THE GENETIC BACKGROUND OF RARE DISEASES – CHAIRPERSONS: FEKETE, GYÖRGY AND TORY, KÁLMÁN		
	BIOMARKER RESEARCH – CHAIRPERSONS: CHINOPOULOS, CHRISTOS AND IGAZ, PÉTER		
	GENETIC AND SOCIOBEHAVIORAL FACTORS PREDISPOSING TO PSYCHIATRIC DISORDERS – CHAIRPERSONS: BITTER, ISTVÁN AND PUREBL, GYÖRGY		

10. NOVEMBER, 2012 (SATURDAY)

08.30-10.00 NEUROPSYCHOPHARMACOLOGY – CHAIRPERSONS: GYÖRGY BAGDY AND KÁROLY MIRNICS

	Mirnics, Karoly (Vanderbilt Kennedy Center, Nashville, USA) – 25 minutes	GABA-ergic dysfunction in schizophrenia: from postmortem studies to animal models	p. 300
	Bagdy, György (Semmelweis University, Budapest, Hungary) – 25 minutes	Monoamine-endocannabinoid interactions in anxiety and depression: relevance to personalized medicine	p. 300
	McKie, Shanke (Neuroscience and Psychiatry Unit, University of Manchester, UK) – 25 minutes	Exploring serotonin function in the human brain using phMRI	p. 301
	Discussion – 15 minutes		
10.00-10.20	**BREAK**		

10.20-12.15 GENOMIC NEUROLOGY: PREDICTION AND TREATMENT OF NEUROLOGICAL DISORDERS – CHAIR PERSONS: ANDRÁS SPAT AND GÁBOR KOVÁCS

	Wartiovara, Anu (University of Helsinki, Finland) – 25 minutes	The expanding fi eld of the mitochondrial medicine	
	Kovács, Gábor (Medical Univesity of Vienna, Vienna, Austria/ Semmelweis University, Budapest, Hungary) – 25 minutes	Lessons from GWAS in cognitive decline	p. 301
	Gulyás, Balázs (Karolinska University, Stockholm, Sweden) – 25 minutes	Translational molecular neuroimaging	p. 302
	Weis, Joachim (RWTH University Hospital, Aachen, Germany) – 25 minutes	Medical and biological insights from genetic studies of rare inherited neurological disorders	p. 302
	Discussion – 15 minutes		
12.15–12.35	**BREAK**		

12.35-14.05 PERSONALIZED DECISION MAKING IN THE ERA OF EVIDENCE-BASED NEUROLOGY – CHAIRPERSONS: DANIEL BERECZKI AND PETER LANGHORNE

	Bereczki, Dániel (Semmelweis University, Budapest, Hungary) – 25 minutes	Personalized medicine and evidence based medicine: enemies or brothers in arms?	p. 303
	Langhorne, Peter (University Glasgow, UK) – 25 minutes	Systematic reviews in stroke care: have they changed clinical practice?	p. 303
	Charland-Verville, Vanessa (University Liege, Belgium) – 25 minutes	Coma and disorders of consciousnes	p. 304
	Discussion – 15 minutes		
14.05	**TEST**		

14:20- CLOSING CEREMONY

ABSTRACTS OF THE PLENARY LECTURES

Predictive diagnostics, targeted preventive measures and personalized treatment: New philosophy in healthcare

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Current healthcare. Currently severe chronic pathologies such as cardiovascular disorders, diabetes and cancer are treated after onset of the disease, frequently at near end-stages. Pessimistic prognosis considers pandemic scenario for type 2 diabetes mellitus, neurodegenerative disorders and some types of cancer over the next 10-20 years followed by the economic disaster of healthcare systems.

Advanced healthcare tailored to the person. Advanced healthcare promotes the paradigm change from delayed interventional to predictive medicine tailored to the person, from reactive to preventive medicine and from disease to wellness. The cost-effective management of diseases and the critical role of predictive, preventive and personalised medicine (PPPM) in modernisation of healthcare have been acknowledged as priorities by global and regional organizations and health-related institutions such as the Organisation of United Nations, the European Union and the National Institutes of Health.

Integrative medical approach by PPPM as the medicine of the future. The integrative concept enables to predict individual predisposition before onset of the disease and provide targeted preventive measures before onset of the disease. The expected outcomes are conducive to more effective population screening, prevention early in childhood, identification of persons at-risk, stratification of patients for the optimal therapy planning, prediction and reduction of adverse drug-drug or drug-disease interactions relying on emerging technologies, such as pharmacogenetics, pathology-specific molecular patters, sub/cellular imaging, disease modelling, individual patient profiles, etc. Integrative approach by PPPM is considered as the medicine of the future. Being at the forefront of the global efforts, the European Association for Predictive, Preventive and Personalised Medicine (EPMA, http://www.epmanet.eu/) promotes the integrative concept of PPPM among healthcare stakeholders, governmental institutions, educators, funding bodies, patient organisations and in the public domain. *The EPMA Journal* (BMC, UK, http://www.epmajournal.com) and *Book Series "Advances in PPPM*" (EPMA / Springer Dordrecht Heidelberg New York London, http://www.springer.com/series/10051) overview multidisciplinary aspects of advanced bio/medical approaches and innovative technologies, integrate individual professional groups and provide expert recommendations for advanced healthcare.

Personalized Medicine: from theory to clinical practice

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President of Hungarian Society of Personalized Medicine, Medical Director Gedeon Richter Plc, Budapest, Hungary

In the recent years increasing number of personalized treatment become available for patients, but so far no breakthrough can be seen in the change of perspective from evidence-based medicine to personalized therapy. The utilisation of biomarkers might be one solution to speed up this slow progress. Therefore biomarkers are becoming increasingly important tools in later-stage research, drug development and in clinical practice, as well. Classification of biomarkers is especially important as different types of biomarkers can carry distinct therapeutic consequences. By utilizing biomarkers patients who are most likely to benefit from a treatment can be identified. Moreover response to therapy could be predicted, or patients who likely to be at increased risk for adverse reactions can be screened beforehand. Furthermore, biomarkers help to shift the emphasis in medicine from reaction to prevention, increase patient adherence and help to control the overall cost of health care. The integration of biomarkers into the clinical research is essential, but to fulfil all the expectations the clinical research has to address many critical and partly unsolved issues.

High-throughput Immunomics: data mining of vaccine targets in viral pathogens

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Advances in genomics, proteomics, and bioinformatics have provided massive amounts of data that offer insights into the diversity of pathogens, their interaction with hosts, and molecular mechanisms that underlie responses to vaccines. Tens of thousands sequence variants are available for rapidly mutating pathogens such as HIV, influenza, or flaviviruses. Reverse vaccinology is an approach where an entire pathogen genome is screened using bioinformatics methods to identify antigenic targets that are subsequently tested using recombinant protein, peptidic, or DNA vaccines based on wet lab testing for immune responses against identified targets. Common approaches to reverse immunology include identification of peptides conserved across vast majority of viral strains followed by their experimental validation and further investigation as universal vaccines. Universal or cross-protective vaccines are effective against majority of viral strains. The disadvantage of this approach is that many promising antigenic sites will be missed because they have more than one antigenic variant.

Large amount of immunological data are scattered in publications, technical reports, and databases using a variety of formats. The extraction of knowledge from the vast amounts of immunological data through data mining remains a challenging task. To bridge the gap between data and knowledge, we developed a framework for fast deployment of Web-accessible databases. This framework enables semi-automated data collection and integration, automated data storage and retrieval, and fast deployment of computational tools for in-depth analysis of various structural and functional properties associated with immune responses and vaccine development. The framework aims to speed up the immunological research and vaccine design by providing specialist databases hosting cleaned, well-annotated and structured data and integrating them into data mining pipeline for the discovery of new knowledge. Furthermore we developed a set of workflows that enable automated analysis of antigen diversity and their immunological potential through identification of potential T- or B-cell epitopes. The visualization tools help display reports and results in an intuitive and easy-to-grasp format. By applying such analyses to influenza A, flaviviruses, and norovirus we extended the antigenic target sets by an order of magnitude relative to previously reported vaccine target sets. Data-mining platforms enable rapid and comprehensive identification of vaccine target sets.

Genomic investigations in asthma

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Objective. Asthma is a multifactorial disease development of which is influenced by genetic and environmental factors. Genetic and genomic methods offer the possibilities of detecting novel genes and pathways important in the pathogenesis of the disease.

Methods. Based on earlier linkage analyses we selected the 11q13 and 14q22 asthma susceptibility regions, for which we designed a partial genome screening study using 145 SNPs in 1201 individuals (436 asthmatic children and 765 controls). We also carried out SNP screenings in several additional asthma candidate genes, gene expression measurements in a mouse model of asthma and in induced sputum in humans. The results were evaluated with traditional frequentist methods and we applied a new statistical method, called Bayesian network based Bayesian multilevel analysis of relevance (BN-BMLA).

Results. With frequentist methods one SNP (rs3751464 in the *FRMD6* gene) provided evidence for an association with asthma (OR=1.43(1.2-1.8); $p=3x10^{-4}$). In the BN-BMLA analysis altogether 5 SNPs in 4 genes were found relevant in connection with asthma phenotype: *PRPF19* on chromosome 11, and *FRMD6*, *PTGER2* and *PTGDR* on chromosome 14. In the mouse model of asthma and in induced sputum of humans we found significant alterations in the expression levels of several genes including *FRMD6, BIRC5*, and YAP1, all of them are members of the Hippo pathway. Furthermore, in a case-control study examining single nucleotide polymorphisms in the *BIRC5* regulatory regions, the minor alleles of rs8073903 and rs8073069 were found to be significantly associated with asthma and especially non-allergic asthma phenotypes. Additionally, with linear regression analysis we showed that rs9904341 in the *BIRC5* gene was significantly correlated with both absolute and relative serum eosinophil levels in humans.

In ***conclusion***, our results suggest a possible role of the Hippo pathway in asthma.

This study was supported by OTKA: K81941 (C. Szalai), and NKTH TECH_08-A1/2-2008-0120 (A. Falus, C. Szalai, P. Antal).

Systems biology approach in tumor genomics and epigenetics

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The role of histamine in tumour growth is far from being fully uncovered. In this lecture two approaches of systems biology will be introduced, in order to resolve the mechanism of action of histamine.

The role of histamine have been demonstrated in tumor (melanoma) growth using mouse melanoma cells manipulated via stable transfection with sense mouse HDC mRNA, a mock control, and an antisense HDC RNA segment, respectively. Gene expression profiles and in silico pathway analysis of transgenic mouse melanomas, secreting different amounts of histamine show a histamine H1 receptor dependent suppression of expression of the tumor suppressor insulin-like growth factor II receptor and the antiangiogenic matrix protein fibulin-5.

Simultaneously, HDC-knockout mice seem to show a high rate of both spontaneous and induced colon and skin carcinogenesis. HDC is expressed primarily in $CD11b^{+}Ly6G^{+}$ immature myeloid cells (IMCs) that are recruited early on in chemical carcinogenesis. Transplant of HDC-deficient bone marrow to wild-type recipients results in increased IMC cell mobilization and reproduces the cancer susceptibility phenotype of HDC-knockout mice. In addition, mouse CT26 colon cancer cells directly downregulate HDC expression at epigenetic manner through promoter hypermethylation and by rinhibition of myeloid cell maturation enhances tumor formation.. These data indicate key protective role of histamine in myeloid cell differentiation and IMCs in early cancer development.

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The modern methods of metabolomics and proteomics

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Atmospheric pressure surface sampling/ionization techniques have become a significant area in mass spectrometry (MS) research. Direct mass spectrometric analysis of surfaces is of considerable interest because of the potential for mass spectrometry to provide low level, specific detection of targeted compounds and molecular mass and structure determination for suspect compounds and unknowns. With these methods, analytes can be directly analyzed from a variety of surface types, in some cases, without the need for extensive sample preparation. However, in some cases some sample preparation or a separation may be necessary to obtain the required analytical data, for example to differentiate between isomeric species.

In this presentation, atmospheric pressure surface sampling/ionization techniques will be overviewed with specific emphasis on desorption electrospray ionization (DESI) and liquid extraction based methodologies. The DESI system utilizes a plume of charged droplets directed at the surface to desorb and ionize analytes from a surface. This technique was applied to detect and identify peptides from two-dimensional separations of cytochrome c and myoglobin tryptic digests on thin-layer chromatography (TLC) sheets and for chemical imaging of whole-body thin tissue sections of mice intravenously dosed with drug. In contrast, the liquid extraction based methodologies create a controlled liquid junction between the probe and the surface in order to extract the analytes of interest from the latter. Different implementations of this surface sampling technology were primarily used for spatial profiling of drugs and corresponding phase II metabolites from whole-body mouse thin tissue sections.

Population level newborn screening for inherited metabolic disorders

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In many European countries neonatal screening for inborn errors of metabolism has been introduced in the last 50 years. In Hungary the national screening program started in 1974. The extension of screening program for 26 diseases was implemented in 01. October 2007.

In a survey conducted in 2010/2011 data from the 28 EU member states, four EU candidate states (Croatia, FYROM, Iceland, Turkey), three potential EU candidate states (Bosnia Herzegovina, Montenegro, Serbia), and two EFTA states (Norway and Switzerland) were collected.

The interval between birth and sampling should not be done before 48 hours post partum in view of physiological variation leading to potential false positive or negative results. Finland and Malta both use cord blood but do not screen for phenylketonuria. The interval between sampling and start of analysis should be as short as possible, but it is often several days. The average annual number of samples per laboratory varies from 2050 (Malta) and 121852 (Greece). The number of screened diseases varies from 1 in Montenegro to 29 in Austria. The conditions most screened are congenital hypothyroidism (37 countries) followed by phenylketonuria (33 countries). Congenital adrenal hyperplasia is in the third place (15 countries). Political support for screening has been reported by all responding countries.

The result of this survey was that there are large variations in the design of the newborn screening programmes in the European countries and the day-to-day practices.

In comparison to previous surveys in Europe (2002, 2007) it is noted that there has been a large increase in the number of conditions screened for at least 11 countries. The major impetus has been the introduction of the tandem mass spectrometry technique making multiplex screening for fatty acid oxidation disorders, amino acidaemias, and organic acidurias possible.

The goal of the EU survey was to implement a uniform screening panel as have been achieved in the USA resulting in equal screening opportunities for all European newborn infant. Out of this goal the newborn screening expansion has brought some new, important problems: (1) lack of knowledge about the natural history of some of the disorders, (2) absence of effective preventive therapy for some disease, (3) identification of seemingly benign disorders or benign variants of severe disorders, (4) resulting parental anxiety.

Mass spectrometry methods as confirmation analyses in the diagnosis of inherited metabolic disorders

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Objectives. The goal of the newborn screening (NBS) for inborn errors of metabolism is the early detection and confirmation of disease, thus enabling early medical intervention, treatment, and improved outcomes. Although the application of electrospray ionization – tandem mass spectrometry (ESI-MS/MS) to NBS permits efficient identification of groups of disorders with acceptable laboratory operating costs, for obtaining a definitive diagnosis detailed confirmation analyses are necessary. In this project several mass spectrometry based enzyme activity measurements were developed as confirmation analyses for suspected inherited metabolic disorders.

Methods. Samples collected from healthy adult volunteers and patients with clinically confirmed metabolic disorders were analysed by liquid chromatography coupled MS, and enzyme activities were assessed by quantitative determination of the specifically chosen artificial substrates and/or respective enzymatic products.

Results. Enzyme activity measurements were developed for fatty acid oxidation disorders, namely medium chain acyl-CoA dehydrogenase (MCAD) deficiency from peripheral blood mononuclear cells (PBMC), for biotinidase activity from serum and specially pretreated dried blood spots (DBS) and for enzymes responsible for lysosomal storage disorders from DBS. The methods were successfully used for confirmation of conditions identified in the neonatal metabolic screening and confirmed by genetical analyses.

Conclusions. The developed enzyme activity determination methods are suitable confirmation analyses for metabolic disorders identified in NBS. The availability of these methods at the Laboratory of Metabolic Screening offers the possibility of fast diagnosis and enables an appropriate personalised therapy.

Mass spectrometric profiling of biological tissues – a comprehensive tool for personalized medicine

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The recent advent of ambient ionization methods enabled the mass spectrometric investigation of a whole new set of sample types, including unmodified biological tissues. Desorption Electrospray Ionization has been successfully employed for the imaging analysis of native, frozen tissue sections, yielding unique information on the spatial distribution of low molecular weight tissue constituents. Besides the fundamental biochemical information, DESI imaging – similarly to MALDI imaging – also allows the histology-level identification of tissues and spectral information shows considerably good correlation with the presence/status of well characterized genetic markers used for predictive cancer diagnostics. While imaging mass spectrometry has the potential of revolutionizing molecular histopathology, this experimental approach still does not solve the problem of rapid tissue identification. In-situ, sometimes in-vivo tissue characterization has critical importance in case of interventional cancer diagnostics (e.g. endoscopy) and surgical treatment of solid tumors. Currently used solutions include intraoperative frozen section histology, confocal laser scanning microscopy, in-vivo fluorescent labeling of tissues and intraoperative medical imaging. Practically all of these technologies provide some level of solution, however the problems regarding specificity, sensitivity, time demand and associated additional costs do not make any of them an ideal and general solution. While imaging mass spectrometry cannot solve this problem due to the incompatibility of ionization method with the physical characteristics of vital biological tissues, the recently developed Rapid Evaporative Ionization Mass Spectrometry (REIMS) method provides a dedicated solution. REIMS technique is based on the observation, that electrosurgical interventions involve the ionization of certain tissue components, especially membrane lipids. As a consequence, the direct combination of electrosurgery with a mass spectrometer results in a tissue dissection technique, which also provides real-time chemical analysis of dissected tissues. Since REIMS profile of biological tissues shows high level of histological specificity, the technology generally termed 'intelligent surgical device' or in a short form 'iKnife' is also able to identify tissues on practically real time, giving a highly valuable tool for cancer surgery. Nevertheless, identification of tissues is only feasible if a large collection of authentic spectra and appropriate search engine are available. Due to the nature of pattern-level tissue specificity, multivariate statistical tools were proven to be the appropriate approach for spectral comparison and identification. However, regarding the construction of spectral library, the REIMS technology is not the ideal tool, since electrosurgical dissection does not feature cellular-level accuracy and also requires large amounts of human tissue samples. At this level, imaging mass spectrometry methods gain importance again, especially due to the high similarity between REIMS, LDI or DESI spectra.

Primary prevention population cohort: Budakalász Epidemiology study

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The Framingham study provides important association data between risk factors and cardiovascular disease in a US cohort. No reliable morbidity data exist however in on Hungarian population. Semmelweis University started a cardiovascular screening programme involving the adult population (>20y) in a Central-Hungarian town. The complete adult population (8.000 inhabitants) of the selected town is targeted in our voluntary programme. Protocol includes health questionaire, non-invasive tests (cardiac- and carotid ultrasound, resting blood pressure and ankle-brachial index) and venous blood biobanking (serum, plasma and DNA), using coded samples linked to a clinical database. Low-dose cardiac CT for coronary calcium and cardiac fat determination is also performed in certain age groups (>35y in males and >40y in females).

By September 2012 the number of inhabitants screened was 1202 (mean age: 55.0±14.4y). Increased body mass index (over 25 kg/m^2) has been detected in 863 inhabitants (71.8%), in 540 (44.9%) enhanced systolic blood pressure, in 514 people (42.7%) elevated HgbA1c (40 mmol/l) has been detected and pathologic LDL cholesterol level has been verified in 556 inhabitants (46,2%). Elevated high-sensitivity CRP (2-10 mg/L) was measured in 490 people (40.7%). Preserved left ventricular ejection fraction with elevated NT-proBNP level (>220 pg/ml) has been measured in 139 inhabitants (11.5%). Average cardiovascular mortality risk is 9.8%. Our goal is complete the protocol on the total adult population in the selected town and to perform longitudinal follow-up studies to analyze associations between cardiovascular risk factors, morbitity and mortality in a primery prevention Hungarian cohort. Prospective screening is being performed in the frame of the Budakalász Epidemiology Study.

Heart failure epidemiology in Hungary

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Purpose. Progressive heart failure (HF) is a significant public health problem of industrialized countries. Incidence of HF in the European population is about 0.4-2% and has a strong correlation to age. There are no data available about the incidence and mortality of HF in Hungary. Moreover there are no data about the regional distribution of end-stage heart failure patients in a single heart transplant center country. A nationwide analysis was made based on the International Statistical Classification of Diseases and Related Health Problems (ICD) codes to summarize incidence and mortality data of HF and heart transplantation (HTX).

Methods. Based on the official administrative data of all the Hungarian hospitals a retrospective study was performed to analyze HF incidence. Mortality data were collected from national registers. Patient data were available at the National Health Insurance Fund register between 2000-2007. A clinically relevant heart failure was represented by the following ICD codes: hypertensive heart disease with heart failure (I 11.), dilated cardiomyopathy (I42.0), heart failure (I50.), cardiogenic shock (R57.0). HTX data were collected in the national HTX center.

Results. The number of lately diagnosed HF cases represented by the above-mentioned ICD codes as main diagnosis tended to 0.3% of the population during the observed years: 35,194 (2004); 32,205 (2005); 30,325 (2006). Incidence of HF as main diagnosis (new cases/10,000 citizen) in 2006 was 28.0 (men) and 32.0 (women). The average of one-year mortality of heart failure patients on active hospital units was ca. 25% during the observation period. During the last 20 years more than 200 patients were transplanted In Hungary. The regional distribution of HF, HTX listed and transplanted patients were overlapping, however did only partially correlate to the regional distribution of the largest cardiac centers.

Conclusion. Analysis official administrative data demonstrated that incidence of HF in Hungary approaches the values of the European population mean. Distribution of end-stage HF patients is not equaled countrywide but independent of the healthcare provider system.

Cardiac-CT in population screening

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Atherosclerosis of the coronary arteries is the leading cause of morbidity and mortality in industrialized nations. Every year, 19 million people worldwide experience a sudden cardiac event. Atherosclerosis, as many other diseases, is the result of complex interactions between genetic susceptibility and environmental factors and it can be asymptomatic and remain undetected for many years. A large portion of this population has no prior symptoms and the first manifestation of coronary artery disease is acute coronary syndrome or sudden cardiac death. Thus, there is a considerable demand for early diagnosis and treatment of the underlying pathological conditions of sudden cardiac events.

Currently, there is no method available for clinicians that can identify asymptomatic patients with vulnerable coronary atherosclerotic lesions and predict the likelihood of a plaque rupture. In addition, not all high-risk plaques rupture and only a fraction of plaque ruptures lead to coronary event. Therefore, it is not clear whether the identification of one or several plaques with vulnerable phenotype, the determination of overall coronary plaque burden, or a combination of both would enhance management of patients with coronary atherosclerosis. Because of its ability to image the coronary vessel wall, cardiac CT is well suited for the non-invasive characterisation of different plaque types. Cardiac CT provides valuable information regarding intraplaque attenuation pattern, Napkin-ring sign, remodelling index, amount of calcification, plaque location and plaque size. In addition, it enables the reproducible quantification of overall calcified plaque burden, which have an important prognostic value.

The identification of vulnerable patients with an elevated calcifies plaque burden and high-risk lesions would enable the early initialisation of therapeutic interventions, therefore represent a major breakthrough in public health.

GABA-ergic dysfunction in schizophrenia: from postmortem studies to animal models

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Objectives. Glutamic acid decarboxylase 67 kDa (GAD67) is the primary GABA-producing enzyme in the brain. GABAergic interneuron subtypes can be classified by molecular markers such as neuropeptide Y (NPY), cholecystokinin (CCK), somatostatin (SOM), or parvalbumin (PV). While GAD67 downregulation is one of the most robust and reproduced findings in post-mortem tissue from schizophrenic patients, the importance of GAD67 downregulation across the various interneuronal cell types and their potential role in regulating behavior remain unclear.

Methods. To determine empirically whether cell-type specific GAD67 downregulation is sufficient to induce alterations in the brain and behavior, we have developed a novel method for silencing GAD67 in distinct subpopulations of interneurons in transgenic mice. We created bacterial artificial chromosome (BAC) constructs containing the NPY or CCK promoter-enhancer elements, an eGFP reporter, and a synthetic microRNA (miRNA) targeted to silence GAD67 mRNA specifically in NPY+ or CCK+ interneurons. Construct efficacy and validity was assessed using immunohistochemistry. Male transgenic mice (n=12) and their wild-type littermates (n=12) were subjected to a broad behavioral testing battery.

Results. NPYBAC and CCKBAC constructs effectively suppressed GAD67 expression in specific cell types. While eGFP was detected in NPY+ cells or CCK+ cells, GAD67 expression could not be detected in the two targeted interneurnal subpopulations. Behavioral testing of transgenic and control mice revealed no differences in general neurological/ neuromuscular functions, fear extinction, or sensorimotor gating. However, GAD67 downregulation led to cell-type driven changes in locomotor activity, social behavior, anxiety, memory, and response to amphetamine (3mg/kg).

Discussion. We conclude that GAD67 downregulation in a single interneuron cell type is sufficient to induce behavioral changes. We report that the NPY+ and CCK+ mouse lines responded oppositely to an amphetamine challenge. These results suggest that GABA system dysfunction and dopaminergic dysregulation are interrelated and warrant further examination. Our observations suggests that dysfunction of particular cell types could underlie particular behavioral dysfunction and may be a promising future avenue for therapeutic targeting of behavioral symptoms associated with various neuropsychiatric disorders, including schizophrenia.

Monoamine-endocannabinoid interactions in anxiety and depression: relevance to personalized medicine

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Cannabinoid type-1 (CB_1) receptor antagonists were among the most promising drug targets in the last decade. They have been explored and found to be effective as therapeutic agents for obesity and related cardiometabolic problems however, use of rimonabant, the first marketed CB_1 receptor antagonist, has been suspended due to its anxiogenic and depressogenic side effects. There is strong evidence that endocannabinoids modulate signaling of serotonin and noradrenaline, which play key roles in the pathophysiology and treatment of anxiety and depression. In addition, most human and rodent studies suggest that the presence of under-functioning endocannabinoid type-1 (CB_1) receptors is associated with increased anxiety and elevated extracellular serotonin concentration. Promoter variants of the serotonin transporter and the CB_1 receptor genes have been shown to modulate anxiety in human studies. In contrast, noradrenaline is presumably implicated in the mediation of depression-type symptoms of CB_1 receptor antagonists. Evidence show that most CB_1 receptors located on axons and terminals of GABA-ergic, serotonergic or glutamatergic neurons stimulate the activity of noradrenergic neurons, while those located on noradrenergic axons and terminals inhibit noradrenaline release. In this latter process, excitatory ionotropic or G protein-coupled receptors, such as the NMDA, alpha1 and beta1 adrenergic receptors, activate local endocannabinoid synthesis at postsynaptic sites and stimulate retrograde endocannabinoid neurotransmission acting on CB_1 receptors of noradrenergic terminals. The underlying mechanisms include calcium signal generation, which activates enzymes that increase the synthesis of endocannabiboids, while $G_{q/11}$ protein activation primarily increases the formation of 2-arachidonoylglycerol from diacylglycerol during the signaling process. Based on these data and our existing knowledge concerning the role of genetic, phenotypic and environmental factors the selection of persons who are at no or low risk for psychiatric adverse effects may be possible.

Studies were supported by EU, LSHM-CT-2004-503474, TAMOP-4.2.1.B-09/1/KMR-2010-0001 and ETT318/041/2009.

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Exploring serotonin function in the human brain using phMRI

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Pharmacological-challenge magnetic resonance imaging (phMRI) is a recently developed MRI technique designed to visualise the acute action of drugs on the human brain. In this talk I will explain the principles behind phMRI and why it is a cheaper, and in some cases more viable, alternative to Positron Emission Tomography (PET). I will then highlight the results of several studies designed to investigate serotonin (5-HT) function in healthy volunteers. Selective serotonin reuptake inhibitor (SSRI) citalopram is known to increase the amount of serotonin released into the synapse and is a well establish treatment for depression. I will show the results of three studies using citalopram phMRI in a) healthy volunteers as a proof of principle, b) healthy volunteers who have remitted from depression and c) healthy volunteers after 11 days chronic citalopram treatment. The results of the studies implicate the ventral striatum in the mechanism for remission from depression. The 5-HT_{2C} receptor subtype has also been implicated in the pathogenesis of mood and anxiety disorders. I will show the results of a complex study, using mirtazapine (5-HT_2 antagonist) pre-treatment with m-chlorophenylpiperazine (mCPP; 5-HT_{2C} agonist) phMRI, in order to dissect the brain regions specific to 5-HT_{2C}. The results of this highlight a possible link to the citalopram-induced responses discussed earlier. I will then discuss some current studies in which serotonin phMRI has been taken into specific patient populations. If these studies are successful, serotonin phMRI could provide an exciting opportunity for the rapid development of better treatments for psychiatric conditions and psychiatric drug development.

Lessons from GWAS in cognitive decline

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The recent sequencing of the human genome has revolutionized biomedical research. Understanding genetic contributions to non-pathological cognitive ageing is based on heritability, candidate gene, and genome wide association studies. Candidate gene studies have suggested several genes as associated with cognitive ageing, however, many of these lack replication. Another study performed genetic association analysis of cognitive ability and cognitive ageing, using markers for genes associated with oxidative stress or cognition and suggested a possible role of *APP* in normal cognitive ageing. Genomic studies in Alzheimer`s disease (AD) are also emerging. More than two-dozen novel potential susceptibility loci were highlighted beyond the well-established *APOE* association. However, for none of the novel candidate genes do we have conclusive functional genetic evidence that would allow establish any of these loci as AD risk genes. Further approaches in GWAS include the definition of a diagnostic marker and then evaluation of the influence of a genetic variation on these markers. One potential reason for the various results is the miscellaneous criteria used to define cases. Application of a simplified concept, defining AD as a single entity, could potentially jeopardize GWAS as well as therapy trials. Recent molecular neuropathologic investigations have increased the number of neurodegenerative conditions with different protein depositions in the brain, moreover, their concomitant presence in the same brains, as morphological substrates for dementia. Thus, further development and better specification of genomic observations can be achieved when evaluating very strictly defined cases that have undergone neuropathological/biochemical classification.

Supported by the European Commission's 7th Framework Programme under GA No 278486, "DEVELAGE".

Translational molecular neuroimaging

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The human genome contains approximately 23.000 genes determining structural and/or functional proteins. According to recent estimates, ~4.000 of them can be targeted at ~12.000 various target sites. Using advanced molecular imaging techniques and molecular imaging biomarkers molecular targets, including disease biomarkers and/or disease modifiers, can be visualized *in vivo*. Complemented with animal disease models, with special regard to transgenic models, this approach has significant benefits in diagnostic biomarker development and therapeutic drug development. "Humanised" small animal disease models, new technological solutions in the radiochemistry of molecular imaging biomarkers, and technological developments in multimodal human and dedicated animal scanners (multimodal scanners with high resolution and sensitivity) have contributed to the recent advancements of translational molecular neuroimaging with the help of which the development of CNS drugs and molecular imaging biomarkers can be facilitated and accelerated.

The development of novel diagnostic imaging biomarkers for neuroinflammation and neurodegeneration has been and is in the forefront of translational molecular neuroimaging research. The social burden of various neurodegenerative diseases, including MCI (mild cognitive impairment), Alzheimer's disease, Parkinson's disease and Huntington's disease, puts a special emphasis on the problem. The early diagnosis of neurodegenerative diseases is hampered by the lack dedicated diagnostic markers that may specifically label alterations in the ailing brain's biochemical processes in a way that by using molecular imaging techniques the disease can be recognised distinctively in its early phase with high sensitivity and specificity. The search for dedicated molecular imaging biomarkers of neuroinflammation and neurodegeneration has a major emphasis in now-a-days' PET radioligand development. The presence and progression of both neurodegenerative and neuroinflammatory processes can be visualized and quantitatively assessed by various ways, including the targeting of classical neuroreceptor systems, the TSPO system, metabolic enzymes or amyloid plaques with molecular imaging biomarkers.

The lecture will give an outline of the recent status of translational molecular neuroimaging, with special regard to the development of molecular imaging biomarkers for neuroinflammation and neurodegeneration.

Medical and biological insights from genetic studies of rare inherited neurological disorders

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The classification and diagnosis of neuromuscular diseases has relied on the analysis of the morphological phenotype of the biopsied muscle and nerve for decades. Molecular genetic analysis has identified the underlying mutations in a large number of hereditary neuromuscular diseases during recent years. Genotype-phenotype studies correlating molecular genetic findings in human patients to nerve and muscle biopsy phenotypes as well as studies using genetically engineered mouse models have contributed greatly to the understanding of the pathogenesis of neuromuscular disorders. I´ll present exemplary results obtained during the course of our analysis of gene mutations and their phenotypes on the molecular genetic, cellular and ultrastructural level in paradigmatic hereditary neuromuscular disorders, including Charcot-Marie-Tooth disease and Marinesco-Sjögren syndrome, and discuss the diagnostic and pathophysiological relevance of the typical morphological features. Our data contribute to the understanding of the function of the affected genes in the development and maintenance of human peripheral nerve and skeletal muscle.

Personalized medicine and evidence based medicine: enemies or brothers in arms?

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The most important sources of evidence in modern medicine are human studies using epidemiological approach – like randomized controlled trials –, and results obtained by molecular biological and genetic methods. The practice of *evidence-based medicine* combines the most reliable scientific information with individual expertise and patient preferences to offer the optimal diagnostic and therapeutic option for the patient. *Personalized medicine* represents an approach considering well defined, biomarker-based differences among individual patients in decision making. Whether the evidences used in patient care are of statistical nature derived from a large number of clinical observations or personalized ones based on biomarkers – both should be scientifically sound to apply in patient care. *Evidence based medicine* could be confronted with *personalized medicine* only if the former is misinterpreted and restricted only to the use of randomized trials and their systematic reviews. It should be recognized that the practice of personalized medicine is also based on evidences, and on the other hand evidences from molecular research are also statistical in nature in many instances. In health care decision making about an individual patient, whether using evidences from randomized trials or from molecular studies of biomarkers, we have to base our decisions on reliable, good quality evidences. Evidences from molecular and genetic medicine therefore improve the armament of evidence based medicine, yielding a more reliable support for our decisions in everyday practice.

Systematic reviews in stroke care: Have they changed clinical practice?

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Systematic reviews have been developed over the last 20 years to provide reliable summaries of evidence in healthcare. It is generally accepted that a good systematic review is comparable in quality and reliability to a large multicentre randomised trial. In stroke care a large number of new treatments have come into use over the last 25 years, almost all of which are underpinned by multicentre randomised trials or systematic reviews.

Systematics reviews in stroke care – A large number of systematic reviews have been carried out in stroke care both within the Cochrane Collaboration (by the Cochrane Stroke Group) and through conventional journals. A particular strength is that many areas of stroke care have not been subjected to large randomised trials and systematic reviews provide the best summary of evidence. A survey of recommendations across a variety of clinical practice guidelines in stroke indicate that systematic reviews (and Cochrane Reviews in particular) are the commonest source of evidence in these guidelines.

Have systematic reviews changed clinical practice? - It is more difficult to demonstrate a clear effect of a systematic review on changing clinical practice but there are several examples (e.g. stroke unit care, thrombolysis, early supported discharge services) where large systematic reviews have almost certainly been influential in changing practice.

Systematic reviews have underpinned evidence in stroke care for over 20 years and have almost certainly been a major influence in modernising practice.

Coma and disorders of consciousness

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When patients in „persistent vegetative state" (recently coined unresponsive wakefulness syndrome) show minimal signs of consciousness but are unable to reliably communicate – the term minimally responsive or minimally conscious state (MCS) is used. MCS was recently subcategorized based on the complexity of patients' behaviors: MCS+ describes high-level behavioral responses (i.e., command following, intelligible verbalizations or non-functional communication) and MCS- describes low-level behavioral responses (i.e., visual pursuit, localization of noxious stimulation or contingent behavior such as appropriate smiling or crying to emotional stimuli). Patients who show non-behavioral evidence of consciousness or communication only measurable via ancillary testing (i.e., functional MRI, positron emission tomography, EEG or evoked potentials) can be considered to be in a functional locked-in syndrome.

Taken together, recent studies show that awareness is an emergent property of the collective behavior of frontoparietal top-down connectivity. Within this network, external (sensory) awareness depends on lateral prefrontal/parietal cortices while internal (self) awareness correlates with precuneal/mesiofrontal midline activity. Of clinical importance, this knowledge now permits to improve the care of patients with disorders of consciousness.

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ABSTRACTS OF THE POSTERS

I. Prediction of treatment effectivity and side effects

I.1. The activation of melanin-concentrating hormone (MCH) producing neurons correlates with the number of rems episodes

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Objectives. Rapid eye movement sleep (REMS) rebound after REMS deprivation is characterized by increased time spent in REMS and the activation of melanin-concentrating hormone (MCH) expressing neurons of the hypothalamus, known to have a central role in the regulation of REMS regulation. The time spent in REMS shows a strong correlation with the activation of MCH cells of the hypothalamus.

Methods. Male Wistar rats were selectively REMS deprived for 72h by the classic flower-pot method, followed by a 3h rebound sleep, during which EEG, EMG and motility were recorded. Immediately after rebound, each animal was perfused. The MCH/cFos double immunohistochemistry (cFos as an indicator of neuronal activation) was performed on hypothalamic sections. Home cage animals were used as controls. Data were evaluated by Spearman-correlation.

Results. During REMS rebound, both the number of REMS episodes and the average duration of REMS items increased. However, a positive correlation with MCH/cFos double staining was shown only in case of the number of REMS episodes.

Conclusion. The activation of MCH-expressing neurons of the hypothalamus seems to be in a strong connection with the number of REMS, suggesting that REMS deficiency may be compensated *via* the enhancement of the number during the rebound rather than the average duration of REMS items.

This work was supported by
TAMOP-4.2.1. B-09/1/KMR-2010-0001

I.2. Genetic risk factors of anthracycline cardiotoxicity – relevant polymorphisms identified in enzymes and transporters of anthracycline pharmacokinetics

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Objectives. The main dose limiting side-effect of anthracyclines is late cardiotoxicity. Survivors of anticancer therapy have increased risk for cardiovascular problems and have higher such mortality. Subclinical changes may become crucial in case of later accompanying diseases affecting the cardiovascular system, or these changes can precede severe late onset cardiac failure. Identifying patients with altered tolerance to anthracyclines would provide great clinical benefit.

Methods. We studied 164 paediatric acute lymphoblastic leukaemia (ALL) patients who had been treated with ALL BFM protocols. They had cardiac ultrasound scans with a mean follow up of 6.4 years after anthracycline therapy. Left ventricular function was assessed as fractional shortening (LVSF). Germline genotypes of 19 single nucleotide polymorphisms (SNPs) in the *ABCC1*, *CBR1*, *CBR3* and *AKR1A1* genes were measured. Logistic regression analyses were performed to test for associations.

Results. Patients with *ABCC1* rs246221CT/TT genotype had lower LVFS at the time of the latest echocardiography compared to CC patients (38.4% and 40.7% respectively, p=0.027). Those with *AKR1A1* rs2088102CC genotype had lower LVSF than those harbouring at least one T allele (36.9% and 39.1% respectively, p=0.013). Further SNPs showed no association with left ventricular function.

Conclusion. Our results suggest that the *ABCC1* rs246221 and the *AKR1A1* rs2088102 variations are associated with altered left ventricular function in late survivors of childhood acute lymphoblastic leukaemia. The identified early subclinical changes may contribute to a polygenic disorder that evolves over a longer time and manifests in congestive heart disease later. A concise overview of the literature will be presented on gene polymorphisms found in association with anthracycline-induced cardiotoxicity.

I.3. Genetic risk factors of neurotoxicity during chemotherapy

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Among the numerous cytostatic drugs used in childhood acute lymphoblastic leukemia (ALL) treatment, vincristine, methotrexate, cytarabine and glucocorticoids are thought to exert most acute side effects on the central nervous system (CNS). Genetic variations can modify the function of enzimes and transporters involved in pharmacokinetics and pharmacodynamics. We hypothetize that these variations may influence toxicity.

DNA samples of 354 children with ALL were genotyped for 47 single nucleotide polimorphisms (SNP) in 15 genes coding transporters and metabolizing enzimes of chemotherapeutic agents. Clinical data was collected retrospectiveliy form the patients' medical records. Among the patients 31 (7%) suffered acute CNS toxicity of grade II or above according to the Common Terminology Criteria for Adverse Events v3.0. Logistic regression adjusted for potential confouders was performed using SPSS 19.1 software.

When analysing SNPs separately, association of grade II-IV neurotoxitiy was found with *ABCC1* rs246219 CC genotype (OR=5.1, CI 95%=1.15-25, p=0.032) and with *MVP* rs4788186 G allele (OR=0.38, CI 95%=0.17-0.88, p=0.024). The population was also searched for potential interaction of the two SNPs individually influencing neurotoxicity. In our cohort patients with *ABCC1* rs246219 CC and *MVP* rs4788186 AA genotypes were more prone to suffer neurotoxicity compared to patients with other genotypes (OR=3.43, CI 95%=1.41-8.33, p=0.0064). The *ABCC1* (ATP-binding cassette, sub-family C member 1) protein transports both methotrexate and vincristine. The *MVP* gene encodes the major vault protein which mediates drug resistance, perhaps via a transport process. According to our results genetic variations alone might have only little impact on the side effects of chemotherapy, but the combination of genotypes is strongly associated with neurotoxicity.

Our results indicate that genetic variations in genes involved in drug resistance may have high impact on the acute CNS toxicity of chemotherapy. SNPs can be used as genetic markers to identify patients at risk. This approach has a potential for individualizing therapy in the future.

I.4. Examination of individual sensitivity for the acute ototoxic side effects of cisplatin treatment in the light of the glutathione S-transferase enzyme polymorphism

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Objectives. We elaborated a prospective research protocol looking for whether the polymorphism of the glutathione-S-transferase (GST) T1, M1 and P1 enzyme coding genes is related to the early or late appearance of ototoxic side effects, and whether there is such a genotype which increases or decreases the chances of the ototoxic side effects. An aim of ours was that in contrast to the examinations found in the literature, in addition to subjective hearing tests we collected data with objective methods too(tympanometry, distorsion product otoacoustic emission(DPOAE), and we examined the patients before and after each treatment cycle.

Methods. We examined 86 testicular-tumour patients before they started their first chemotherapy and who had never had any known ototoxic treatment. The patients received daily 20mg/m^2 cisplatin for 5 days (100 mg/m^2/each cycle). We made detailed anamnesis and an otoscopy was done. We took blood from the patients, and the DNA patterns isolated from the separated lymphocytes, were genetically examined. To define the gene polymorphism of GSTT1, GSTM1, GSTP1, restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) methods were used.

Results. For variance analysis we used the ANOVA test and the significant values (level of significance at $p<0.05$) were examined post hoc (Scheffe and LSD tests). During the treatment or in the period leading up to the next chemotherapy more than 10% of patients complained of hearing problems. After the first cycle of cisplatin we did not find any significant amplitude reduction either doing the DPOAE or the threshold audiometry between subgroups created on the basis of GST genotype.

Conclusions. With the longitudinal following of patients we could get information both of early and late ototoxic changes and the eventual individual sensitivities. We have found patients who had hearing complaints indicative of ototoxic side effects as early as after the first cisplatin cycle, which calls attention to the importance of selecting "genetically high risk patients". This selection may guide the individualised and more accurate planning of cisplatin treatment. Patients with complaints must be sent for hearing tests the earlier the better so that we can avoid the irreversible impairment of the inner ear, or as feasible, depending on the oncological stage, less ototoxic treatment (e.g. carboplatin) could be introduced.

I.5. Examination of the genetic background of aminoglycoside induced, and non-syndromic, hereditary sensorineural hearing loss

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Objectives. With the development of molecular genomics we can recognise the connection between more and more genetic variations and medication side effects and we can discover an increasing number of genetic differences behind idiopathic symptoms. The joint occurrence of non-syndromic inheritable sensorineural and aminoglycoside induced hearing loss with the substitution of certain nucleotids of mitochondrial DNA has already been described. The aim of our present examination was to define the frequency of occurrence in the Hungarian population of mtDNA 1555 A>G és 1494 C>T nucleotid substitution in mitochondiral patients who suffer from maternally inheritable hearing loss and in healthy volunteers.

Methods. The participants of the research were: 30 patients suffering from aminoglycoside induced hearing loss (17 men, 13 women–average age: 47±6 years), 160 mitochondrial patients, among whom maternally inheritable hypacusis is frequent (75 men, 85 women–average age: 43±8 years), and 150 volunteering control participants (67 men, 83 women–average age: 46±5 years). For the genetic analysis the DNA was isolated from blood, and from postmitotic tissue (skeletal muscles). The nt. 1229-1832 segment of the isolated mtDNA was amplified by polymerase chain reaction (PCR), and then sliced with restriction fragment length polymorphism (RFLP). The intensity of the bands and the absence of heteroplasmia ratio was measured using Quantity One Software.

Results. Out of the examined 340 participants m.1555 A>G the heteroplasmic substitution appeared in 12 cases (5 mitochondrial patients and 7 control participants), in the patients examined the frequency of mutation was: 3,53%. No m.1494 C>T mutation was found. In the case of 2 patients maternally inheritable hearing loss was discovered.

Conclusions. The targeted examination of mtDNA may render the screening of carriers possible in the future, which may serve as the basis for the appearance of personalized therapies and, among others, it may contribute to avoiding undesirable ototoxic side effects.

I.6. The impact of race on interferon-α responsiveness: a genome-wide study

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In chronic HCV-infection, African Americans show sharply decreased responsiveness to interferon alpha (IFNα) therapy compared to Caucasians. In this study, we sought to analyze the molecular background of this phenomenon and identify disease-independent and race-associated response markers of IFNα responsiveness in healthy blood donors.

We analyzed T cells isolated from healthy Caucasian and African Americans by comparing IFNα-induced activation of STAT1,-2,-3,-4,-5, performing whole genome gene expression analysis of interferon stimulated genes (ISGs), and about one million single nucleotide polymorphisms (SNPs) of the two ethnicities. Surprisingly, we found that in contrast to HCV-infected individuals, healthy African Americans do not have impaired IFNα responsiveness either in terms of STAT activation, or IFNα-induced gene expression response. Although approximately 200 ISGs reacted to IFNα, we observed no significant differences in baseline gene expression or activation of ISGs by IFNα between healthy Caucasians and African Americans. The only gene affected by race (NUDT3, $p<10\text{-}7$), was not affected by IFNα, or known to be related to IFNα signal transduction, HCV infection, or immunity against HCV. Although race-associated SNPs were found to be numerous, and many polymorphisms were associated with ISGs, these differences did not affect responsiveness to IFNα in the absence of HCV infection.

We conclude that in chronic HCV infection, race does not affect IFNα signaling directly, but rather HCV pathophysiology or viral interference with IFNα signaling, which are ultimately responsible for altered effectiveness of IFNα therapy. Further studies are ongoing to analyze this mechanism focusing on the interaction of plasmacytoid dendritic cells and HCV.

I.7. The predictive role of Myxovirus resistance protein A (MxA) in Multiple Sclerosis treatment

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Objectives. Multiple sclerosis (MS) is an autoimmune disease of the central nervous system. The four main clinical courses of disease are relapsing remitting, secondary progressive, primary progressive, and progressive relapsing. Myxovirus resistance protein A (MxA) is an antiviral protein which is produced by the immune system after the Myxovirus A caused infection. The mRNA level of MxA has been shown to be a prognostic biomarker of MS. Furthermore it has been suggested, that the level of MxA expression may indicate the level of serum neutralizing anti-drug antibodies in patients treated with IFNβ. According to the literature 3-45% of the MS patients treated with IFNβ develop neutralizing anti-drug antibodies, which can inhibit the clinical effect of these therapies.

Aims: In this study we tested the MxA gene expression in MS patients (therapy nad've and previously treated patients) and healthy controls in order to determine the predictive role of MxA gene expression.

Patients and methods: 81 MS patients (drug nad've: 23, treated with IFNβ: 30, treated with natalizumab: 15 and treated with glatiramer-acetate: 13) and 56 healthy controls were tested for MxA expression. The mRNA was isolated from blood samples with PAXGene system. The MxA expression was measured by real-time PCR and the degree of the expression was established by ddCT method.

Results. In the IFNβ treated group the MxA gene expression levels were increased compared to healthy controls. In this IFNβ group two distinct subgroups could be identified based on the results gained. In the first subgroup of IFNβ treated patients the mRNA level was similar to the control values measured in healthy subjects. This subgroup is supposed to produce anti-IFNβ antibodies. Meanwhile the second subgroup has significantly higher MxA mRNA expression level as compared to the control group, and these patients may not develop anti-IFNβ antibodies.

Conclusion. Monitoring the MxA mRNA expression during IFNβ treatment may reliably predict the efficiency of the therapy. Consequently, it has the potential to improve the personalized treatment of patients suffering in MS.

This work was supported by TAMOP-4.2.1/ B-09/1/KMR-2010-0001.

I.8. Interaction of orthosteric and allosteric binding sites on the alpha7 nicotinic receptor: Modulation of choline-evoked currents by PNU 120596.

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We investigated the kinetics of choline evoked currents, and the effect of the positive modulator PNU 120596 on alpha7 nicotinic acetylcholine receptors expressed by GH4C1 cells (obtained from Siena Biotech SpA) in whole-cell and outside-out patch-clamp experiments. Using a theta-tube system for rapid application of agonists, we measured the dependence of current kinetics on the solution exchange rate. By extrapolation and by kinetic modeling we estimated the intrinsic kinetics of the receptor: what would be the amplitude and kinetics of choline-evoked currents at instantaneous agonist application. In the presence of PNU 120596 the single channel mean open time is drastically prolonged, this allowed us to determine the ratio of simultaneously open channels using nonstationary fluctuation analysis. By determining the approximate number of channels in a patch, we could determine the peak open probability of 10 mM choline-evoked currents in the absence of the modulator (0.0333 ± 0.0056), as well as the open probability in the presence of 10 mM choline and 10 μM PNU 120596 (0.632 ± 0.065). We performed kinetic experiments to determine the affinity of PNU 120596 to three different conformational states of the receptor: resting state, desensitized state and a slowly developing second desensitized state. We found that PNU 120596 was ineffective at both the resting and the slow desensitized states, while it bound to the desensitised state and re-activated the receptors. We investigated the nature of cooperativity between the agonist and the modulator. We found that while the agonist increases the apparent affinity of the modulator only by inducing desensitized conformation (which is preferred by the modulator), the modulator induces a true increase of agonist affinity probably by allosterically affecting the conformation of the agonist binding site itself.

I.9. Candidate gene association study in pediatric acute lymphoblastic leukemia

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Objectives. Childhood acute lymphoblastic leukemia (ALL) is a clonal disease of lymphoblasts and the most common malignancy of all childhood cancers. In gene association studies, due to the high number of inconclusive results, it is generally accepted that the role of a gene or a genetic variation can only be acknowledged, if it is confirmed by independent studies. We selected genes that are important in transcriptional regulation and differentiation of lymphocyte progenitors. *ARID5B* (AT-rich interactive domain 5B) is a novel susceptibility factor for childhood ALL and plays a crucial role in the regulation of embryonic development, cell growth and differentiation. Ikaros proteins (Ikaros zinc finger 1 gene) are master regulators of lymphocyte development, and differentiation. Our study provides more detailed analysis to identify the causal variants of *ARID5B* and *IKZF1* susceptibility to childhood ALL.

Methods. In 543 children with ALL and 529 controls we genotyped 62 single nucleotide polymorphisms (SNPs) in 20 candidate genes and investigated whether the presence of these polymorphisms was associated with the disease. The genomic DNA was obtained retrospectively from whole, peripheral blood in remission. Genotyping of the selected SNPs was carried out by Sequenom iPLEX Gold MassARRAY technology (McGill University and Génome Québec Innovation Centre, Canada). The results were evaluated with traditional frequentist-based methods and a new one, called Bayesian network based Bayesian multilevel analysis of relevance (BN-BMLA).

Results. We found that 6 polymorphisms in 2 genes influenced the risk of ALL significantly. The most relevant SNPs were: rs10821936 ($p=7.31*10^{-5}$) in *ARID5B* and rs6964969 ($p=1.67*10^{-5}$) in *IKZF1* in the whole population and even in the subgroups. With BN-BMLA we also computed the a posteriori probability of the different association types with respect to ALL susceptibility in all sample groups. According to the BN-BMLA method the major SNPs with high posteriors (P) for strong relevance and genes were rs10821936 in *ARID5B* (P=0.76) and rs4132601 (P=0.97) in *IKZF1*. In our presentation we also show some examples how the different BN BMLA results can be interpreted.

Conclusion. Our results contribute to the understanding of genetic basis of ALL development. Better elucidation of the mechanisms through which *ARID5B* and *IKZF1* variants are involved in childhood ALL could be of great diagnostic value, and help to improve risk directed therapy and disease outcome.

I.10. CD3zeta-chain expression is regulated by tumor necrosis factor via Src-like adaptor protein dependent proteasomal degradation

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Introduction. Rheumatoid arthritis (RA) is characterized by overproduction of proinflammatory cytokines, cartilage destruction and bone erosions. T cell involvement in the pathology of RA is compelling and points to a local dysregulation of T cell function in the inflamed joint. Synovial T lymphocytes of patients with RA display severe hyporesponsiveness upon antigenic stimulation, the expression of T cell receptor (TCR) zeta chain is downregulated in these cells. The precise molecular mechanism of T cell dysfunction in RA is not understood. Src-like adapter protein (SLAP) is a negative regulator of T cell activation and acts as an adaptor between E3 ubiquitin ligase c-Cbl and the TCR complex, thus, it controls the degradation. According to very recent data SLAP deficiency can prevent zymosan-induced arthritis in mice.

Objectives. Our aim was to investigate the possible association between the elevated level of TNF and T cell hyporesponsiveness.

Methods. CD3zeta expression was determined by Western blot and real-time RT-PCR. Degradation was studied in the presence of appropriate inhibitors. SLAP expression was also assessed with Western blot, and SLAP was silenced with siRNAs. The colocalization of SLAP and CD3zeta was examined with confocal microscopy. Five out of 10 RA patients were treated with disease modifying drug, while five received anti-TNF therapy.

Results. TNF treatment (15-40 ng/ml) selectively downregulates CD3zeta chain in a dose dependent manner on human T lymphocytes, without affecting the mRNS expression. Blocking of the lysosomal compartment or selective inhibition of NF-kB fails to restore the TNF induced CD3zeta downregulation, while inhibition of the proteasome prevented the effect of TNF. Both SLAP expression and the colocalization with CD3zeta were enhanced by TNF treatment, while zeta expression was recruited by silencing SLAP. Elevated SLAP expression was observed in human RA T lymphocytes, while TNF treatment of RA T cells showed different SLAP-regulation according to the type of the therapy.

Conclusions. Our present data suggest that TNF modulates T cell activation during inflammatory processes, by regulation the amount of zeta-chain via SLAP.

II. Prediction and prevention of cardiovascular and cerebrovascular disorders

II.1. Rate of atrial fibrillation in a neurology - stroke department. Role of the neurologist in vascular prevention

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Objectives. Atrial fibrillation (AF) is the most common chronic cardiac arhythmia, and a very important risk factor for stroke (STR), too. In 20 % of the patients stroke is caused by AF. AF is associated with a five times higher risk of stroke, compared to patients without atrial fibrillation. STR has a worse prognosis when atrial fibrillation is present: the probability of death within one year is 50%. Therefore, the detection and treatment of atrial fibrillation is essential.

Methods. The authors reviewed the history of patients with AF treated in a neurology department – providing management for patients with acute stroke – in 2010. AF was present in 13,4 % of 2313 patients. These 310 patients were divided into five groups: 1) patients admitted for first acute stroke, 2) patients with previous stroke admitted for a new STR event, 3) patients with a history of STR, but admitted for a different reason, 4) transient ischemic attack (TIA), 5) patient history or reason for hospitalization did not include cerebrovascular disease. Patients were further classified into subgroups according to whether they had a previously known/ treated AF, and based on CAH_2DS_2-VASc-score.

Results. In 2,81% of patients, AF was revealed during neurological care. Groups 1 and 2 included 69,7% of patients, group 4 16,9%. The STR-negative group 5 included 10,6% of patients. The CAH_2DS_2-VASc-score was highest in group 2, and the lowest in group 5.

Conclusion. The number of patients diagnosed with AF at our department is higher then what AF's stroke promotive effect would suggest. These data suggest that neurology departments have an important role in the diagnosis of AF, thereby in efficient vascular prevention, even among patients who seem to have no vascular risk.

II.2. Chronic quercetin administration results in morphological and functional remodeling of small coronary vessels

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Objectives. Fruits and vegetables contain several types of polyphenols, quercetin and its glycosylated products seem to be the most important. Vasodilatory actions of these products have been successfully demonstrated, also in our laboratory, but no publications deal with their long term effects on microvascular networks. In the present work we targeted this topic.

Methods. Twenty four male Wistar rats were distributed into two groups. In the first group, quercetin was added to the drinking water (1 mM/L). The second group was kept in parallel without quercetin (control). After 8 weeks, the animals were sacrificed and intramural coronary arteriole segments were prepared from the heart. Segments, with around 200 μm outer diameter were cannulated at both ends put in a glass-bottomed tissue bath of a pressure angiometer device. The active and passive biomechanical properties of the vessels were measured.

Results. Arterioles from the quercetin treated group had 143±14μm inner diameter when immersed in nKR solution, at 50mmHg, while those from the control group had 185±14μm under the same conditions. The difference in spontaneous tone of the two groups proved to be significant, p=0.0389. Thus, arterioles from the treated group had higher spontaneous tone than controls (18 vs. 11%), providing a higher dilation reserve for these segments. Wall thickness was significantly larger in the quercetin treated group (24.9±1.0μm vs. 17.8±0.7μm) resulting in a reduced isobaric wall stress (17.2±1.8 kPa vs. 21.3±1.9 kPa in nKR, at 50mmHg). No significant difference in isobaric incremental elastic modulus was found between treated and control groups (2.32±0.09lg (kPa) vs. 2.47±0.04lg (kPa)).

Conclusion. It was found, that in addition to acute vasodilatory effects on resistance sized coronary vessels, demonstrated by us earlier, chronic administration of quercetin is effective in inducing morphological and functional remodeling of these vessels. This remodelling involves elevated dilation reserve of the vessel wall and reduction of wall tension without changes in stiffness of the vessel wall.

Supported by OTKA 32019, 42670, the Hungarian Hypertension Society, and the Hungarian Kidney Foundation.

II.3. Smoking habits in adults of a Hungarian suburban town

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Objectives. Our goal was to analyse smoking habits of the adult population of Budakalász Health Examination Survey (BHES) and to compare our results to a former survey performed by the Hungarian National Institute for Health Development (NIHD) and to the WHO's survey in Poland, 2010.

Methods. BHES is a population-based screening program designed to define the prevalence and risk factors of cardiovascular disease in our region. We analysed the data of 1202 adults (older than 20 years). A health interview survey contains questionnaire about previous medical history and lifestyle including precise description of smoking habits (start date, duration, cigarettes/day, quitting).

Results. The mean age was 55+/-14, 482 men (40%) and 720 women (60%) completed the survey. The rate of smokers was 15.6 %. Daily smokers are 87.7% of the total smokers, the rest smokes only occasionally. In the smoker group 3.2 % started within a year and the others have smoked for about 18 years in both genders. In the currently non-smoker group 69.9% has never smoked, 26.6% quit more than a year ago, 3,5% quit in a year. Smoking is more frequent in men (18.7% vs. 13,8%, p=0,02). Quantity of smoked cigarettes is higher in men (23.2 pack-year vs. 11.7 pack-year, p<0,001). BMI (body mass index) is elevated in most men irrespective to smoking habits, but in women elevated BMI was more frequent in non-smokers (69.2% versus 51%, p<0,001). The smoking rate was highest in the 20-30 age group (30.4%). It seems that smoking rate drops in women at the age 30-40 and it rises again in the 40-50 group. This phenomenon is observed mainly in mothers (rates by age group: 20-30: 25%, 30-40: 14.5%, 40-50: 25.3%). Above the age of 50 the proportion of smokers decreases gradually in both genders. We compared groups by different educational level: low, intermediate and high. Smoking rates in men were 28.4%, 18.5% and 10.5%, in women 17.5%, 13.8% and 10.5%, respectively. Smoking rate is lower in the highly educated group (p<0,001).

Conclusion. The results show that the prevalence of smoking is lower comparing to the data of the NIHD survey. This may be caused by the fact that our health survey is voluntary, so we could presume that the voluntaries are more health conscious than the average. The lower smoking rate in women in the 30-40 age group and the higher rate in the age group 40-50 could be explained by childbearing and post partum relapse.

II.4. The cardiovascular target-values in a primery prevention cohort based Hungarian screening programme

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Objectives. Our goal was to estimate the patients' knowledge about their own health and the effectivity of certain therapies in participants of the Budakalász Cardiovascular Health Examination Survey.

Methods. The Budakalász Cardiovascular Health Examination Survey is aimed to perform a comprehensive cardiovascular screening programme, including health questionnaire and non-invasive tests targeting the adult population (>20y) in a Central-Hungarian town. In this voluntary programme non-invasive tests (cardiac- and carotid ultrasound, resting blood pressure and ankle-brachial pressure index), venous blood biobanking and laboratory analysis is being performed.

Results. 1202 inhabitants have been screened until September 2012 (male: 482 (40.0%), avr. age: 55.0 (+/- 14.4) years. Medical history included hypertension in 563 patients (46.8%), among them 511 (90,7%) are on anti-hypertensive regime, the measured blood pressure, however was in the normal range only in 209 persons (40,9%). Hyperlipidaemia was previously known in 431 persons (35.8%), among them 219 persons (50.8%) takes statin daily. In contrast, at the time of the screening 696 persons had elevated total cholesterol level (57.9%) and only 131 person, 59.8% of the treated group had the cholesterol in the normal range. Diabetes was known in 145 persons (12.0%), and was previously unknown in 45 (4.2%) patient (Hba1c>6.5%). Pathological ankle-brachial index, an indicator of potential peripheral artery disease (PAD, normal range: 0.9-1.2) was measured in 163 persons (13.5%), but only 73 (6.0%) had PAD in the medical history.

The average Framingham risk score for cardiovascular disease for the next 10 years in the population was 16.2%, and for CV death risk was 9.8%.

Conclusion. Our screening programme is effective to detect the relevant cardiovascular risk factors and this could ameliorate the population's health-consciousness.

II.5. Is the cooperation between healthcare participants more difficult in poor social environment associated with a higher risk of stroke?

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Objectives. Low standard of living plays a crucial role in poor morbidity and mortality rates of cerebrovascular diseases. It is associated with a lower education level and health awareness of the population, and a looser relationship of people with the healthcare system. By investigating stroke patients in two districts of Budapest with extremely different life circumstances, our team has demonstrated that stroke occurs at a significantly earlier age (68,1±15,0 vs. 74,4±12,3 years) in poorer (VIII.) district. In this study, we investigated the role of the quality of relationship between stroke center, specialist outpatient department, GP-s and ambulance service.

Methods. Our anonymous database consisted of residents of the two districts who suffered a stroke in 2007. Pre-stroke history, risk factors, treatment after the cerebrovascular accident and adherence to therapy were also included. At the same time, data for 2010 were evaluated. We investigated the rate of patients referred to hospital by GP-s with a diagnosis of stroke/TIA.

Results. The rate of patients referred to hospital by GP-s or a specialist outpatient department with a diagnosis of cerebrovascular disease was significantly higher in (the wealthier) district XII with a more favourable stroke epidemiology (85,21 vs. 58,85%), whereas the ratio of referrals with completed stroke vs. TIA was significantly lower (1,20 vs. 2,96).

Conclusion. At a lower standard of living, the efficacy of cerebrovascular prevention, access to acute stroke care and patient follow-up is decreased. There is a looser relationship between the patient and his/her GP.

II.6. The Budapest Districts 8-12 project. Follow-up of stroke patients in two districts of the Hungarian capital. The role of local environmental factors in stroke epidemiology

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Objectives. The so-called Budapest Districts 8-12 project was started in 2010, in which demographic data of patients treated with acute stroke in 2007 from two districts of the capital with significantly different life circumstances (from district VIII with lower standard of living and from district XII) were collected, and clinical course of patients was analyzed. A data base was created and used for several substudies. Our team has demonstrated that the onset of stroke and associated mortality occurs at a significantly earlier age in district VIII. In this study, we investigated whether there are any differences in epidemiologic data of stroke within each district. We assumed that these differences are not due to socio-cultural factors, but to different local environmental effects.

Methods. We contacted GP-s of all stroke patients. An anonymized data form was filled out for every patient, including pre-stroke history, risk factors, therapy of cerebrovascular event, adherence to therapy, and health status in 2010. In this substudy the same demographic data were analyzed by district zones, based on postal code.

Results. Our substudy demonstrated only slight differences – mainly in stroke mortality, survival and number of risk factors – between zones of each district. These differences were less marked than in the comparison of districts.

Conclusion. The slight differences within a district may be associated with various environmental effects.

II.7. Ischemic stroke of the „hand knob" area – 6 years and 12 cases

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Background. Isolated weakness of the hand due to stroke is rare, and can be a clinically challenging syndrome.

Methods. Stroke admissions to our department with close to a thousand annual stroke cases have been carefully followed. All patients to this study were recruited prospectively during clinical rounds. Clinical signs, results of neuroimaging (brain CT or MRI) were recorded, and etiologies were evaluated by the TOAST criteria. All patients had ECG, carotid duplex sonography and transthoracic echocardiography.

Results. Between 2005 and 2011 we identified 12 patients (6 women, 6 men, mean age: 67 ± 11 years) for this study. In 11/12 patients this was a first-ever stroke. All patients presented isolated weakness of the distal upper limb. Associated signs were increased brachial and brachioradial reflexes (7/12 patients), and mostly transient upper limb numbness (8/12 patients). Small infarction in the corresponding hand knob area of the precentral gyrus was found by CT scans in 5 cases and by MRI scans in 7 patients. One patient had bilateral isolated hand palsy and the MRI confirmed symmetrical infarctions in the precentral gyri. Symptomatic high grade internal carotid artery stenosis was found in four patients, four had cerebral small vessel disease, and cardioembolic mechanism was responsible for the signs in 2 cases. Two cases had unknown etiology. Hypertension was the most prevalent vascular risk factor (10/12), followed by smoking (8/12) and hyperlipidemia (7/12).

Discussion. Small infarct in the hand knob area presenting as a distal upper limb weakness is a rare syndrome – appearing in less than 0.5% of all ischemic strokes. Associating reflex- or sensory signs may help to differentiate from peripheral neuropathy. Precise evaluation of the clinical and radiological profiles proves that a cortical lesion is responsible for the syndrome.

II.8. Ischaemic stroke in patients with Fabry Disease in the central region of Hungary

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Objectives. Fabry disease (FD) is a rare X-linked lysosomal storage disease with multiorgan involvement. Deficiency of alpha-galactosidase A activity leads to the accumulation of neutral glycosphingolipids in various tissues, particularly in blood vessels, kidneys, myocardium, cornea and in the peripheral and autonomic nervous system. The prevalence of ischaemic stroke is increased in patients with FD. Our aim was to screen the clinical and subclinical manifestations of central nervous system lesions in Fabry patients (FP) in the central region of Hungary.

Methods. Participants completed a focused questionnaire, they underwent clinical neurological examination, and quality-of-life assessments. Central nervous system involvement was examined by brain MRI/MRA or CT. Carotid duplex ultrasonography and carotid intima-media thickness (cIMT) measurement were also performed.

Results. Eight FP (4 males, 4 females) and three female carriers of the Fabry gene were recruited. FPs were collected from the central region of Hungary. Enzyme replacement therapy (ERT) was introduced in every FPs. The female carriers were free of neurological signs and symptoms. A 33 year-old male FP had severe ischemic stroke, dementia, and hearing loss before the diagnosis and the introduction of ERT. Three FPs (1 male, 2 females) have asymptomatic multiple small white matter lesions on MRI scans. Two FPs (1 male, 1 female) have increased cIMT. Normal, tortuosity-free cerebral arteries were detected by MRA.

Conclusion. Neurological complications in FD are common, complex and may be devastating, as ischaemic stroke. Early diagnosis is very important to introduce ERT as soon as possible. The long-term follow-up and treatment of patients are elementary in FD to prevent complications.

II.9. New familial vasculopathy resembling COL4A1 spectrum disease

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Background. Mutations in the COL4A1 gene encoding type IV collagen a1 chain, a component of basement membranes in various organs, have been recently identified as a cause of autosomal dominant cerebral small vessel disease (cSVD) along with a whole spectrum of widely variable manifestations in the eyes, kidneys and muscles. The most frequent phenotype in adults is characterized by subcortical intracerebral hemorrhages (ICH), diffuse cSVD and retinal arteriolar changes. HANAC syndrome (hereditary angiopathy with nephropathy, aneurysm and cramps) -a distinct phenotype- is dominated by systemic manifestations: hematuria, renal cysts, muscle cramps with elevated creatine-kinase and frequently bilateral aneurysms of the intracranial carotid artery (ICA). Hereby we present a young patient with a specific combination of these abnormalities and a seemingly positive family history but with a non-mutated COL4A1 gene.

Methods. We examined a 29 year old male patient with a history of recurrent minor ICHs causing mild motor and sensory hemisyndromes on alternating sides. His father had a major subarachnoid hemorrhage from an ACoA aneurysm at the age of 23 years. General physical and neurological examination, brain MRI (with T1, T2, FLAIR, GRE, DWI, MRA), laboratory tests, ophthalmologic examination and genetic testing for the entire COL4A1 gene have been performed.

Results. MRI showed subcortical ICHs of different age; severe cSVD with diffuse white matter damage and multiple cerebral microbleeds; and small aneurysms of the intracranial ICA bilaterally. No abnormality was seen on fundoscopy. Laboratory workup revealed significant microscopic hematuria and elevation of CK. Genetic testing for COL4A1 was negative.

Conclusions. Our patient showed an unprecedented combination of manifestations of the COL4A1 disease spectrum without mutation in the gene. Further genetic studies of the patient and family members are planned to search for a possibly new genetic cause of a systemic vasculopathy.

II.10. Human Embroynic stem cell-derived endothelial cells: a Future source of new vessels?

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Objectives. Cardiovascular derivatives of stem cells are promising candidates for future personalized tissue-specific therapy, disease modelling and drug testing. Various stem cell types are being investigated in cardiovascular field.

The aim of this study was to develop human embryonic stem cell derived endothelial cells (hESC-ECs) *in vitro* and to characterize their gene expression and immunocytochemistry profile.

Methods. Undifferentiated H7 hESCs were differentiated into mesodermal and endothelial lineage. After two weeks CD31-positive cells were separated from the differentiating culture using FACS (fluorescence activated cell sorting). HUVECs were used as positive control. The hESC-ECs along with Matrigel extracellular matrix were transplanted subcutaneously into 3 months old athymic nude rats.

Results. hESC-ECs were stained positive for endothelial markers such as CD31, vWF, ve-cadherin, formed capillary-like tubules on Matrigel, and took up ac-LDL. Investigating the gene expression profile with quantitative PCR, hESC-ECs expressed a range of endothelial genes. Both arterial (EphrinB2 and Notch1-2) and venous (EphB4 and FLT4) genes were expressed, suggesting the presence of different subpopulations in culture. In vivo, two weeks after the implantation, immunohistochemistry proved that hESC-ECs form capillary-like structures. The hESC-ECs remain viable in culture after re-isolated from rats. The mRNA levels of angiogenesis genes were significantly increased during engraftment of hESC-ECs (angiopoietin mRNA levels after implantation: 86.23±26.34-fold increase versus preimplantation; apelin mRNA levels after implantation: 1197.42±703.75-fold increase versus preimplantation, n=5, both p<0.05).

Conclusion. Our data show that hESC-ECs form capillary-like structures both in vitro and in vivo. In vivo hESC-ECs continue their development and may support angiogenesis. The separation of arterial and venous subpopulation of hESC-ECs warrants further investigations. Use of hESC-ECs may provide us with a tissue-specific proangiogenic therapy in cardiovascular diseases.

This study was supported by Hungarian Scientific Research Fund (OTKA MB08A 81237), Semmelweis University Kerpel-Fronius Program Wekerle Project, TÁMOP-4.2.2/B-10/1/2010-0013

II.11. Time course, distribution and cell types of induction of transforming growth factor betas in a rat model of ischemic stroke

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Objectives. Transforming growth factor betas (TGFβ1-3) form a small group of related proteins involved in the regulation of cellular proliferation, differentiation, and survival of various cell types. TGF-beta injection decreased while its antagonism increased the infarct size following middle cerebral artery occlusion (MCAO) in rats supporting its neuroprotective function.

Methods. The present study describes the induction of TGFβ1-3 in the rat after focal ischemia at 3h, 24h, 72h and 1 month after transient (1h) or permanent (24h) MCAO using in situ hybridization histochemistry and quantitative analysis. Double labeling with different markers was used to identify the localization of TGFβ mRNA relative to the penumbra and glial scar, and the types of cells expressing TGF-betas.

Results. TGF-beta1 expression increased 3h after MCAO in the penumbra and was further elevated 24h after MCAO. TGFβ1 was present mostly in microglial cells but also in some astrocytes. By 72h and 1 month after the occlusion, TGFβ1 mRNA-expressing cells also appeared in microglia within the ischemic core and in the glial scar. In contrast, TGFβ2 mRNA level was increased in neurons but not in astrocytes or microglial cells in layers II, III, and V of the ipsilateral cerebral cortex 24h after MCAO. TGFβ3 was not induced in cells around the penumbra. Its expression increased in only a few cells in layer II of the cerebral cortex 24h after MCAO. The levels of TGFβ2 and -β3 decreased at subsequent time points. Permanent MCAO further elevated the levels of all 3 subtypes of TGFβ-s suggesting that reperfusion is not a major factor in their induction. TGF-beta1 did not co-localize with either Fos or ATF-3, while the co-localization of TGFβ2 with Fos but not with ATF-3 suggests that cortical spreading depolarization, but not damage to neural processes, might be the mechanism of induction for TGFβ2.

Conclusion. These data suggest that TGFβ-s are produced in different cell types at different time points after an ischemic attack, which may contribute to spatially and timely regulated neuroprotective and inflammatory processes.

Support was provided by Hungarian Scientific Research Fund Research Grants OTKA NNF2 85612

II.12. Remodeling of the rat saphenous vein wall and its tributary network after partial occlusion. A quantitative histochemical evaluation

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Background, aims. Gravitational load and flow disturbance acting in concert is in the background of the human chronic venous disease of the leg. Remodeling processes in the wall of the rat saphenous vein main branch and its tributary network were studied in our lab after chronic partial occlusion. Our present target was a quantitative histochemical description of how the expression of some typical proteins with key functions in vascular biomechanics is altered. My specific task was to develop a computational technique for quantitative evaluation of histochemical sections.

Methods. A 500 μm chronic clip was put around the main saphenous vein of young male rats. 4-8-12 weeks later tissue samples were taken from the thigh. To follow wall and network remodeling, smooth muscle actin (SMA immuno-histochemistry with DAB), elastin (RF) and collagen (Picro-Syrius) stainings were used. In addition, cellular division processes were also spotted (Ki67, DAB). Automatically stained, scanned, digitalized tissue sections were examined. The territory of the brown color (SMA and Ki67, DAB) had to be defined by us. We tried more opportunities for evaluation. An image analyzing program was used to get pixel matrices of the pictures in red (R) and blue (B) colors. The inequity of R/B>1.2 identified the brown spots with sufficient exactness. To improve the specificity and sensitivity of pixel identification, a series of light brown and dark brown spots were chosen by the eye. A MATLAB program was written that could mark and identify all the tones between the two border colors defined by us. To identify the two border colors was a long iteration process. More than 100 opportunities had to be tried out of the potential 1 million options to get the most accurate results. After that the purple territories of the RF elastica stain were identified in a similar manner.

Results and Conclusion. The quantitative alterations in contractile and connective tissue protein were in accord with earlier biomechanical (pressure angiography) and anatomical (casts) observations. Ki67 activity and newly formed smooth muscle actin marked the site of collateral network development. A delayed appearance of elastic tissue in developing vessels while being in the process of morphological flow dilation, might expose them to gravitational stress inducing pathologic varicous remodeling of their walls.

Supported by OTKA TO32019, The Hungarian Hypertension Society and the Hungarian Kidney Foundation.

III. The genetic background of rare diseases

III.1. Cerebrotendinous xanthomatosis with the c.379C>T mutation in the CYP27A1 gene associated with premature age-associated limbic tauopathy

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Objective. Cerebrotendinous xanthomatosis is a rare autosomal recessive disease associated with mutations in the mitochondrial *CYP27A1* gene. Our aim is to report the clinical, biochemical and neuropathological features of two individuals homozygous for the mutation c.379C>T (p.R94W).

Methods. **C**linical, biochemical, genetic and neuropathologic examination and magnetic resonance imaging. The patients are 43 (patient 1) and 46-year-old (patient 2) brothers with similar progressive and multisystemic neurological symptoms including cognitive decline.

Results. Plasma cholestanol concentration of patient 2 (76.4 μmol/L) was highly above the normal range (2-12.6 μmol/L) and was in the diagnostic range (36-102 μmol/L). Patient 2 was homozygous for the mutation c.379C>T (p.R94W). Neuropathological examination of Patient 1 revealed leukodystrophy and xanthomatous changes, furthermore, systematic evaluation of neurodegeneration-related proteins showed neuron-predominant tau pathology predominating in the limbic system that was compatible with the age-dependent argyrophilic grain disease.

Conclusion. Our observations implicate a role for the deficiency of sterol 27-hydroxylase in the development of a limbic predominant tauopathy and argues that premature ageing of the brain associates with the phenotype of argyrophilic grain disease, which is observed only in the elderly.

Supported by the European Commission's 7th Framework Programme under GA No 278486, "DEVELAGE", TÁMOP-4-2-1/B-03/1/KMR-2010-001 and TÁMOP-4.2.2.A-11/1/KONV-2012-0031.

III.2. Genetic basis and phenotype of early onset and familial Parkinson's disease in the Hungarian population

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Objectives. To evaluate the phenotype and the frequencies of mutations in PRKN, LRRK2, PINK1 and SNCA genes in patients with early onset Parkinson's disease (PD) in Hungary.

Methods. 164 PD patients participated in the study, of whom genetic examination was performed for LRRK2 p.G2019S mutation hotspot at 118 patients using restriction fragment length polymorphism technique, and 62, 44, 34, 13 patients underwent sequence analysis with Sanger's methodology for PRKN, LRRK2, PINK1, SNCA genes respectively. All patients were originating from Hungary, and underwent detailed neurological examination. The selection criterias were positive family history for PD or young age at onset. We've recorded detailed datas from 144 patients in the webdatabase of the NEPSYBANK biobank system.

Results. The mean age at onset was 46 years. Family anamnesis was positive at 31 patients. In the LRRK2 gene the **p.G2019S** substitution in 118 patients was not present. Sequencing this gene 17 intronic variant, 1 very likely pathogenic splice site mutation, and 6 exonic polymorphisms were detected. Two intronic SNPs (single nucleotide polymorphism) were also detected in a cohort of german patients. The **rs33958906** SNP, detected in two patients, had a high score by the protein prediction software PolyPhen2, however association analysis, performed by others, did not indicate pathogenicity. The **rs1156418** SNP was present in 4 patients homozygously. Previously it showed associaton with increased risk of PD in Asian population, but not in European. In the PRKN gene we've found 1 pathogenic (**p.R402C**), 1 exonic, and 4 intronic variants. In the PINK1 gene we've detected 6 intronic and 4 exonic, and in the SNCA gene 2 intronic polymorhisms. The detected SNPs in the PINK1 gene, **rs45530340**, and **rs3738136** were reported in Chinese han population as risk factors for PD.

Conclusions. We've detected 1 possibly new pathogenic substitution, 3 substitutions associated with PD in Asian population, and 2 substitutions predicted probably damaging in silico. Our data suggest that substitutions in the LRRK2 gene are frequent among early onset and familial Parkinson's dis-

ease patients in Hungary. The very common LRRK2 point-mutation (p.G2019S) was not present in the Hungarian population. The genetic etiologic factors and phenotypic characteristics in PD need to be further studied to evaluate the pathogenesis, genetic risk factors shared with Asian populations, and to stratificate the patient population for personalised therapy.

This work was supported by BIOINF009-TÉT_10_1_2011_0058.

III.3. The genetic background of the hereditary neuropathies in Hungary

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Objectives. Charcot-Marie-Tooth neuropathies (CMT) are a clinically and genetically heterogeneous group of rare inherited neuropathies. Their prevalence is 1:2,500. Over the past years, research on Charcot-Marie-Tooth neuropathies (CMT) has undergone a change from largely phenomenological descriptions to molecular genetics and functional studies. Until now 40 CMT genes and over 50 additional loci have been identified.

Aims. In this study we analysed the mutation frequencies of the most common genes (PMP22 duplication, deletion, MPZ, MFN2, Connexin 32 and EGR2 mutations) in an electrophysiologically well-characterized group of patients. In patients with gypsy origin the R148X mutation in the NDRG1 gene, which is typically alteration in LOM type of neuropathy, and the IVS6 + 389C>T founder mutation, which is a frequent cause of congenital cataracts, facial dysmorphism and neuropathy (CCFDN), were also tested.

Patients and Methods. 450 (256 male and 194 female) clinically and electrophysiologically characterized patients with neuropathy have been investigated. The mutation analysis was performed by real-time PCR, PCR, RFLP and bidirectional sequencing.

Results. Family history was positive in 40 index patients. The electrophysiological examinations of the patients showed axonal, demyelinating, intermedier and undeterminate type of neuropathy in 41 percent, 21 percent, 28 percent and 10 percent of cases, respectively. During the quantitative analysis of the PMP22 gene, 84 duplications and 65 deletions have been observed. Pathogenic mutation in the MPZ gene was detected in 5, in the EGR2 gene in 2, in the MFN2 gene in 5 and in the Connexin32 gene in 4 cases. Regarding the investigations of patients with gipsy origin, LOM type of neuropathy was present in 8, while the CCFDN mutation in 4 cases. In one family the PMP22 deletion and a pathogenic EGR2 mutation were both present.

Conclusion. Pathogenic mutation has been identified in 37.5% of the investigated patients. In our presentation we would like to emphasize that mutation analysis of clinically and electrophysiologically well-characterized neuropathic patients reveals the genetic etiology in a relatively large percent of cases.

This work was supported by TAMOP-4.2.1/ B-09/1/KMR-2010-0001.

III.4. GJB2 gene mutation in patients with nonsyndromic hereditary hearing loss

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Genetic testing is a powerful tool for the etiologic diagnosis of hearing loss. In our patients with sensorineural hearing loss 35DELG mutation of the GJB2 gene was tested to provide prognostic information about the hearing loss and aid in the establishment of treatment. A great part of autosomal recessive nonsyndromic hereditary hearing loss is caused by GJB2 and GJB6 gene mutations. These genes encode the protein connexin 26 and 30. The onset of severe to profound sensorineural hearing loss is pre-lingual, results serious default in speech understanding and production. Early diagnosis and treatment (cochlear implantion) is substantial for our child patients to provide speech learning and education in integrated kindergarten and schools.

Among our 44 pre-lingual patients with profound sensorineural hearing loss 17 had homozygous, 12 heterozygous 35DELG mutation and in 15 patients this mutation was not detected. Hearing of these patients was rehabilitated with cochlear implantation, so genetic testing played an important role in the early diagnosis.

III.5. Premature ovarian failure (POF/POI) and detection of CGG repeat number in FMR1 gene promoter region by Repeat Prime PCR (RP-PCR) method

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Background. The premature ovarian failure (POF), also called primary ovarian insufficiency (POI) in women in fertile age can significantly influence the reproductive chances.

Objective. Introduction of a molecular genetic test method in the routine diagnostics of the POF/POI patients by which accurate and reliable results can be predicted concerned about the disease. The main research targets in patients of premature ovarian failure are the detection of the increase in the number of (CGG)n repeats in the FMR1 gene promoter region, confirmation of the premutation status and determination of the trinucleotide repeat number.

Patients and methods. There was molecular genetic testing in 52 patients of the 1st Department of Obstetrics and Gynecology among the patients examined with suspected POF. Early ovarian depletion criteria were consistent with international protocols: secondary amenorrhea, ovarian failure up to the age of 40 years, levels of FSH≥40 IU/L in two different measurements, and low estrogen levels. As a first step the patients were tested by Southern blot analysis and we subsequently investigated the CGG trinucleotide repeats in the FMR1 promoter region by hybridization with radiolabeled DNA probes. In all cases, which confirmed the pre-mutation status by the Southern blot, we completed the so-called Repeat Prime PCR (RP-PCR) method used to determine the exact number of CGG repeats.

Results. In 6 cases out of 52 patients we could verify the increase of the CGG repeat number, it is nearly 11% of the cases. In one cases we found mosaic form.

Conclusions. The genetic examination of the premature ovarian failure is very important for the patient and her family also, because the genetic results have serious influence on the reproductive possibilities and family planning of the premutation carriers.

III.6. Newborn metabolic disease in adulthood: Phenylketonuria – case report

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Objectives. Phenylketonuria (PKU) is a recessively inherited metabolic disorder, with an incidence of 10-15/100.000 in Hungary. Newborn are routinely screened for the disease on their 3. day of life among 25 other diseases. Thanks to the newborn screening programme and adequate treatment (lifelong diet), PKU is rare in adults. Aim of the poster is to highlight the importance of metabolic screening in adults, who present with mental retardation and neurological involvement, and brain MRI reveals diffuse white matter lesion.

Methods and Results. 39-year old male was seen at our outpatient clinic for diffuse white matter lesions. Family history was unremarkable for inherited metabolic diseases. Symptoms started at the age of 8 years as bilateral hand-tremor and learning difficulties, followed by anxiety and erectile dysfunction in early adulthood. Previous investigations revealed decreased IQ (98), anxiety, elevated serum prolactine. Brain MRI and MR spectroscopy detected diffuse white matter lesion, neither typical for demyelinating disorders nor for cerebrovascular events. Metabolic screening revealed high serum concentration of phenylalanin, suggesting PKU.

Conclusion. Metabolic disorders such as phenylketonuria should be considered as differential diagnosis in case of neuropsychological problems and white matter lesions in adults. Early dietary restriction, vitamin and amino acid supplementation except phenlyalanin is the cornerstone of PKU treatment. Further investigation is needed to follow-up the patient and white matter lesions.

This work was supported by
BIOINF009-TÉT_10_1_2011_0058.

III.7. UMOD mutations in familial juvenile hyperuricaemic nephropathy

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Familiar juvenile hyperuricaemic nephropathy is a chronic tubulointerstitial nephropathy characterized by autosomal dominant inheritance, medullary cysts, association of gout and progression to end-stage renal disease (ESRD) between the 3th and the 7th decades of life. Mutations of the *UMOD* gene are responsible for 30-70% of the cases. The majority (95%) of the mutations are missense mutations located in exons 4 and 5.

The mutation screening of the *UMOD* gene has been introduced by the direct sequencing of exons 4 and 5. Seven families have been tested.

Mutations were found in two families. Both of them carried an unknown heterozygous missense mutation. The first, a c.179G>A variant affected a glycine conserved even in arthropods (p.Gly60Asp). The second variant, c.742T>C, affected a cysteine conserved in vertebrates (p.Cys248Arg). Both amino acid changes were predicted to be pathogenic by SIFT and Polyphen-2 softwares. The mutations segretated with the disease, and none of them were found in 100 ethnically-matched controls. Three family members in the first family have developed ESRD, between the ages of 35 and 40. In the second family, nine family members have progressed to ESRD between the ages of 24 and 62 years. An asymptomatic family member, considered to be a potential donor, was tested on his request and found to carry the mutation.

In accordance with the literature data, *UMOD* mutations are missense mutations unique for a single family. Their identification helps the differential diagnosis of chronic nephropathies, and the assessment of potential kidney donors.

This work was supported by
TÁMOP-4.2.1/B-09/1/KMR-2010-0001 and by János Bolyai Scholarship (KT).

III.8. Analysis of the mitochondrial DNA and it's mutational „hot spots" in patients with mitochondrial disease

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Objectives. The mutation rate of the maternally inherited mitochondrial genome is ten times larger than the nuclear genome due to the lack of an efficient DNA repair system. The mitochondrial DNA (mtDNA) has several mutational hot spots, e.g. in the RNA genes or in the genes encoding subunits of NADH. Our aim was to compare methods used in the diagnosis of mtDNA diseases.

Methods. PCR-RFLP was used to detect known pathogenic mutations (MERRF, MELAS, NARP, LHON). Genes encoding tRNA's and their boundary regions were sequenced bidirectionally (ABI Prism 3500). In case of 17 patients, the resequencing of the complete mitochondrial DNA (MitoChip v.2.0, Affymetrix) was also done. "Common" deletions and multiple deletions were determined by long PCR technique.

Results. MERRF (A8344G) mutation screening of 890 patients was performed, out of which the A8344G substitution was found in 10 cases. The pathogenic A3243G substitution of MELAS (A3243G) was also detected in 10 patients from the investigated 1001 patients. NARP syndrome is caused by two mutations (T8993G, T8993C). The T8993G mutation was found in two cases and the T8993C mutation was detected only in one case out of 890 patients. The three point mutations described in the background of LHON (G3460A, G11778A and T14484C) were investigated in 65 patients. The G11778A mutation was found in five cases, while we could not detect the other two mutations in our cohort.

We have found two heteroplasmic pathogenic mutations in the tRNA: the $tRNA^{Asn}$ G5698A and $tRNA^{Leu1}$G4298A. From the 17 patients in whom the entire mtDNA was sequenced, a heteroplasmic pathogenic mutation was found in one case (A12770G).

We investigated 981 patients for the "common" deletion and multiple deletions. We have found multiple deletions in 61 cases, while the "common" deletion was identified in 146 patients. In 31 cases we have not found mtDNA deletions in the blood sample, but could detect deletions in the muscle sample of which single deletions were found in 16 cases and multiple deletions in 15 patients.

Conclusion. The detection of mtDNA diseases is highly method-dependent. The different heteroplasmic rates and the postmitotic nature of the different tissues strongly influence the probability of whether the presence of the mtDNA mutation can be detected in the given tissue.

This work was supported by
TAMOP-4.2.1/ B-09/1/KMR-2010-0001.

III.9. POLG1 gene analysis in patients with multiple mtDNA deletions

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Introduction. Mutations present in nuclear genes responsible for the intergenomial communication may result in multiple deletions of the mtDNA. In many of the cases the mitochondrial polymerase gamma (POLG1) gene is affected by these deletions. To date a wide variety of clinical syndromes have been observed due to POLG1 mutations. Relying solely on the clinical phenotype it is challenging to determine whether the mutations of POLG1 gene is the underlying cause of a clinical syndrome.

Aims. The investigation of the frequency of POLG1 gene mutations in patients with mitochondrial disease and analyse the phenotype-genotype correlation.

Methods. The mtDNA deletions were investigated by long PCR, performed on DNA sample isolated from postmitotic muscle biopsy specimen. The POLG1 gene was sequenced bidirectionally.. Segregation analysis of the observed mutations was also performed.

Results. 430 patients with multisystemic syndromes were tested for POLG1 gene mutations. Among them, in 50 patients multiple deletions were detected in the mitochondrial DNA. Muscle biopsy showed dysfunctions characteristic of mitochondrial diseases. In the POLG1 gene analysis showed 31 different alterations (8 missense, 3 samesense mutations, 3 intronic frame shift, 16 intronic substitutions and 1 trinucleotide expansion). Some of these alterations were pathogenic, genetic modifier factor, associated with valproate toxicity. In a single case the coexistence of one pathogenic mutation and a modifier factor was detected. During segregation analysis existence of pathogenic mutation characteristic for the given family could be showed in further eight family members

Conclusion. In our cohort using the above mentioned strategy in 7% of the patients pathogenic mutations in the POLG1 gene were detected. We assume that in patients with Mendelian inherited mitochondrial diseases mtDNA isolated from muscle biopsy specimen multiple deletion analysis may provide useful information on proper genetic diagnosis.

In the patients with multiple deletions and positive family anamnesis we recommend to perform POLG1 gene sequence analysis. In the case of negative results further investigation of nuclear genes responsible for intergenomial communication is deemed necessary.

This work was supported by
TAMOP-4.2.1/ B-09/1/KMR-2010-0001.

III.10. Mutation rate of NPHS2 and WT1 in Hungarian children with steroid-resistant nephrotic syndrome

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Objectives. Steroid-resistant nephrotic syndrome (SRNS) represents a common cause of ESRD in childhood. The differentiation of its genetic and immune form is essential for the therapy, but only possible by mutational screening of SRNS genes. The most frequently mutated gene is *NPHS2*. Mutations of *WT1* cause isolated SRNS in girls and associated genital abnormalities in boys. Our aim was to introduce the mutational screening of these two genes.

Methods. Out of a cohort of 49 Hungarian unrelated patients with SRNS and/or nephrotic-range proteinuria, 40 patients were screened for *NPHS2,* and 23 (15 girls) for *WT1* mutations.

Results. *NPHS2* and *WT1* mutations were found in 11/40 and 4/23 patients, respectively. Five of the 11 patients with *NPHS2* mutations were either homozygous or compound heterozygous for truncating mutations or the severe p.R138Q mutation. Three patients carried the p.V290M mutation. While the 5 patients with severe mutations progressed to ESRD before the age of 10 years, two patients carrying p.V290M showed an extremely mild clinical course, as they have not developed nephrotic syndrome below the age of 18 years. Haplotype analysis of the p.V290M alleles revealed that a founder effect can explain its high frequency in Hungary.

Four of the 11 patients with *NPHS2* mutations were found to carry only a single heterozygous mutation. Their pathogenicity is indeed questionable, as one of the patients was in complete remission after cyclosporine therapy and a second patient was also found to carry a heterozygous splice mutation in *WT1* (c.1228+4C>T), which can entirely explain his phenotype. In addition, *WT1* mutation was found in a boy with Denys-Drash syndrome and in two girls with isolated SRNS. Immunosuppressive treatment was stopped in all patients with identified mutations.

Conclusion. The mutation rate of *NPHS2* and *WT1* in Hungary is similarly high to that of other cohorts. Their identification is therefore essential for the personalized therapy. We propose that not only the *NPHS2* p.R229Q variant, but also the p.V290M mutation should be screened in Central European patients with an onset of SRNS below the age of 30.

This work was supported by TÁMOP-4.2.1/B-09/1/KMR-2010-0001 and by János Bolyai Scholarship (KT).

III.11. Mutations of NPHP1 are responsible for 60% of the nephronophthisis cases in Hungarian children

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Objectives. Juvenile nephronophthisis (NPH), an autosomal recessive chronic tubulointerstitial nephropathy, is responsible for 6-10% of ESRD in childhood. Genetically it is highly heterogeneous, with more than 10 genes identified thus far. Out of them, *NPHP1* is the most commonly mutated secondary to its frequent mutation, a deletion of the whole gene. Our aim was to introduce the mutational screening of *NPHP1* in Hungary.

Methods. Within the cohort of 116 Hungarian children and young adults with cystic kidney disease or chronic renal failure without hematuria, proteinuria or urinary tract malformation, 25 patients from 21 families have been diagnosed with NPH, based on clinical symptoms, recessive inheritance and renal morphology. Six cases were syndromic with either associated neurologic symptoms (Joubert syndorme – JS), retinopathy (Senior-Loken syndrome – SLS) or hearing impairment. No family was consanguineous. *NPHP1* homozygous deletion was screened first by the amplification of exons 7, 19, introns 2, 18 and a control region. Second, a heterozygous deletion was screened by MLPA. Once a heterozygous deletion identified, the 20 coding exons and the intronic junctions were directly sequenced.

Results. Thirteen of 21 families carried *NPHP1* mutations (62%). Nine of them harboured a homozygous deletion of the whole *NPHP1* gene. Four patients carried a heterozygous deletion associated with either a point mutation (c.489delT, p.Phe163Leufs*19), a short deletion (c.84_87delTTCT, p. Ser29Argfs*4) or a deletion of exons 18-20. All these associated mutations were novel. The clinical phenotype was particular in three cases. The retinopathy of a patient with SLS is extremely severe: his visual acuity at the age of 29 years is lost one one side and 0,25 on the other. One patient with JS was treated with autism, which is not exceptional in JS, but has not been reported in patients carrying *NPHP1* mutations. Finally, one child is treated with sensorineural hearing impairment which is rarely associated to NPH.

Conclusion. The mutation rate of *NPHP1* is high in Hungarian children with nephronophthisis. Its screening thus greatly helps the differential diagnosis of cystic kidney diseases. The associated extra-renal symptoms can be more extreme than previously found.

This work was supported by TÁMOP-4.2.1/B-09/1/KMR-2010-0001 and by János Bolyai Scholarship (KT).

III.12. The first family with NPHS2 homozygous p.R229Q and family members without steroid-resistant nephrotic syndrome: the missing evidence

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NPHS2 is the most frequently mutated gene in steroid-resistant nephrotic syndrome (SRNS) in both child- and young adulthood. Its mutations are inherited in an autosomal recessive fashion. Patients with *NPHS2* mutations typically develop SRNS during the first decade of life. A later onset can be associated with the c.686G>A (p.R229Q) variant. The p.R229Q allele has been reported to be pathogenic in association to an *NPHS2* mutation. However, it is difficult to assess the pathogenicity of homozygous p.R229Q. Several studies have identified homozygous p.R229Q in patients with late-onset SRNS and reported these patients as *NPHS2*-associated, suggesting that homozygous p.R229Q can cause SRNS in itself.

A 37-year-old patient with focal segmental glomerulosclerosis progressing to end-stage renal disease at the age of 33 was found to carry the *NPHS2* p.R229Q variant in homozygous state by direct sequencing. The allele frequency of p.R229Q was ascertained in 212 Hungarian controls and found to be 3% (13/424), giving a chance of 1 in 1100 for finding a homozygous p.R229Q accidentally. However, both the father and the brother were homozygous for p.R229Q with no proteinuria at the age of 59 and 40 years (<2mg/m^2/hour), proving the lack of its pathogenicity.

Based on the associated ocular involvement, the causative role of the *LAMB2* gene was considered. An unreported heterozygous non-silent variant c.724A>T (p.I242F) was found not only in the index patient but also in the father and brother. Nevertheless, the brother and the patient were haploidentical for both *LAMB2* alleles, excluding the pathogenicity of *LAMB2* as well.

A detailed ophthalmological evaluation has been performed and a morning glory anomaly was detected. Therefore, the *PAX2* gene has been sequenced, and a de novo, truncating mutation (c.76dupG) was detected. This mutation explained the phenotype of the patient, proving again the lack of pathogenicity of homozygous p.R229Q. The identification of the causative mutation made possible to personalize the immunosuppressive regime after renal transplantation.

We conclude that the homozygous variant p.R229Q should not be considered pathogenic in late-onset SRNS.

This work was supported by TÁMOP-4.2.1/B-09/1/KMR-2010-0001 and by János Bolyai Scholarship (KT).

III.13. Asian-specific mitochondrial genom polymorphism (9 bp deletion) in the Hungarian population

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Objectives. Several polymorphisms in the mitochondrial genome have population-genetically and anthropologically interest. The 9 bp deletion is anthropological marker for people of East-Asian origin.

Methods. The mitochondrial A8344G mutation was investigated by PCR-RFLP, performed on DNA samples isolated from blood and postmitotic muscle biopsy specimens. The mitochondrial COII/tRNS^{Lys} and hypervariable regions were sequenced bidirectionally.

Results. From 890 patients we found 13 cases with 9 bp deletion (CCCCCTCTA) in the mitochondrial hipervariable non-coding region. Among them in 11 cases the 9 bp deletion was present with homoplasmic C8270T substitution. Their coexistence determines the M haplogroup. In one family (3 patients) beside these alterations we found a new heteroplasmic A8332G mutation in the tRNA^{Lys} gene, wich was absent in 150 normal controls.

Conclusion. M haplogroup is in European populations very rare. It is mainly present in Asia, America and Australia, because of the human migration directions, these populations migrated Eastwards. The frequency of 9 bp deletion in the Hungarian population is 1,5%. This polymorphism can be explained by the Westward migration of Hungarians from Siberia (in the matriarchal lineage). The deletion induces instability of this mitochondrial DNA-region like enough, and provoke the conformation other pathogen mutations.

This work was supported by
BIOINF009-TÉT_10_1_2011_0058.

IV. Biomarker research

IV.1. The impact of SUCLA2 mutations on mitochondrial phosphorylation potential

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Objectives. Succinyl-CoA ligase catalyzes the only reaction in the mitochondrial matrix capable of ATP (or GTP) generation by substrate-level phosphorylation in the absence of oxygen. SUCLA2 encodes for the subunit that mediates ATP formation in the beta isoform of this enzyme.

Methods. Fibroblasts from patients suffering from four different splice mutations and one with a complete deletion of the SUCLA2 gene were collected, and in situ mitochondrial phosphorylation potentials were compared to those obtained from age-matched individuals. Electron transport of in situ mitochondria was inhibited by either rotenone or stigmatellin, and the directionality of the adenine nucleotide translocase was probed by recording the effect of its inhibitor, bongkrekic acid while measuring mitochondrial membrane potential in intact cells. Furthermore, adenine nucleotide exchange rates as a function of mitochondrial membrane potential were measured in permeabilized cells.

Results. Fibroblasts obtained from patients suffering from SUCLA2 mutations exhibited premature reversals of the mitochondrial adenine nucleotide translocase, as well as diminished adenine nucleotide exchange rates as a function of mitochondrial membrane potential, compared to age-matched individuals.

Conclusions. The ATP-forming succinyl-CoA ligase plays a critical role in the maintenance of adenine nucleotides in the mitochondrial matrix, and as such may prevent a bioenergetic collapse in the absence of oxidative phosphorylation.

IV.2. Exclusive neuronal expression of SUCLA2 in the human brain

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Objectives. SUCLA2 encodes for the ADP-forming, β-subunit (A-SUCL-β) of succinyl CoA ligase, an enzyme of the citric acid cycle. Mutations in SUCLA2 lead to a mitochondrial disorder, stemming from the depletion of mitochondrial DNA. This mitochondrial disorder manifests as neonatal encephalomyopathy associated with dystonia, deafness and pronounced lesions in the basal ganglia. Despite that a SUCLA2 gene defect results in distinct brain pathology, precise localization of the encoded protein has never been investigated.

Methods. A-SUCL-β immunoreactivity in human brains was examined by immunohistochemistry.

Results. Immunoreactivity of A-SUCL-β in the human cerebral cortex was present exclusively in neurons, identified by their morphology and visualized by double labelling with a fluorescent Nissl dye. The A-SUCL-β immunoreactivity co-localized >99% with that of the d subunit of the mitochondrial F0-F1 ATP synthase. Specificity of the anti-A-SUCL-β antiserum was verified by the absence of labelling in fibroblasts from a patient with complete deletion of SUCLA2. A-SUCL-β immunoreactivity was absent in glial cells, identified by antibodies directed against the glial markers GFAP and S100B.

Conclusions. Our work establishes that SUCLA2 is expressed exclusively in neurons in the human cerebral cortex. Therefore, all encephalopathic features of the disease emerging by mutations in this gene originate solely from the neuronal cell population.

IV.3. Calcium indicator loading of different cell types in the organ of Corti by single-cell electroporation in hearing mice

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Objectives. Hearing loss is the most common human sensory deficit. Impairments of the cochlea or the auditory nerve, i.e., sensoneuronal hearing losses (SNHLs; e.g. presbycusis or noise-induced hearing loss) are not curable by pharmacological tools at present. Invention of effective drug therapy needs deepening of our pathophysiological knowledge at the cellular level. However, investigations are set back largely by the paucity of applicable preparations from mice of mature hearing. Hemicochlea preparation from hearing mice (>P15) meets the requirements, but have not been used for functional imaging studies so far. Our aim was to set up a reliable Ca^{2+} indicator dye loading method suitable for investigating mechanism of SNHLs in different cell types of the organ of Corti by functional imaging in the hemicochlea.

Methods. Cochlea of P15-27 mice were cut in half by a vibratome. The preparation was fixed in an epifluorescent microscope and perfused with oxygenated buffer solution. The selected and identified cells were touched by the fluorescent dye filled glass pipette guided by a micromanipulator. The indicator was injected into the cells by a current pulse (electroporation). We tested Oregon Green 488 BAPTA- and the ratiometric dye Fura-2. Fluorescence changes were followed by a cooled CCD camera based imaging system.

Results. We defined the optimal dye concentration in the pipette (1 mM), the amplitude (10 μA) and width (10 ms) of the square-wave current pulse and parameters of the pipette (4-6 MOhm). We could fill up 2-6 cells in parallel in one organ of Corti, including both sensory- and supporting cells. The background staining remained low. Although in different distribution, but all cell types of the organ of Corti have purinergic receptors (P2X and P2Y). For validation of the method, we evoked intracellular Ca^{2+} responses in the cells by ATP application. The responses were ATP concentration dependend and repeatable several times.

Conclusion. We set up a method of single-cell electroporation of Ca^{2+} indicators in hemicochlea preparation of hearing mice for the first time. The method supports high spatial- and temporal resolution functional imaging in both wild type and genetical modified mice. Since it is based on a cochlear preparation from mice with mature hearing, the patomechanism of adult SNHLs can be studied without contamination of developmental changes.

This work was supported by the NIH-ANR Collaborative R&I Programme, TÉT_10-1-2011-0421.

IV.4. The negative impact of alpha-ketoglutarate dehydrogenase complex deficiency on matrix substrate-level phosphorylation

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Objectives. Provision of succinyl-CoA by the alpha-ketoglutarate dehydrogenase complex (KGDHC) is essential for generation of matrix ATP (or GTP) by substrate-level phosphorylation catalyzed by succinyl-CoA ligase. A decline in KGDHC activity has been associated with neurodegeneration.

Methods. Mitochondrial phosphorylation was investigated in tissues of transgenic mice with deficiencies in KGDHC subunits.

Results. We demonstrate ATP consumption in respiration-impaired isolated and *in situ* neuronal somal mitochondria from transgenic mice with a deficiency of either dihydrolipoyl succinyltransferase (DLST) or dihydrolipoyl dehydrogenase (DLD) exhibiting a 20-48% decrease in KGDHC activity. Import of ATP into the matrix of mitochondria from transgenic mice was attributed to a shift in the reversal potential of the adenine nucleotide translocase towards more negative values due to diminished matrix substrate-level phosphorylation, causing the translocase to reverse prematurely. Immunoreactivity of all three subunits of succinyl-CoA ligase and maximal enzymatic activity were unaffected in transgenic mice as compared to wild-type littermates. Therefore, decreased matrix substrate-level phosphorylation was due to diminished provision of succinyl-CoA. These results were further corroborated by the finding that mitochondria from wild-type mice respiring on substrates supporting substrate-level phosphorylation exhibited ~30% higher ADP-ATP exchange rates compared to those obtained from DLST+/- or DLD+/- littermates.

Conclusions. We propose that KGDHC-associated pathologies are subserved by the inability of respiration-impaired mitochondria to rely on "in-house" mitochondrial ATP reserves.

IV.5. Absence of Ca2+-induced mitochondrial permeability transition but presence of bongkrekate-sensitive nucleotide exchange in Crangon crangon and Palaemon serratus

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Objectives: mitochondria from the embryos of brine shrimp (Artemia franciscana) do not undergo Ca(2+)-induced permeability transition in the presence of a profound Ca(2+) uptake capacity. Furthermore, this crustacean is the only organism known to exhibit bongkrekate-insensitive mitochondrial adenine nucleotide exchange, prompting the conjecture that refractoriness to bongkrekate and absence of Ca(2+)-induced permeability transition are somehow related phenomena.

Methods. PTP in two crustaceans were examined by maximum Ca^{2+} uptake capacity, swelling, and visualization by electron microscopy. Their ANT sequences were evaluated by alignment analysis.

Results. Mitochondria isolated from two other crustaceans, brown shrimp (Crangon crangon) and common prawn (Palaemon serratus) exhibited bongkrekate-sensitive mitochondrial adenine nucleotide transport, but lacked a Ca(2+)-induced permeability transition. Ca(2+) uptake capacity was robust in the absence of adenine nucleotides in both crustaceans, unaffected by either bongkrekate or cyclosporin A. Transmission electron microscopy images of Ca(2+)-loaded mitochondria showed needle-like formations of electron-dense material strikingly similar to those observed in mitochondria from the hepatopancreas of blue crab (Callinectes sapidus) and the embryos of Artemia franciscana. Alignment analysis of the partial coding sequences of the adenine nucleotide translocase (ANT) expressed in Crangon crangon and Palaemon serratus versus the complete sequence expressed in Artemia franciscana reappraised the possibility of the 208-214 amino acid region for conferring sensitivity to bongkrekate.

Conclusions. Our findings suggest that the ability to undergo Ca(2+)-induced mitochondrial permeability transition and the sensitivity of adenine nucleotide translocase to bongkrekate are not necessarily related phenomena.

IV.6. Properties of the mitochondrial permeability transition in human cells lacking the adenine nucleotide translocase isoform 1

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Objectives: In pathological conditions, mitochondria recruit proteins to the inner membrane to form a pore that disrupts membrane integrity. This pore, termed "permeability transition pore" (PTP) is of a sufficient size to allow the passage of solutes and water since the mitochondrial matrix is hyperosmotic to the cytosol that may also result in rupture of the outer membrane. For long, the adenine nucleotide translocase (ANT) was suspected to be a structural component of the pore; however, recent experiments showed that mitochondria obtained from ANT knock out mice were still capable of demonstrating pore formation; it was therefore, inferred that ANT modulates the opening of the mitochondrial permeability transition pore, but it is not a structural element per se. Hereby we investigated the properties of in situ permeability transition in human fibroblasts obtained from a patient with a complete absence of ANT isoform 1.

Methods. In situ fibroblast mitochondria of the patient and a disease-free related individual were visualized by mito-DsRed2 targeting and challenged by calcimycin. The effects of glucose, NaCN, and an uncoupler were evaluated by measuring mitochondrial volume using the thinness ratio technique.

Results. The absence of ANT1 abolished the hastening of the swelling of in situ mitochondria by glucose deprivation and NaCN co-application, upon calcimycin exposure. Furthermore, the number of PTP-exhibiting cells obtained from the patient with the ANT1 deletion was significantly less than that obtained from the disease-free related individual. As expected, the extent of swelling quantitated by the thinness ratio technique was the same for both cell types.

Conclusions. The impact of a lack of an electrochemical gradient substantiated by absence of glucose and presence of cyanide predisposing to pore opening is mediated by ANT1.

IV.7. Combined effects of extracellular vesicles and cytokines on the gene expression profile of a human monocyte cell line

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Introduction. The immunomodulatory effects of extracellular vesicles (EVs) on monocytes are well established. However, no studies have so far addressed the combined effects of EVs and cytokines, even though under physiologival conditions, EVs occur together with cytokines. We hypothesized that EVs in combination with cytokines *vs.* EVs and cytokines alone may induce different responses of monocyte cells.

Methods. The supernatant of serum-starved (24 h) CCRF human T lymphoma cells was used as the source of extracellular vesicles. The supernatant was spun at 300 g for 10 minutes and the remaining cell-free supernatant containing the total EV pool (apoptotic bodies, microvesicles and exosomes) was used in downstream experiments. Vesicle-free supernatant was used as control. Human monocyte U937 cells were incubated for 24 h with EV-containing or EV-free supernatant, with or without 10 ng/ml TNFα or IL-17. This was followed by RNA isolation, microarray analysis and real-time PCR.

Results. According to GeneSpring hierarchical clustering, the gene expression pattern of the monocytes differed in the combined 'EV+cytokine' treatment compared to the 'EV only' or 'cytokine only' treaments. Gene set enrichment analysis showed that most genes induced by EVs were connected to IL-8. Mainly genes with functions in inflammation, cellular stress response, phagocytosis and cell migration were induced. A selection of genes - IL-8, CD36, CCL-2, cannabinoid receptor 2, chitinase 3-like protein 1, peroxiredoxin 1, beta-hexosaminidase, tissue and plasma alpha fucosidase – were also analysed by Taqman assays in order to confirm the findings of the array analysis.

Conclusion. This work supports the hypothesis that the combined paracrine effects of EVs and cytokines on the gene expression of target cells may be independent, additive, synergistical or opposite, depending on the analysed gene. The novel approach of analysing the combined effects of cytokines and EVs may provide a more accurate mean to model the complexity of the *in vivo* environment compared with analysing the effects of EVs and cytokines independently.

IV.8. Identification of amylin as a novel neuropeptide activated in the brain of mother rats

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Objectives. To investigate adaptations of the maternal brain for taking care of the offspring and to identify potential targets in postpartum depression, we investigated gene expressional changes by microarray in the preoptic area, a brain region whose lesion abolishes maternal behaviours. Amylin was identified as a regulatory peptide present only in mothers taking care of their pups. Thus, the objective of the present study was to investigate amylin in rat dams.

Methods. The expression pattern of amylin during late pregnancy and throughout lactation was examined using quantitative RT-PCR, *in situ* hybridization and immunohistochemistry. The induction of amylin was also investigated in maternally behaving sensitized virgin females with or without ovariectomy. The activation of amylin neurons in response to their pups was examined using the Fos technique.

Results. Amylin is not expressed in the brain during pregnancy but a significant increase in amylin expression is found immediately after parturition in the preoptic area. Amylin expression was also induced in virgin but maternally behaving (sensitized) non-lactating but not in non-sensitized virgin females or in females who did not become maternal despite the sensitization procedure. Immunohistochemistry verified the increased amylin peptide expression in maternally behaving rats and demonstrated the same expression pattern of amylin in dams as *in situ* hybridization histochemistry. Ovariectomy had no effect on the activation of amylin neurons suggesting sexual steroid independent mechanisms. Upon returning the pups after a day separation, neuronal activation was found in the mothers´ preoptic area with a distribution pattern similar to amylin-expressing neurons situated in the medial preoptic nucleus, parts of the medial preotic area, and the ventral part of the bed nucleus of the stria terminalis. Double labeling revealed that 86-93% of amylin neurons were activated by pup exposure.

Conclusion. The results implicate that amylin is a novel neuropeptide with specific maternal functions possibly exerting its actions on maternal behaviors *via* amylin receptors known to be present in brain regions to which preoptic neurons project.

Support: Bolyai János Fellowship of the HAS, OTKA NNF2 85612 and K100319 research grants.

IV.9. Metabolic phenotyping by automated chip-based nanoelectrospray ionization high resolution mass spectrometry

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Objectives. Recent developments on instrumentation have enabled high resolution mass spectrometry (HR-MS) to become a widely available tool in clinical chemistry. This technique provides accurate mass data, which allows the simultaneous identification of several components via determination of their elemental composition. Application of single-stage HR-MS to samples of biological origin provides tremendous amount of biochemical information. The metabonomic approach of data processing supports and simplifies the evaluation; hence more specific characterization of metabolic differences is feasible.

Methods. The method developed is an effective combination of HR-MS (Exactive instrument, provided by Thermo) and nanoelectrospray (nS) ionization (TriVersa NanoMate device, provided by Advion) for the determination of various metabolites. The analysis requires minimal sample preparation and acquisition time is 1 minute. In addition to concentration determination, mass spectrometry data were evaluated with linear statistical pattern recognition methods.

Results. Dried blood spot (DBS) samples were extracted with the methanolic solution of stable isotope-labeled amino acid and acylcarnitine internal standards. After filtration and dilution the samples were analyzed. More than 300 metabolic constituents were identified in newborn DBS samples. Concentration values of substances were determined and correlated with the results of the traditional electrospray tandem mass spectrometry method and linear correlations were found. Validation of the method was performed on 10,000 healthy newborn samples and 500 abnormal samples. Calibration curves, inter- and intraday variability were determined, and statistical methods were applied to the data in order to identify new biomarkers for different metabolic diseases. Urine samples from volunteers were also examined and spectral characteristics were determined in cases of different metabolic conditions. Bacterial cells were disrupted by sonication and extracted with organic solvent mixture. The extracts were analyzed with nS-HR-MS method. Different strains were characterized by different spectral patterns of the small mass range as well as the phospholipid mass range.

Conclusion. It was shown that the developed nS-HR-MS method is a versatile and robust technique. In combination with linear statistical pattern recognition methods it is a promising alternative to the chromatographic separation-enhanced mass spectrometry methods.

IV.10. New quantitative analytical method for biotinidase activity measurement

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Objectives. Biotinidase activity measurement is included in most newborn screening (NBS) protocols, the positive results being confirmed by quantitative enzyme activity measurements. There are various methods for determination of biotinidase activity with different substrates, in different biological samples, and with various techniques. The disadvantages of the presently used quantitative methods are very similar, such as selectivity and sensitivity is relatively low, causing an increased false positive ratio; enzymes in dried blood spots (DBS) are not stable; and these enzyme assays require long incubation times and laborious sample preparation. Liquid-chromatography (LC) in combination with mass spectrometry (MS) detection is, in general, more sensitive than UV or fluorescence methods, and suitable for the high throughput analysis of large sample numbers.

Methods. A new quantitative analytical method for biotinidase activity measurement in DBS by LC-MS was developed. This alternative method contains only one extraction step using pretreated sample collection paper. The enzymatic hydrolysis occurs on the filter paper impregnated with arbitrary substrate, B-PABA. After a simple extraction, the quantity of obtained product, PABA is measured by mass spectrometry. The assignments of this technique were to eliminate the instability of the enzyme in DBS and to reduce the preparation time.

Results. Biotinidase activity was measured both in DBS and in serum samples and was compared with the colorimetric method used in NBS in healthy volunteers and in patients with biotin cycle related metabolic disorders, as well. Biotinidase activity values using the newly developed method were in good comparison to values obtained from serum and enzyme activity data correlated with the results obtained in NBS. According to the measurements' statistical parameters (min, max and quartile values) the biotinidase enzyme activity determination with mass spectrometry proved to be more precise.

Conclusions. We have developed a new robust, quantitative analytical method for measurement of biotinidase activity in pretreated dried blood spot samples, which resulted in decreased false negative and false positive rates. The developed method can be customized for further enzyme activity measurements as well, e.g GGT, GOT, GPT. By introduction of pretreated filter papers for DBS sampling new prospects of enzyme activity measurements might emerge in clinical diagnosis.

IV.11. Novel mass spectrometry method for rapid analysis of native biopsy samples

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Objectives. The aim of our study was to develop a mass spectrometric approach for analysis of biopsy samples based on the newly developed Rapid Evaporative Ionization Mass Spectrometry (REIMS) using a LTQ linear ion trap mass spectrometer.

Methods. The developed method is based on the rapid evaporization of cells producing ionised aerosol rich in the gaseous ions of membrane constituent lipids, suitable to be analysed by mass spectrometry. The collected mass spectra are highly specific for a certain tissue types, thus enables identification of tissues.

A dedicated ion source setup was constructed specifically for the analysis of core biopsy samples. The ion source comprises a stainless steel biopsy needle holder mounted on a 3 dimensional moving stage system an ion transfer device and a standard surgical laser unit.

Due to the laser settings we could collect a sufficient amount of data (about 250 data points) from a single biopsy sample. The mass spectra were collected in single stage MS, negative ion mode, in the mass range of 600-900 *m/z* at unit mass resolution. The samples were collected after surgical interventions, from healthy and cancerous part of each surgical dissection using a 19 Ga biopsy needle. The collected data was processed with the combination of principal component analysis (PCA) and linear discriminant analysis (LDA).

Results. Various tissue types were collected, liver metastasises from various tumours (14 samples, from breast, lung and colorectal cancers), primary liver tumours (5 samples), renal tumours (3 samples), primary colorectal tumours (25 samples), various non-tumorous liver disease biopsies (73 samples), and also healthy tissue biopsies. Based on the combination of PCA and LDA an identification method for biopsy samples was developed.

Using primary tumours and healthy tissues as learning sets the accuracy of identification procedure was higher than 95% for liver metastases. At renal cell carcinoma we could identify not just the healthy and cancerous tissues but the healthy-cancerous margin was also identifiable. The non-cancerous liver diseases were identified also successfully up to 90% probability from biopsy samples.

Conclusion. The newly developed biopsy analysis system is capable to combine the obtained chemical information with histological section, thus decreasing the inter-observer variability during the diagnostic process, thus offering a fast, reliable and accurate diagnostic tool.

IV.12. Real-time tissue identification in the neurosurgical theatre

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Objectives. The recently developed rapid evaporative ionization mass spectrometry is an emerging technique allowing quasi real-time, in-situ tissue characterization during MS-guided surgical interventions. The aim of this study was to test the novel REIMS method coupled with commercially available bipolar forceps during neurosurgical interventions.

Methods. Commercially available bipolar forceps with irrigation was used for ionization in-vivo during surgical interventions and ex-vivo after surgery. The generated surgical smoke is lead away through the gap of the forceps originally created for irrigation to the capillary inlet of a Mass Spectrometer. A home-built SQL database and software was created for data interpretation and an algorithm using principal component analysis (PCA) and linear discriminant analysis (LDA) is used. During the surgical intervention, the acquired new spectra is transformed to the LDA-space of the loaded "PCA+LDA model" and the spectrum is assigned to the closest class, if the distance does not exceed a previously defined threshold value.

Results. Data was collected during 43 surgical interventions and the tissue specific database was tested on 11 additional patients (5 glioblastoma multiforme, 3 metastases, 3 meningiomas). The species in both healthy and cancerous tissue mostly overlap, however the ratio of each characteristic species is significantly different and show a tissue specific distribution. The peak distribution in metastatic cancer tissue differs more from the brain tissue, than the primer brain cancer tissue compared to healthy brain tissue. The separation of all tissues using PCA+LDA algorithm has proven to be successful (100% sensitivity and 83.33% specificity using validation set, and 95.65% sensitivity and 95.35% specificity using cross-validation). Our algorithm using multivariate statistical data processing is capable of separating healthy and cancerous brain tissue, primer and metastatic brain tumors and also has a potential in separating different grade primer brain tumors.

Conclusions. The main goal of this study was to test the possibility of an in-situ, in-vivo neurosurgical tool created to assist the neurosurgeon during surgery. Statistical analysis of in-vivo and ex-vivo brain tissues proved that the mass spectrometer coupled intelligent surgical knife is capable of providing real-time identification of intra-operative pathology which could significantly influence 'on table' decision-making.

V. Genetic and sociobehavioral factors predisposing to psychiatric disorders

V.1. Association of the TPH2 gene, suicidal behaviour and cardiovascular comorbidity is mediated by hopelessness

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Objectives. Hopelessness is one of the strongest rsik factor for suicidal behavior but related genetic studies are poorly available. Tryptophan hydroxylase (TPH) is widely considered to be a good candidate for depression and suicide, however, relevant studies on direct associations have resulted in conflicting data. We hypothesized that hopelessness could be a mediating phenotype between TPH2 gene and suicidal behavior. On the other hand, cardiovascular disorders (CVD) are common comorbidity with depression but there are no genetic studies of the relationship between hopelessness and CVD.

Methods. We involved 760 individuals into our study. Depressive symptoms and hopelessness were investigated the by Zung Self Rating Depression Scale (ZSDS) and the Beck's Hopelessness Scale (BHS). Based on international studies more than 9 points of BHS was regarded as an increased risk of suicide, while above 15 points indicated a serious danger of suicide. Seven tag SNPs (rs1843809, rs1386493, rs6582078, rs10506645, rs1352250, rs1386485, rs1487275) across the TPH2 gene. The cardiovascular comorbodity was measured by a self-rating test.

Results. One SNP of the TPH2 gene, namely the rs6582078, had a significant effect on the BHS scores. Subjects with homozygous GG genotype had significantly higher BHS scores, while subjects with GT and TT genotypes had intermediate and lower BHS scores, respectively. Compared with other genotypes, homozygous GG individuals also had almost three times greater suicidal risk, as did carriers of the AA genotype of SNP rs6582078rs and of 1352250. Moreover, we identify a risk (BHS=+2.544; p_{perm}= 0.0159; freq= 3.22%) and a protective (freq=12.5%; BHS=-2.078; p_{perm}= 0.0402) haplotype of the TPH2 gene in association with BHS score. The cardiovascular comorbidity was associated with the TPH2 gene variants only with a trend but a significant relationship has been found between CVD and hopelessness in GG genotype carriers. Surpriseingly, the TPH2 gene variants did not show association with ZSDS score in any of models and CVD was independent from ZSDS score in our sample.

Conclusions. We found that the TPH2 gene is asociated with hopelessness, with its allied increased suicidal risk and cardiovascular morbidity but not with general depressive symptoms. These results can be possible explanation for earlier conflicting data on direct association of TPH2 and suicide and depression. Our findings suggest that cardiovascular comorbidity of depression can be genetically predisposed.

These studies were supported by the Sixth Framework Programme of the EU, LSHM-CT-2004-503474, HRF T03298/2000, Hungarian Ministry of Health RG 318-041-2009 and TAMOP 2010.

V.2. Considering some important factors in the background of cross-cultural variability of unipolar major depression

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Objectives. Major depression is a prevalent illness worldwide, present in every country and culture, although prevalence data and symptomatic manifestation shows significant differences. The background factors of these differences, whether psychological, social, or biological-genetic, are not yet sufficiently explored and understood.

Our objectives were to promote better understanding of the possible background factors of cross-cultural diversity of unipolar major depression, by finding some candidate variables and visualising their distributions in a map. Visualising results also helps us to identify possible patterns of certain values of these variables.

Methods. We performed a literature search of cross-country variability of unipolar major depression and related variables linked to symptomatology and etiopathology of this disorder. Among all the putative factors previously related to depression, we selected the ones that were measured in several countries around the world within the same study in order to diminish the occurrence of biases and first type errors.

Results. We identified important cross-country differences in socioeconomic variables (Steptoe et al., 2007), Hofstede's individualism-collectivism dimension, and allele frequencies of 5-HTTLPR (serotonin transporter promoter repeat length polymorphism) (Chiao and Blizinsky, 2010) possibly related to the observed different depression prevalences and characteristics.

Conclusion. We may conclude that these variables are only a few of the countless factors underlying the experienced cross-country variabilities in unipolar major depression. Apart from the possible factors acting in etiopathology, associations and interactions between these factors may also be various.

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V.3. From genetic evolution to sociocultural characteristics: cross-country association between distribution of dominant affective temperaments and Hofstede's cultural indeces

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Objectives. Although psychiatric disorders carry a reproductive disadvantage, they do not become extinct over evolution, probably due to their association with genes encoding for otherwise adaptive traits. This adaptive component in case of mood disorders may be affective temperaments, which carry an evolutional advantage either on the individual or group level. One polymorphism associated with affective temperaments is the 5-HTTLPR which shows uneven geographical distribution, and this phenomenon may be related to cross-cultural differences in mood disorder prevalences.In our study we investigated the distribution of dominant affective temperaments in national studies on general non-clinical populations.

Methods. Dominant affective temperament frequencies extracted from six large nonclinical population studies (Argentina, Germany, Hungary, Korea, Portugal, and Lebanon) were compared using chi square tests.

Results. We found a significant difference in dominant affective temperament frequency for Cyclothymic ($p=0.0363$, significantly higher in Korea compared to Lebanon and significantly lower in Argentina compared to all other samples), Hyperthymic ($p<0.0001$, significantly lower in Lebanon compared to other samples except for Argentina and significantly lower in Argentina compared to Portugal, Hungary, Korea and Germany) and Irritable temperament ($p<0.0001$, significantly higher in Lebanon compared to Portugal, Hungary, Argentina and Germany and significantly lower in Hungary compared to Germany and Argentina). Pairwise significant differences also emerged for the Depressive temperament.

Conclusions. We identified intriguing parallels between distribution patterns of dominant affective temperaments and cultural dimensions described by Hofstede. Frequency pattern of Hypethymic, Irritable and Depressive dominant temperaments in samples paralleled the order of these countries on the Uncertainty Avoidance, Power Distance and Individualism-Collectivism index, respectively. Furthermore, characteristics encompassed by these affective temperaments are remarkably similar to the corresponding cultural dimensions. Our results are also in line with earlier reports concerning the association of the 5-HTTLPR polymorphism with affective temperaments as well as its characteristic geographical distribution, therefore our results also indicate that affective temperaments and cultural dimensions may be different manifestations of the same genetically determined predispositions.

The work described in this paper was supported by TAMOP-4.2.1. B-09/1/KMR-2010-0001, and OTKA 80289. The authors report no conflict of interest.

V.4. Analysis of suicide attempts with a focus on preceding diseases, causes and methods

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The OSPI project (Optimised Suicide Prevention programs and their Implementation in Europe) has a new area of intervention in Hungary, the city of Miskolc. Unfortunately, the prevalence of suicide is particularly high in this community.

Our database that were thoroughly analysed consists of more than 2500 well-detailed cases recorded at the departments of toxicology, neurology and psychiatry in the last 3 years. The main goal was to reveal those physical diseases that are most commonly related to suicide attempts. We found that disc disorders, schizophrenia, epilepsy are significantly more frequent between suicidal people regardless of their gender. Alcohol dependency is significantly more often recorded among suicidal women.

Furthermore, we examined the reason of the suicide attempts. We can categorise the patients based on their motivation. The most common category is the "parasuicid pause", when the person only tries to escape from a certain situation (39%). The "serious intent to die" is the second most common category (38%). The patients in the third group do not have any real suicidal motivation, it is just a parasuicid gesture (5%). Since the direct self-injury is hardly to be separated from other suicidal acts our research include those as well (10%).

The most common method of suicidal attempts is medication. We found that the quantity of the swallowed pills is highly correlated with the seriousness of the intention. The most common drugs are anxiolytics, psycholeptiys and antiepileptycs (Rivotril, Frontin, Xanax). Women often use some sort of cardiovascular medication as well.

V.5. Psychosocial issues and changes during adjuvant low-dosis interferon therapy in malignant melanoma

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Objectives. Cancer has severe psychosocial impact involving not only the patients, but their relatives and caregivers, as well. Psychosocial support is necessitated by the ever increasing incidence of melanoma, well demonstrated by the annual 400 new cases diagnosed in the National Institute of Oncology (Budapest).

According to chronic stress-related mechanisms and other neurobiological aspects, the deeper psychological contents, like coping mechanisms, general psychological preparedness, social support, etc. may have an influence on tumor progression.

Methods. In a longitudinal study with tumor-free, high risk melanoma patients treated with adjuvant interferon the possible relationship between the psychological constructs and the primary tumor characteristics and the progression rate was examined.

Results. In our sample (N= 49) increased level of distress (Beck Depression Inventory and State Trait Anxiety Inventory) were found. The extent of social support and the thickness of primary tumor (Breslow-scale) showed significant negative correlation ($p<0,05$), just like the trait-anxiety and the thickness ($p<0,05$). We also found significant negative correlation between social support and illness intrusiveness, and the level of anxiety ($p<0,05$). There were significant ($t=2,789$, $p<0,01$) differences in the anxiety patterns of men and women, but in other psychological constructs no differences were found.

Conclusion. Treatment may result in longer symptom-free survival providing better quality of life. Social support may be an important field of psychological intervention. Exploration of the possible role of psychosocial preparedness requires further long term research.

V.6. Mitochondrial psychiatry: clinical practice supports the hypothesis

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Objective. The central nervous system (CNS) depends heavily on an intact mitochondrial apparatus for its metabolism, including the maintenance of the transmembrane potential, calcium homeostasis, signal transduction and synaptic plasticity. Mitochondrial dysfunction tends to manifest in neurologic, psychiatric and neuropsychologic symptomatology.

Methods. We assessed 19 patients (13 female, 6 male, mean age: 34±8.43 years) with genetically proven primary mitochondrial DNA (mtDNA) mutation. Psychiatric examination used the Symptom Check List-90-Revised (SCL-90-R), the Beck Depression Inventory-Short Form (BDI-SF), the Hamilton Depression Rating Scale (HDRS) and the clinical version of the Structured Clinical Interview for the the DSM-IV (SCID-I and SCID-II). Neuropsychologic assessment consisted of the Stroop Test, the Rey Auditory-Verbal Learning Test, the Trail Making Test Part A and B (tmtA, tmtB) as well as letter and category fluency tests. Full Scale Intelligence (FSIQ), Verbal and Perfomance Quotients were measured using the Hungarian validated version (MAWI) of the Wechsler Adult Intelligence Scale (WAIS-IV).

Results. The patients scored significantly higher than controls on the BDI-SF, the HDRS and the Global Severity Index (GSI) of SCL-90-R (12.85 versus 4.40, p<= 0.031; 15.62 vs 7.30, p<= 0.043 and 1.44 vs 0.46, p<= 0.013, respectively). SCID yielded a lifetime prevalence of 9/19 (47%) for mood disorders and 8/19 (42%) for personality disorders. Weaker performance on most cognitive tests has been detected compared to matched healthy controls (Stroop 45 sec: 50.54 vs 69.67,p<=0.0142; stroop 60 sec 62.69 vs 81.67, p<=0.0303; Rey test: 7.15 vs 12.15, p<0.0001; tmtA: 96.39 vs 34.15, p<=0.0016, tmtB:186.08 sec vs 64.39 sec, p<=0.0007, FSIQ: 95.2 vs 123.7, p<= 0.003, VQ: 97 vs 117.6, p <= 0.006 PQ: 94.1 vs 127.2, p<= 0.007).

Conclusion. Compared to controls, patients with mtDNA mutation display significantly weaker cognitive performance and higher prevalence of mood disorders. Mood disorders do not correlate with the somatic symptoms implying that they are independent manifestations of the mitochondrial dysfunction and not the consequence of the chronic somatic disease. They tend not to have a classic course and to be treatment-resistant. A complex metabolic disturbance of the CNS, including mitochondrial dysfunction, has been reasoned in a variety of psychiatric disorders. Further studies may lead us to the new era of 'mitochondrial psychiatry'.

This work was supported by TAMOP-4.2.1/ B-09/1/KMR-2010-0001.

V.7. The significance of quality assurance of clinical data and biological samples in the life of biobanks

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Well established biobanks are the main pillars of personalized medicine. The data obtained from the examination of blood and tissue samples, and the DNA, RNA and proteins extracted from them can only be interpreted properly if a detailed clinical database linked with the biological samples is also available.

In Hungary several institutes runs a biobank or possess biological sample collections. The national schizophrenia biobank (SCHIZOBANK) was established as a result of the cooperation of the national academic sphere and the pharmaceutical industry and the subsidy of the former National Office of Research and Technology (currently National Development Agency). SCHIZOBANK is a collection of the DNA, RNA and plasma samples of schizophrenic patients and healthy control persons.

A detailed clinical database forms an integral part of the SCHIZOBANK, in which the individuals' case history, family anamnesis, physical status, former medication, the results of laboratory examinations, important environmental-social factors, the scores of side effect evaluating scales and standard clinical interviews are recorded.

The continuous quality assurance of the clinical database and the biological samples is essential requirement of the long-term storage and high-quality research. Standard operating procedures regulates the whole process e.g. the responsibilites of the single subtasks, the steps of sample moving, the daily control of the biobank. Continuous monitoring of the database and the quality control of the DNA and RNA samples provide the appropriate quality.

Presently 515 patients with schizophrenia diagnosis and 225 control persons are in the biobank. The strengths of this SCHIZOBANK is, that beside the patients sample their 2 healthy family members are involved in 16 cases as well. The investigation of this trios is very importnat in the postgenomic era.

In this poster we present our experiences related to the establishment of the first national schizophrenia biobank, the SCHIZOBANK.

This work was supported by SCHIZO-08 Consortium.

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V.8. Effects of escitalopram on rem sleep: comparison of acute and chronic administration in rats

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Objectives. There is a marked overlap between the neuronal pathways involved in sleep/wake regulation and depression. Both the structure and the organization of sleep are altered in depressed patients; they enter the rapid eye movement sleep (REMS) earlier, the amount of REMS is increased and also the non-REMS is reduced in the first sleep cycle.

Serotonin reuptake inhibitor antidepressants (SSRIs) exert their therapeutic effect on the serotonergic system, which has a central role in the modulation of mood and vigilance.

The aim of this study was to investigate the acute and long-term effect of the SSRI escitalopram on REMS in rats.

Methods. The effect of a single (10 mg/kg i.p.) and chronically administered (10 mg/kg/day, released by an osmotic mini pump for 21-day-long) escitalopram was studied in male Wistar rats. Electroencephalogram, electromyogram and motility were recorded for three hours starting at light onset.

Results. The acutely administered escitalopram significantly reduced the time spent in REMS in the first three hours, compared to control. However, this REMS-reducing effect was abolished after chronic administration.

Conclusion. There was a clear difference in the effect of escitalopram on REMS following acute and chronic administration, providing evidence for the adaptive changes of serotonin receptors, which take several weeks to evolve, and considered to have a role in the development of therapeutic effect of SSRIs in the treatment of depression.

This study was supported by the TAMOP-4.2.1. B-09/1/KMR-2010-0001 and by Richter Gedeon Plc.

V.9. MDMA-induced serotonergic damage: reduction and recovery of the hippocampal theta activity in rats

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Objectives. The amphetamine derivate MDMA (3,4-methylenedioxymethamphetamine, „ecstasy") is used widespread as a recreational drug. There is evidence, that MDMA causes selective damage of the serotonergic neurons in experimental animals and possibly in humans. Serotonin (5-HT) plays an important role in cognition and memory. The wakefulness, active and passive wake (AW, PW), are characterized by hippocampal theta oscillations (5-9 Hz) are of hippocampal origin, and during wakefulness they are associated with attentive and exploratory behaviour. Our aim was to study the theta activity after a single dose of MDMA treatment with quantitative-electroencephalography (Q-EEG) in AW and PW, moreover to compare the results with serotonin transporter (5-HTT) immunohystochemistry.

Methods. Experiment was carried out on adult male Dark Agouti rats. Animals were equipped with electroencephalographic (EEG) and electromyographic (EMG) electrodes. After the recovery and the habituation, rats were injected i.p. with MDMA or saline. The EEG recordings were made 7, 21 and 180 days after a single dose of MDMA (15 mg/kg, i.p.) treatment and Q-EEG was analysed for the first two hours starting at light onset. The EEG power spectrum of AW and PW was calculated. The density of serotonin transporter (5-HTT) immunoreactive fibres was measured with immunohistochemistry at the same days.

Results. Our main result shows that there is a significant decrease in cumulative power (μV^2) of theta band 21 days after the treatment in PW. Significant, 30-40% reductions in serotonin transporter density were detected in the hippocampus 7, 21 and 180 days after the treatment.

Conclusion. In spite of fact that the hippocampal damage was maintained during the 180 days, reduction of theta power was observed only 21 days after the treatment. These data suggest that accomodations and adaptive changes may occur that compensate for the loss of serotonergic function after MDMA-induced damage.

This work was supported by the 6th Framework Program of the EU, LSHM-CT-2004-503474.

V.10. Escitalopram treatment increased deep slow wave sleep duration following REM sleep deprivation

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Objectives. The firing of serotonergic neurons increases extracellular serotonine (5-HT) concentration, which consequently depresses the duration of all sleep stages, and promotes wakefulness. The firing frequency of the serotonergic neurons is highest in active wake. Accordingly selective serotonine reuptake inhibitor (SSRI) administration causing elevated 5-HT levels, increases both rapid eye movement sleep (REM) and non-REM (NREM) latencies. After highly selective REM deprivation by the flower pot method an increase in REM sleep can be observed, called REM rebound. Our aim was to study the effect of increased extracellular 5-HT concentration by the SSRI escitalopram during the rebound sleep after flower pot REM deprivation.

Methods. Male Wistar rats surgically equipped with electroencephalographic (EEG) and electromyographic (EMG) electrodes were placed onto flower pots (FP) or into home cages (HC) for 72 hours. After the REM sleep deprivation 10 mg/kg escitalopram or vehicle (saline) was administered i.p. Thereafter frontoparietal EEG, EMG and motility recordings were made in the first three hours of the rebound sleep starting at the beginning of the passive phase. The EEG power spectra were analyzed of the following sleep stages: active and passive wake, light and deep slow wave sleep (SWS-1, SWS-2) and REM. The results of HC, FP, vehicle and escitalopram treated groups were compared to each other.

Results. Escitalopram in the HC animals decreased the duration of both REM and NREM stages. In REM deprived animals, however, the effects of escitalopram were adverse: while REM duration was decreased, SWS-2 was increased significantly in the first two hours of rebound sleep.

Conclusion. These results suggest an unexpected regulatory effect of serotonin after long-term, selective REM deprivation, namely the possible promotion of SWS-2.

This work was supported by the 6th Framework Program of the EU, LSHM-CT-2004-503474.

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TÁMOP-4-2-1/B-09/1/KMR-2010-0001