

DBM

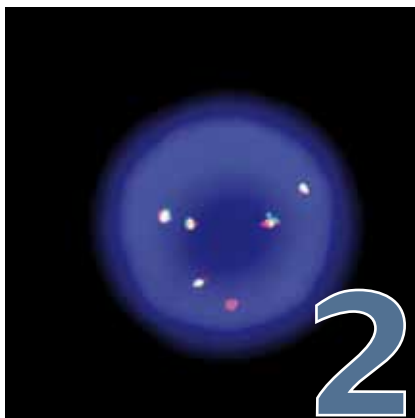
FACTS

Periodisches Informationsblatt des Departementes Biomedizin
Universität Basel, Universitätsspital Basel und
Universitäts-Kinderspital beider Basel



**Medical Genetics: Linking genes, genomes and diseases |
Christmas in Poland | The Art of Fencing**

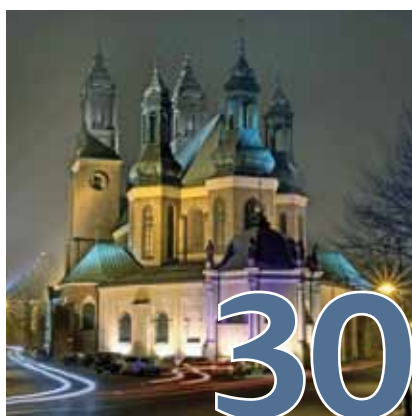
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**Medical Genetics: Linking genes,
 genomes and diseases**
 from Sven Cichon and his team



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IMPRESSUM

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Sunset landscape in winter with snow in Holland
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EDITORIAL



Radek Skoda
Leiter DBM

Liebe Leserinnen und Leser

2013 neigt sich seinem Ende zu. Der Umbau der Bibliothek im zweiten Stock ZLF ist pünktlich fertig gestellt worden, so dass die DBM-Forschungsgruppen per 1. Januar 2014 die neuen Laborräumlichkeiten im 2. Stock im ZLF beziehen können. Seminarräume und Sitzungszimmer sind nun ebenfalls im 2. Stock untergebracht. Die baulichen Anpassungen im 3. und 4. Stock (GMP Labor) werden 2014 noch fortgesetzt. Herzlichen Dank an alle, die diesen Umbau möglich gemacht haben!

Schon warten neue Herausforderungen: Am 23. Januar 2014 finden der DBM Research Day und der Besuch des Advisory Boards statt. Mike Recher verstärkt als SNF-Professor mit seiner Forschungsgruppe «Immunodeficiency» das DBM Hebelstrasse ab dem 1. Januar 2014. Am 1. März 2014 kommt Lukas Jeker ebenfalls als SNF-Professor mit seinem Labor dazu. Ein SCORE Ambizione hat Christoph Berger erhalten. Wir heissen alle herzlich willkommen und wünschen Ihnen viel Erfolg!

Die letzte Ausgabe dieses Jahres ist ganz der Medizinischen Genetik gewidmet, deren Leitung zu Jahresbeginn mit Sven Cichon neu besetzt wurde und die seitdem zum USB gehört. Im ersten Beitrag erfahren wir mehr über den aktuellen Stand in der Forschung. Im zweiten Beitrag steht die Dienstleistung der so wichtigen Disziplin im Mittelpunkt. Die neuesten Publikationen am DBM finden Sie ab Seite 15.

Wie man in Polen Weihnachten feiert, wie es sich mit fleischfressenden Pflanzen lebt, und warum Fechten zur Passion werden kann, erfahren Sie ab Seite 30.

Schöne Festtage und einen guten Rutsch ins 2014!

Dear Readers

2013 is drawing to a close. The renovation of the library on the second floor of the ZLF has run to schedule and the DBM research groups will be able to move into the new laboratory space on 1st January 2014. Seminar rooms and meeting rooms have also been moved to the second floor. The modifications to the third and fourth floors (GMP laboratory) will begin in 2014. Many thanks to all who have made this renovation possible!

New challenges are already waiting us: On the 23rd January 2014 the DBM Research Day will take place along with the visit of the Advisory Board. From the 1st January 2014 SNF Professor Mike Recher will strengthen the DBM Hebelstrasse with his research group "Immunodeficiency". On 1st March Lukas Jeker will also join as an SNF-Professor along with his research group. Christoph Berger has received a SCORE Ambizione. We welcome them all and wish them every success.

This final edition of the year is fully devoted to Medical Genetics, which has been under the direction of Sven Cichon since the start of the year, and which has been part of the USB since then. In the first article we find out about the current status of research. In the second article the services of this very important discipline are highlighted. The latest publications from the DBM can be found on page 15.

From page 30 on you can read how Christmas is celebrated in Poland, how one lives with carnivorous plants and how fencing can become a passion.

Happy holidays and all the best for 2014!

Medical Genetics:

Linking genes, genomes and diseases

On New Year's Day 2013, Medical Genetics was formally transitioned from the Children's Hospital (UKBB) to the University Hospital (USB). This step reflects the overall development of this relatively young medical specialty over the past decades. Medical Genetics originated in the 1960s as a special discipline of pediatrics. At that time, human chromosomes could be stained and viewed under the microscope and initial work therefore focused on the detection of chromosomal aberrations in (malformed) fetuses as well as children with severe disease symptoms. Driven by the development of molecular genetic techniques in the 1970s and 1980s, the detection of (submicroscopic) DNA mutations gradually became possible. It led to the identification of genetic defects in thousands of rare, Mendelian diseases, and -more recently- the recognition of genetic risk factors in an increasing number of common diseases. It is generally perceived today that the majority of diseases and traits in humans are influenced by genetic factors and that genetics has become a key discipline in medicine.

In the following article, we will give an overview of the work covered by Medical Genetics. There are basically two major areas: the research group "Human Genomics" which aims at identifying so far unknown disease-causing or -influencing genetic factors as an essential prerequisite to understanding the biology of these diseases; and the clinical genetics and diagnostics part which supports clinicians in substantiating suspected diagnoses and offers genetic counselling and genetic diagnostics to individuals and families with hereditary diseases and congenital malformations.

Research Group "Human Genomics"

Identification of the genetic changes that cause diseases is probably the shortest way of describing the major goal of our research group. While it sounds straightforward the devil is often in the details, or -rather- in the nature of the genetic changes. The best scenario is when a mutation alone is sufficient to cause a particular disease and is present in the affected individuals of one or more families with multiple patients. This scenario is found in many rare, monogenic diseases. The greatest portion of the disease cases in a population, however, is represented by common, multifactorial diseases. In these diseases, one mutation alone does not cause

the disease. Instead, we have dozens to thousands of genetic risk variants that each contribute a bit to the overall disease risk of an individual. In most cases, environmental factors are also involved, leading to a complex mixture of genetic and non-genetic factors that altogether determine whether an individual gets the disease or not. The identification of these factors is still a major challenge for genetic research. In human cancer syndromes, there often is a hereditary mutation that mediates a high risk to develop cancer at some point in life. Specific somatic mutations are necessary, however, to actually generate a tumor.

The research group has three major research areas: Research into hereditary cancer syndromes has a long-standing tradition in the group, with Karl Heinimann being the principle investigator for many years. Another area, clinical research which focuses on pre- and post-natal characterization of rare neurodevelopmental phenotypes or multiple congenital anomalies, is covered by Peter Miny and Isabel Filges. A third research area has recently been established with the start of Sven Cichon, Per Hoffmann and Stefan Herms: the work on complex or multifactorial phenotypes, with special focus on common neuropsychiatric disorders. In parallel, this group is developing an increasing interest in the analysis of rare diseases (e.g. dermatological phenotypes such as hereditary angioedema).

Hereditary cancer syndromes

Since 1979, the activities have focused on the continuous collection and genetic study of families exhibiting hereditary colorectal cancer (CRC) predisposition syndromes, which account for about 2–5% of the total CRC burden. The register actually consists of more than 820 index patients and families referred from all parts of Switzerland creating the basis for many of our clinically-oriented research projects. Besides familial adenomatous polyposis (FAP) and the hamartomatous polyposis syndromes, consisting of Juvenile Polyposis (JPS), Peutz-Jeghers (PJS) and PTEN-Hamartoma-Tumor (PHTS) syndrome, Lynch syndrome (formerly known as hereditary non-polyposis colon cancer, HNPCC), representing



Medical Genetics people

Front row (from left): Martine Meichsner, Sibylle Bertschin, Clarisse Wermuth, Eliane Straumann, Beate Blankstein, Marisa Wenzel, Silvia Hofmann

Middle row: Birgit Füessl, Robert Blatter, Hansjakob Müller, Marianne Häusler, Per Hoffmann, Margrit Bernoulli, Sven Cichon, Nemya Bösch, Karl Heinimann, Joëlle Jenni, Barbara Thüning

Back row: Sandra Jenni, Henriette Kurth, Peter Miny, Friedel Wenzel, Stefan Herms

Missing: Isabel Filges, Carole Egenter, Michèle Attenhofer

the most common cancer predisposition world-wide, has been a main research topic for several epidemiological and molecular genetic studies since 1995. For many years we have cultivated close research collaborations with local (i.a. L. Terracciano, P. Itin, M. Zavolan), national (i.a. G. Marra and J. Jiricny, IMCR Zurich; P. Hutter, Geneva) and international research groups (I. Tomlinson, Oxford, UK; P. Peltomaki, Helsinki, FIN).

The research activities can be broadly grouped into a) identification and genetic (germ line) characterization of hereditary CRC patients b) assessment of genotype-phenotype correlations and c) characterisation of (epi) genetic somatic alterations in cancers from mutation carriers. Applying novel, often cutting-edge, molecular biology technologies (from protein truncation analysis, real time-PCR and MLPA assays to next generation sequencing) our group contributed to the discovery and characterization of novel hereditary CRC genes like *MUTYH*-associated polyposis (Sieber et al., 2003; Russell et al., 2006) and hereditary mixed polyposis (Jaeger et al., 2008, 2012). The identification of *APC* germ line mosaicism in two unrelated, “*APC* mutation-negative” patients with classical polyposis coli emphasized the need to keep a panoply of molecular diagnostic tools in one’s diagnostic repertoire: Using the protein truncation test, a technique largely replaced by DNA-sequencing and gene dosage analysis, we were able to identify two novel, pathogenic *APC* alterations present in a mosaic

state, at blood levels (1–15%) below the detection limits of conventional Sanger sequencing consequently allowing carrier testing in both families (Necker et al., 2011); *Figure 2*. Moreover, our group was able to further characterize some of the somatic genetic alterations occurring in *APC*-related FAP (Heinimann et al., 2001; Sieber et al., 2006) as well as in Lynch syndrome (Zhang et al., 2006). In the latter we also found its molecular hallmark, microsatellite instability (MSI) and loss of mismatch repair (MMR) expression, to be present in about 70% of breast cancer specimens from proven MMR mutation carriers which strongly suggests that MMR deficiency also plays a crucial role for breast cancer development in Lynch syndrome (Buerki et al., 2012). The genetic characterization of hereditary CRC patients provided the basis for observational studies on genetic anticipation (Westphalen et al., 2005) and genotype-phenotype correlations in Lynch syndrome (Kovac et al., 2011) as well as the prevalence of skin lesions in FAP patients (Burger et al., 2011). Very recently, we identified a novel MSI target locus, EWS16T, located in the 3’ untranslated region of the Ewing sarcoma breakpoint region 1 (*EWSR1*) gene, which identifies both, hereditary and sporadic, MMR-deficient cancers with perfect sensitivity and specificity (100%; n=319). As we could show, contractions therein affect multiple regulatory mechanisms implicating the RNA-/DNA-binding Ewing sarcoma protein in MSI-associated colorectal tumorigenesis (Kishore et al., *in press*).

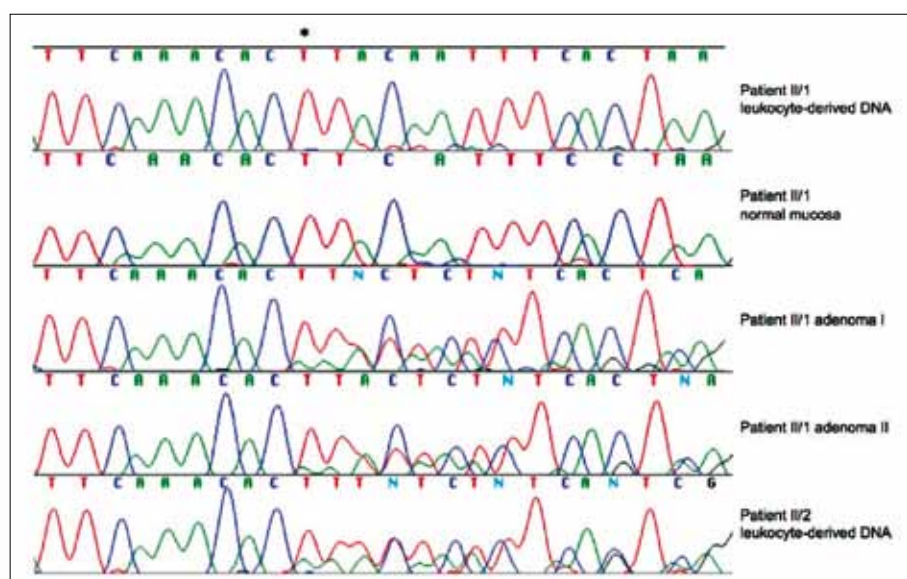


Figure 2: Sanger sequencing has been the preferred method of detecting small base pair mutations (single base exchanges, small deletions or duplications, etc.) since almost 30 years. This example shows the detection of a mutation causing colorectal cancer in the *APC* gene. It depicts sequencing of *APC* exon 15c in different tissues from patient II/1 and his daughter, patient II/2. The asterisks denote the start of the disease-causing four base pair deletion c.2802_2805delTTAC.

Common neuropsychiatric disorders

The World Health Organization (WHO) ranks many neuropsychiatric disorders, such as schizophrenia and affective disorders (bipolar disorder, major depression), among the largest contributors to the global burden of disease. Research has demonstrated that these disorders have a strong genetic component, and heritability estimates range between 50% and 80%. Environmental factors also play an important role. Furthermore, individuals with psychiatric disorders frequently display serious co-morbid medical disorders, such as cardiovascular disease. At present, causal therapy for psychiatric disorders is lacking, and pharmaceutical companies have shown increasing reluctance to develop new drug therapies due to the absence of biological markers and insufficient etiological data. Identification of the causal mechanisms that underlie psychiatric disorders is therefore of major medical and societal importance. Recent progress in our understanding of causal factors has been achieved through systematic genetic methods, including genome-wide association studies (GWAS) and genome-wide analysis of copy number variations (CNVs). This may mark an important turning point, which will stimulate continued pharmaceutical developments.

In the last six years, we have systematically sought after genetic risk factors for schizophrenia, bipolar disorder, and major depression by GWAS, in close collaboration with the Institute of Human Genetics at the University of Bonn, Germany (Prof. Markus M. Nöthen), and the Central Institute of Mental Health in Mannheim (Prof. Marcella Rietschel). This method uses DNA microarrays that allow to genotype hundreds of thousands of common single nucleotide polymorphisms (SNPs) across the whole genome within a reasonable time frame and at relatively low costs in a large number of patients and healthy controls and compare these SNPs between the groups. SNPs that are involved in the disease process (e.g. by changing the function or expression of certain genes in an allele- or genotype-dependent manner) show significant differences in genotype distributions between patients and healthy controls. Our recent GWAS of bipolar disorder is a successful example of this strategy (Cichon et al., 2011) and it identified genetic variation in the gene *neurocan* (*NCAN*) as contributing to the disease (Figure 3). The gene is highly expressed in cortical and hippocampal areas in mice, regions previously implicated in bipolar disorder in a variety of neuropsychological, neuroimaging, and postmortem studies. *NCAN* expression peaks during embryonal development

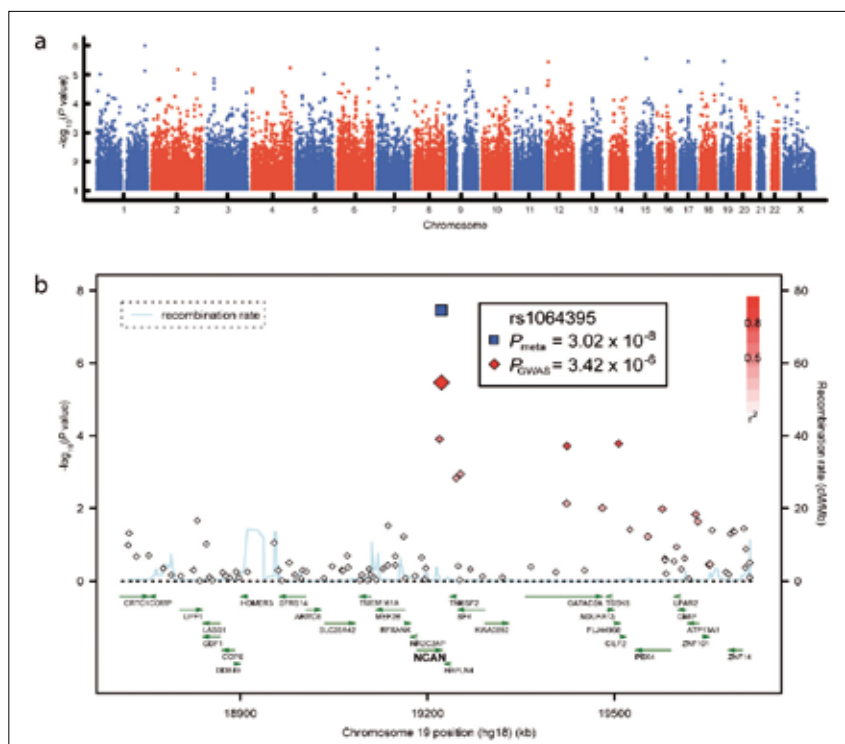


Figure 3: Results of a genome-wide association study (GWAS) for bipolar disorder. (a) Manhattan plot showing a genome-wide overview of association results for SNPs. The x-axis depicts all 23 chromosomes (chr. 1 – chr. X) that are investigated during a GWAS using hundreds of thousands of common SNPs. The y-axis shows the negative decadic logarithm of the p-value for each tested SNP (b) Regional association plots of the strongest associated region around the neurodevelopment gene *Neurocan* (*NCAN*) on chromosome 19. The most associated marker from the GWAS (enlarged red diamond) is centered in a genomic window of 1 Mb (hg18, RefSeq genes). The p-value of the same marker is given after inclusion of all replication samples (blue square). The linkage disequilibrium strength (r^2) between the sentinel SNP from the GWAS and its flanking markers is demonstrated by the red (high) to white (low) color bar. The recombination rate (cM/Mb; second y-axis) is plotted in blue, according to HapMap-CEU.

and significantly drops after birth. The gene product obviously plays a crucial role in adhesion and migration of neuronal cells (Cichon et al., 2011). In follow-up experiments, we performed genotype-phenotype correlations and explored the behavioural phenotype of *Ncan* knock-out mice (*Ncan*^{-/-}). Our results strongly suggest that the genetic risk variant in *NCAN* impacts on mania symptoms in humans (Miró et al., 2012). In further follow-up work, we could show that genetic variation in *NCAN* not only influences the risk of developing bipolar disorder but also schizophrenia (Mühleisen et al., 2012). These results suggest that there is a stronger genetic overlap between schizophrenia and bipolar disorder than previously thought. In fact, recent collaborative studies (including our own patient and control samples) that systematically looked for shared risk variants between five different common psychiatric disorders found that a relatively high proportion of specific risk variants confers a risk to different phenotypes (Cross-Disorder Group of the Psychiatric Genetics Consortium, 2013).

The identification and characterization of the *NCAN* gene in the context of neuropsychiatric disorders is just an example of the potential of modern human genomics concepts to identify genes for common diseases. Meanwhile, hundreds of common risk variants have already been identified in a large number of common diseases, and their number is constantly increasing. The data show that hundreds of common risk factors together explain a substantial proportion of the genetic basis of each common disease. It is expected that rare genetic variation explains most of the rest of the genetic basis. These rare variants are not represented on DNA microarrays used for GWAS, they can only be identified through systematic sequencing. With the development of new sequencing technologies (Next Generation Sequencing (NGS)), this work has recently become much faster and cheaper and it will become soon be feasible to generate whole genome sequences of many individuals in a short period of time at relatively low costs. We are now moving in this direction by using NGS technologies but also establishing newest third generation long read sequencing technology (Pacific Biosciences) in our group. Potential challenges of these methods, in particular the

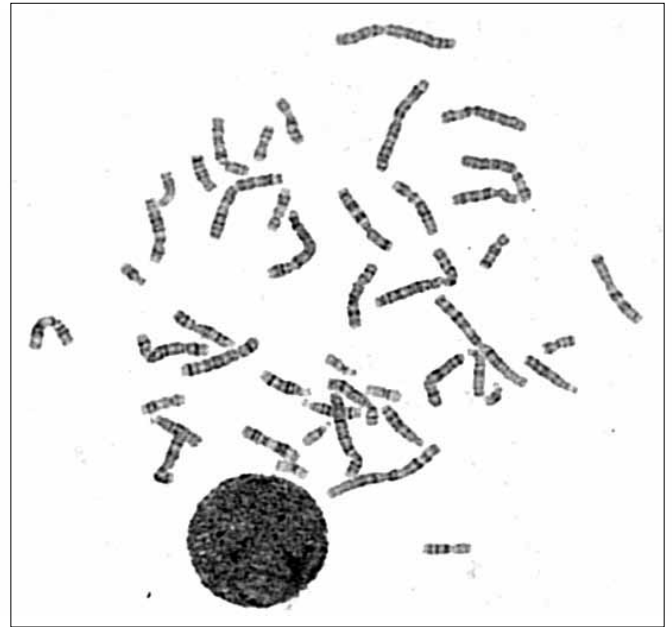


Figure 4: Human metaphase chromosomes, as appearing under the microscope after conventional chromosome preparation.

recognition of the disease-relevant rare variants among the large number of functionally neutral “background genetic variation” are currently being explored.

Another important aspect of our work is the functional characterization of risk variants. How do these variants contribute to disease development at the molecular level? In collaboration with the University of Bonn (Prof. Albert Becker) in Germany, we look into human hippocampus tissue and correlate gene expression and methylation patterns with DNA variants (SNPs, CNVs, rare single nucleotide variants). Together with the Research Center Jülich (Prof. Katrin Amunts), we perform “genomic imaging” studies that provide insights how risk variants for neuropsychiatric disorders influence brain structure and function in healthy individuals.

Clinical research with focus on neurodevelopmental delay or multiple congenital malformations

In the last decade, research in this area has focused on gene identification in developmental disorders (Multiple Congenital Anomalies (MCA), and Developmental Delay (DD)/Intellectual Disability (ID) phenotypes) by using novel genomic technologies such as DNA microarrays and, more recently, whole exome sequencing. We

also have a strong interest in translating those research approaches into clinical genomics diagnostic practice.

The availability of array-based technologies had significantly extended the resolution of classical cytogenetic methods such as microscopic chromosome analysis (see *Figure 4*) and led to the identification of submicroscopic chromosomal imbalances in patients with MCA and/or DD/ID. The development of array-based technologies provides a relatively rapid method to scan the genome for gains and losses of chromosomal material with significantly higher resolution, so-called copy number variants (CNVs), defined as deletions and duplications of DNA segments larger than 1000 bases (1 kb) and up to several Mb in size that are present in variable copy number compared with a reference genome. These have been shown to arise from dosage imbalance of one or more developmentally important genes, caused by structural rearrangements of the genome as a result of chromosomal microdeletions, microduplications, translocations or inversions. Diseases arising from such structural chromosomal rearrangements have been now designated “genomic disorders” or “pathogenic CNVs”. (for example, see *Figure 5*). They are considered to be a major cause of MCA/DD/ID, and we and others have shown that they collectively account for about 10–20% in MCA/DD/ID and autism spectrum disorders, depending on the clinical indication (Filges et al., 2012a). Thus, chromosomal microarrays have revolutionized studies on selected patients with chromosomal phenotypes and normal standard karyotypes offering the per-

spective of a new understanding of chromosomal disease. Within our past project on “Phenotype-genotype correlations of copy number variants” we were the first to define the developmental phenotype caused by a so far undescribed deletion of the *SETBP1*-gene on the long arm of chromosome 18 (18q12.3; Filges et al., 2011a). We could correlate loss of one entire copy (haploinsufficiency) of *SETBP1* with a unique phenotype with mild global developmental delay, a peculiar discrepancy between the absence of expressive language and conserved receptive speech and minor facial anomalies. This phenotype is entirely different and much milder than Schinzel-Giedion syndrome (SGS), a severe malformation syndrome, for which causative mutations in *SETBP1* were identified very recently through exome sequencing (Hoischen et al., 2010). We therefore delimit a phenotype for haploinsufficiency of *SETBP1* distinct from the phenotype of SGS in patients with mutations in the same gene, suggesting a gain-of function or a dominant negative effect of the mutations described. These reports thus illustrate the power of both approaches, array-methodologies for the detection of CNVs and exome sequencing for the detection of Mendelian mutations, and demonstrate how both can contribute to improved understanding of molecular mechanisms underlying the origin of congenital diseases. We were also among the first to identify *PTCHD1* as a candidate gene for ID and autism in a family compatible with X-linked inheritance (Filges et al., 2011b), to link a deletion of *LHX4* to pituitary agenesis and heart failure in a neonate (Filges et al., 2012b), and characterized one of the larg-

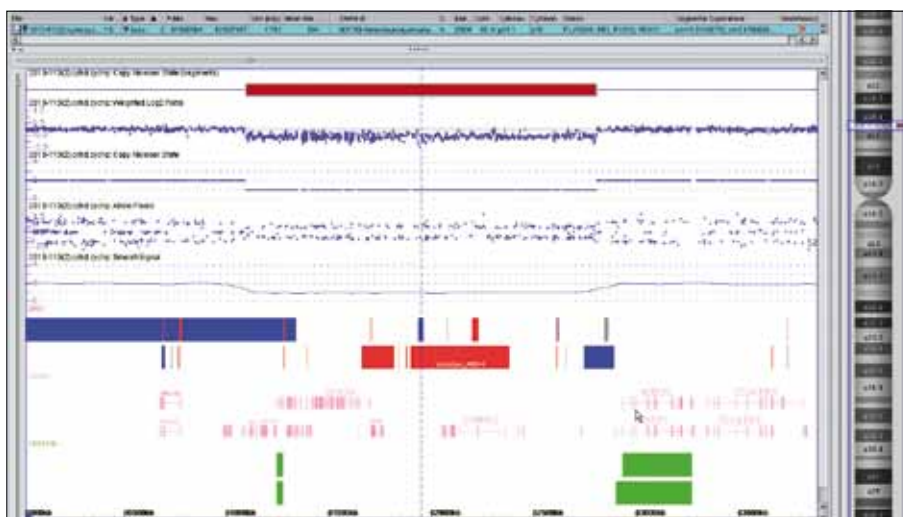


Figure 5: DNA microarray analysis of fetal DNA in search of small (submicroscopic) genetic changes, after sonographic diagnosis of fetal malformations. The right part shows chromosome 2 and the small part on the short arm that is blown up on the left. According to the signal intensity of the probes on the microarray, a de novo microdeletion of about 1.6 Mb (2p15p16.1) was noted (region marked by the red bar; genes included in the depicted chromosomal region are shown below).

est benign deletions without clinical consequences ever reported within the long arm of chromosome 13 (Filges et al., 2009).

Although chromosomal microarray analysis identified a previously unrecognized genetic cause in almost 20% of our patients with an unknown diagnosis, the cause of the MCA or DD/ID is still unknown in the remaining 80%. Most of these patients may have disorders that result from mutations in single genes that have not yet been identified. These families are candidates for various approaches using whole genome/exome sequencing for the identification of causal mutations in candidate genes. As described above, massively parallel sequencing technology is rapidly advancing and is capable of identifying high risk alleles with causative, diagnostic and prognostic relevance by using well selected patients with rare phenotypes. There is accumulating evidence that a spectrum of different alleles -rare variants of medium (or even strong) genetic effect together with common variants of small effect- make up the genetic architecture of many complex diseases. It is therefore hoped that sequencing will have implications for our understanding of both monogenic and complex diseases, with wide implications for health care.

As already mentioned above, data analysis is still a considerable challenge for whole exome/genome approaches. Bioinformatics analysis plays a key role in using the generated large-scale datasets, but besides the pitfalls encountered when calling the variants relevant to disease out of approximately 150.000 variants per individual, each analysis must be tailored to the particular clinical question, familial segregation and inheritance pattern. The identification of causal variants in novel genes is facilitated when several unrelated patients with the same phenotype are sequenced and data can be compared. Some of the patients indeed have a clinically recognizable phenotype, and we recently collaborated on an exome sequencing project identifying the gene for Nicolaides-Baraitser syndrome, a condition with severe impact on intellectual and physical development.

In most families, however, we are dealing with a rare individual disease or genetically heterogeneous diseases

challenging the interpretation of exome data. But we were able to show in a multidisciplinary approach that whole exome sequencing is useful in individual families with undiagnosed multiple congenital anomaly syndromes to discover responsible mutations, provided that prior to data analysis the phenotype can be correlated to a particular developmental pathway. We identified mutations in *KIF14* being causal for a novel lethal fetal malformation syndrome, supported by previous cellular and model organism studies of interacting *KIF14* partners. Studies in a zebrafish knockdown model are currently ongoing.

In the next 10 years the further characterization of CNVs as well as gene identification in monogenic and complex disorders using whole exome and genome sequencing will play a central role in Medical Genetics, and we are excited to work at the clinical and research boundaries to translate basic research into clinical application. Phenotype-genotype correlations using the precise observation and definition of phenotypes in combination with the molecular investigation of rare, often single, affected individuals will lead to the detection of new potentially causative genes for disorders yet to be identified and elucidate new molecular mechanisms, pathways and disease concepts. Novel approaches to detailed phenotyping, functional analysis, animal models and cross-species phenotyping will play an important role in the interpretation and proof of causality for variants in those genes. Sequencing of the genome and bioinformatics alone will not replace clinical approaches – the interpretation of next generation sequencing data also demands next generation phenotyping.

Overall, and this is true for all research areas pursued at Medical Genetics, the identification of rare and common disease alleles will provide valuable clues to the understanding and treatment of the diseases under study and are expected to have far-reaching implications for health care in the future.

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Clinical Genetics and Diagnostics at Medical Genetics

Medical Genetics (also termed Clinical Genetics in many countries) is a comparably young medical specialty committed to providing high quality genetic health care. This includes the evaluation and diagnosis of congenital anomalies and genetic diseases in fetuses, children and adults, genetic counselling regarding the risks of occurrence or recurrence of congenital and multiple congenital anomalies (MCA), developmental delays/intellectual disabilities (DD/ID) and other genetic diseases, as well as their distinction from non-genetic causes such as e.g. teratogens.

Medical Genetics in Basel has a longstanding expertise in the field, and we see patients, their families as well as at risk probands in our outpatient, inpatient and outreach consultations (at our current location in Basel on the Felix-Platter Spital area, through consultant activities at the University Hospital and University Children's Hospital Basel and Hospitals in neighbouring cantons). We also provide comprehensive diagnostic laboratory services in cytogenetics, molecular cytogenetics and molecular genetics. We maintain close relationships

with many other clinical specialties in Basel favouring interdisciplinary approaches in complex questions. Driven by our clinical involvement and the large number of unsolved questions as to the etiology and mechanisms of many developmental disorders in our patients we actively pursue research in this field.

Clinical molecular cytogenetics services

Based on research experience we have successfully transitioned microarrays to our clinical molecular cytogenetics services as a first-tier clinical diagnostic test for individuals with DD/ID/MCA and autism phenotypes in accordance with national and international standards and diagnostic test quality requirements. However, although the knowledge of CNVs is constantly increasing, the interpretation of a CNV's significance for an individual patient can be challenging, as a CNV can be pathogenic, benign or of so far unknown clinical significance. Precise clinical phenotyping is of hallmark importance to address these questions. We are collectively working with the international CNV community (DECIPHER and ISCA consortium) on those important issues.

In the section providing an overview of the research activities of the Division of Medical Genetics, we have already described the development of array-based technologies to scan the genome for CNVs, i.e. gains and losses of chromosomal material. As our division is the only center in Northwest-Switzerland to provide comprehensive genetic prenatal diagnosis services, we are obviously interested in evaluating genomic approaches in a prenatal setting while considering the specific needs of this sensitive field which significantly differs from postnatal care. We have worked intensively on the implementation of array approaches as an adjunct tool to conventional chromosome analysis in pregnancies at high risk for chromosomal anomalies. These patients are often undergoing invasive procedures (chorionic villous sampling or amniocentesis), but lack further diagnostic options when conventional chromosome count turns out to be normal. In the beginning experience in implementing array technology in prenatal diagnosis was limited, and important questions were unanswered

such as reasonable clinical indications, detection rate for pathogenic imbalances and CNVs with unknown significance, use of the appropriate platform and resolution as well as cost-benefit and standardisation aspects. The goal of our studies was to evaluate technical feasibility and the detection rate of pathogenic variants in relation to the rate of undesirable variants of unknown significance. The specific issue of placental mosaicism and its consequences for the diagnostic accuracy has been addressed. We can now offer array analysis if required, accompanied by individual counselling for patients. The field of prenatal care is becoming increasingly complex: the number of pregnancies with advanced maternal age and higher risk for chromosomal anomalies is high and constantly increasing, risk assessment using first trimester test approaches apply to any pregnancy independent of maternal age and significant advances in technologies – ultrasound for the detection of fetal anomalies as well as genomic technologies – offer unprecedented diagnostic options. These possibilities allow for a variety of risk assessment and diagnostic concepts, each with its advantages and limitations in individual situations. We work in close collaboration together with other specialists in prenatal and perinatal care at hospitals and private practice in order to provide personalized approaches to patients. Recently, non-invasive prenatal testing (NIPT) on cell-free fetal DNA in maternal blood has become an option as an advanced screening tool for the common aneuploidies, but experience is limited to pregnancies with increased risk. NIPT cannot replace current standards in prenatal diagnosis. The test is so far mainly provided by large international private laboratories/companies, but we are working on concepts which may allow an implementation in our local laboratories.

Tumorcytogenetics services

The tumorcytogenetic group within Medical Genetics is predominantly engaged in diagnostic procedures in hematological neoplasia; in contrast to the so called "constitutional" part of genetics (involving inherited mutations) these patients are mainly affected by somatic mutations. The continuously increasing knowl-

edge about clinical significance of underlying genetic anomalies on therapeutic decisions as well as on prognostic evaluation correlates with a similarly increasing relevance in tumorcytogenetic diagnostics (WHO 2008).

Conventional cytogenetic analysis based on microscopic evaluation of chromosomes used to be the gold standard for a long time. Approximately 15 years ago, however, additional molecular cytogenetic techniques were developed in routine diagnostics: fluorescence-in-situ-hybridisation (FISH) on metaphases as well as on interphase-nuclei and microarrays. Conventional cytogenetic analysis depends on successful in-vitro cell culture; this dependence may result in modified proliferation and over- or underestimation to the point of non-detection (false negative) of pathological cell clones. This technique provides a total overview on number and macrostructure of all chromosomes, allows detection of chromosomal rearrangements and of cellular clonality in relation to chromosomal aberration. The results are based on a comparatively low number of analysed cells (25 as standard) and resolution is limited to about 10 megabasepairs (i.e. 10 million basepairs), any aberration below this limit will not be detected.

As already described above, microarrays also provide a genome-wide overview about imbalances (deletions / duplications) at a much higher resolution, up to about 10 kb (depending on the platform and microarray type) as well as copy neutral loss of heterozygosity. Microarrays do not require cell culture and are therefore less prone to proliferation artefacts. They have one important disadvantage though: they can only detect the loss or gain of genetic material, balanced translocations or inversions are not detected. In our lab we routinely use the Affymetrix platform with the CytoScan HD array as well as the CytoScan 750k array.

FISH analysis, on the other hand, allows no genome-wide overview but enables to monitor specific chromosomal regions. Thus, there needs to be a hypothesis, before the FISH analysis starts, about the chromosomal locus to be analyzed. FISH on metaphase spreads allows visual location of the FISH-signal on particular chromosomes. This facilitates interpretation but underlies the

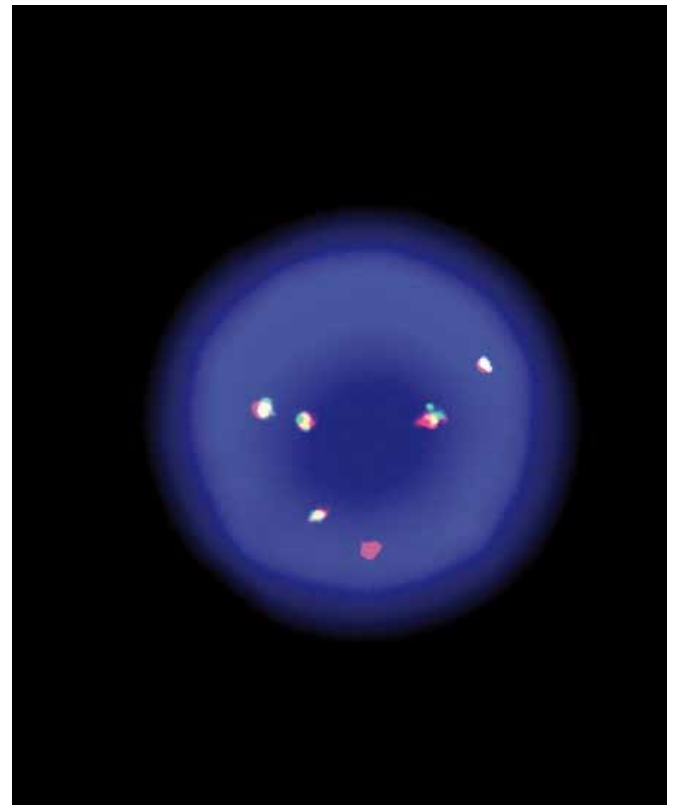


Figure 6: A specific chromosomal translocation between the long arms of chromosomes 11 and 14 is often found in mantle cell lymphoma. Here, the detection of the resulting fusion chromosomes (t(11;14)(q13;q32)) in a nucleus from a patient's bone marrow using interphase-FISH is shown. The red FISH-probe labels the gene CCND1 on 11q13, the green FISH-probe labels the gene IGH on 14q32. As a result of the fusion of these genomic regions by translocation, the red and green signals are observed together and create a mixed colour signal (yellow). It is of note that this particular bone marrow cell shows five fusion signals, indicative of an amplification of that particular genomic region.

limitations introduced by cultured cells, as mentioned above for conventional cytogenetics. FISH on interphase nuclei does not allow pinpointing of the signal to a specific chromosome because the chromosomes are unpacked and almost impossible to identify. This technique requires a larger number of analyzed cells as well as rigorous statistical thresholds to identify pathological cell clones. In turn, however, the detection of rearrangements is possible.

Many hematological neoplasia are associated not only with one specific genetic aberration, but with a higher number of possible genetic anomalies. (for example, see Figure 6). The type of genetic anomaly influences therapy and prognosis. In the lab, the combination of different techniques is often used today in order to increase

sensitivity, but it also means increased time and effort as well as increasing costs. From a clinical hematologic point of view, a test of a few key genetic aberrations may be sufficient in the actual clinical situation when a neoplasia is suspected. However, a complete genetic status is always necessary in case of follow-up examinations to monitor clonal evolution and clonal changes.

Our tumorcytogenetic diagnostics covers myeloid and lymphatic neoplasia with low prevalence to lymphoproliferative disorders. We receive patient samples (~90% bone marrow) from all over Switzerland with a main focus on the Basel area. Our daily challenge is the ambition to deliver high-quality and comprehensive reports within a minimal turn-around-time and to keep close contact and exchange information with the collaborating clinicians.

Molecular genetic diagnostics

Not least, our laboratory offers molecular genetic testing for a multitude of monogenic disorders. Besides analysis of common cancer predisposition genes, such as the mismatch repair (*MSH2*, *MLH1*, *MSH6* and *PMS2*) as well as the colon polyposis genes *APC* and *MUTYH*, the lab also performs routine testing for rare tumor syndromes such as Cowden (*PTEN*), Diffuse Gastric Cancer (*CDH1*), Juvenile Polyposis (*SMAD4*, *BMPR1A*), Li-Fraumeni (*TP53*), naevoid basal cell carcinoma (*PTCH1*), Peutz-Jeghers (*STK11*) and Von Hippel-Lindau (*VHL*) syndrome. Based on individual requests by the clinicians (mainly from in-house gynaecology, neurology, oto-rhino-laryngology, ophthalmology and UKBB pediatric units), we are continuing to establish, first on a research, then, following validation, on a routine basis, genetic testing for the phenotypically very heterogeneous groups of laminopathies (*LMNA*), myofibrillary myopathies (i.a. *DES*, *MYOT*), congenital hypothyroidism, oculopharyngeal muscular dystrophy (*PABPN1*), prelingual non-syndromic deafness (*GJB2*, *GJB6*) and rigid spine muscular dystrophy (*SEPN1*). Finally, the lab offers testing for genetic disorders related to short stature (a-/hypochondroplasia), neurological disorders (Angelman, Fragile X, Prader-Willi, Charcot-Marie-Tooth syndrome,

hereditary neuropathies), cystic fibrosis, infertility (*AZF*, *CFTR*, *SRY*, *ESR1*) and diverse syndromic disorders (i.a. *TWIST1*, *TRPS1*). Last not least, we provide DNA profiling (chimaerism following bone marrow transplantation, maternal contamination of prenatal samples) and make arrangements for genetic testing in other Swiss as well as foreign labs.

Sven Cichon and his team

Announcement



Department of Biomedicine
Research Day 2014
 Thursday, January 23, 08:15 – 12:45 h
 Kleiner Hörsaal, Zentrum für Lehre und Forschung
 Hebelstrasse 20, 4031 Basel

Speakers
 Bernhard Bettler
 Raphael Guzman
 Viola Heinzemann
 Giandomenica Iezzi
 Ivan Martin
 Nina Khanna
 Magdalena Müller-Gerbl
 Jürg Schwaller

Courtesy of Dr. Chiara Rotondo / Taylor lab

Wissenschaftliche Weihnachten

Krefeld. Achim Eickmeier, Professor für Experimentalphysik in Krefeld, bringt seinen Studenten die physikalischen Aspekte der Weihnachtszeit nahe. Eine unterhaltsame Vorlesung und präzise Antworten auf Fragen wie «Wie funktioniert die Sache mit dem Kamin?»

Alle Kinder, ob klein, ob groß, bekommen pünktlich zum Fest ihre Geschenke! Der Weihnachtsmann wird es wieder schaffen, alle Präsente pünktlich abzuliefern. Das ist wissenschaftlich bewiesen. Der Nachweis gelang Professor Dr. Achim Eickmeier von der Fachhochschule Niederrhein in Krefeld. Der 54-Jährige ist seit 1995 Professor für Experimentalphysik an der Fachhochschule Niederrhein in Krefeld.

Das ist sehr unterhaltsam. Der Forscher erklärt in seinen beliebten Weihnachtsvorlesungen seit Jahren Studenten vom Niederrhein «Die physikalischen Aspekte der Weihnachtszeit». Das macht er sehr witzig und anschaulich. In seinen Vorträgen gibt es endlich präzise Antworten auf alle ungeklärten Fragen rund ums Fest: Warum können Rentiere fliegen? Warum klappt die Zustellung der Geschenke immer so reibungslos? Und: Wie funktioniert die Sache mit dem Kamin?

822,6 Besuche pro Sekunde

«Es gibt zwei Milliarden Kinder auf der Welt, die Arbeit des Weihnachtsmannes konzentriert sich freilich auf 378 Millionen Christenkinder weltweit» referiert Eickmeier zu Beginn. «Das sind etwa 15% aller Kinder weltweit. Bei einem Arbeitstag von 31 Stunden (wegen der unterschiedlichen Zeitzonen) muss der Weihnachtsmann also 822,6 Besuche pro Sekunde absolvieren. Bleibt etwa ein Tausendstel Sekunde Zeit pro Kind: Parken, aus dem Schlitten springen, Kamin runter, Geschenke abladen, Kamin rauf, einsteigen, zum nächsten Haus fliegen.»

Der Professor redet – allen Ernstes – weiter: «Dabei legen Rentiere und Schlitten im Schnitt 1,3 Kilometer von Haus zu Haus zurück, insgesamt 120,8 Millionen Kilometer. In einer Sekunde rast der Weihnachtsmann also mit unvorstellbaren 1040 km/sec durch die Lüfte, der dreitausendfachen Schallgeschwindigkeit. Ein gewöhnliches Rentier schafft dagegen höchstens 24 Stundenkilometer...auf dem Boden.

Jeder Schlitten ist mit etwa 378 000 Tonnen Geschenken beladen. Ein Rentier zieht im wahren Leben im Schnitt aber

nur 175 Kilo pro Schlitten. Bei so viel Gewicht braucht Santa Claus also schon 216.000 Rentiere pro Schlitten.»

Professor Eickmeier ist ganz sicher: «Durch die rasende Geschwindigkeit entsteht ein ungeheurer Luftwiderstand, wie ein Shuttle, das in die Erdatmosphäre eintritt. Die armen Rentiere werden kräftig aufgeheizt.» Das vorderste Rentier muss etwa 16,6 Millionen Joule Energie absorbieren, hat der kluge Professor ausgerechnet: «Die ersten Rentiere würden sofort in Flammen aufgehen. Und es gibt einen ohrenbetäubenden Knall», so Eickmeier.

Naturgesetze ausser Kraft setzen

Und der Weihnachtsmann wird der 175.000 fachen Erdbeschleunigung ausgesetzt. «Mit seinen geschätzten 122 Kilo würde der gute Mann an das Ende seines Schlittens genagelt. Wenn der Weihnachtsmann alle Geschenke gebracht hat, ist er praktisch tot», folgert der Physik-Professor. «Das hat mich sehr erschüttert!» Wie ist das alles möglich? Ganz einfach: Die magische Weihnachtsformel, die der Weihnachtsmann Professor Eickmeier exklusiv ins Ohr flüsterte, macht es möglich. Doch diese Formel behält Eickmeier diskret für sich...

Eickmeier: «Das ist die Magie der Weihnacht. Wir können die Naturgesetze mit Hilfe der magischen Weihnachtsformel außer Kraft setzen.» Mit dieser Formel lässt sich auch erklären, so der Krefelder Forscher, warum der Schlitten des Weihnachtsmannes mit ungeheurer Schnelligkeit vom Himmel zur Erde saust. Eigentlich fallen alle Gegenstände in Europa nur mit schlappen 9,81 Metern pro Sekunde Geschwindigkeit zu Boden, so die physikalische Lehre. Eickmeier zeigt das an einem alten Tennisball. Anders der Schlitten: Im Versuch überholt ein Nachbau im Kleinformat den Tennisball und ist viel schneller am Ziel. Denn der Schlitten ist nicht nur schwerer. Er macht während seiner rasanten Sturzfahrt auch eine Drehbewegung, die ihn schneller werden lässt als der freie Fall des Balls.

Wie immer kommt der Erlös aus dem Eintritt der Vorlesung einem guten Zweck zu. Ganz im Sinne des Weihnachtsmannes. Übrigens: Bei seinen normalen Vorlesungen in Krefeld ist er ganz ernsthaft, der Professor Eickmeier.

(Aus der Westfälischen Allgemeinen Zeitung (WAZ))

Dissertationen

Am 12. August 2013 konnte **Daniela Schmid** von der Forschungsgruppe Neurobiology (Departement Biomedizin Hebelstrasse) ihre Dissertation mit Erfolg beenden. Sie befasste sich in ihrer Dissertation mit dem Thema "Functional role of the overexpression of the myelin and lymphocyte protein MAL in Schwann cell differentiation".

Seit dem 23. September 2013 darf sich **Paul Bourguine** von der Forschungsgruppe Tissue Engineering (ICFS/ Departement Biomedizin Hebelstrasse) Herr Dr. nennen. Er befasste sich in seiner Doktorarbeit mit dem Thema: "Combination of cell immortalization and apoptosis induction to engineer decellularized matrices as bone graft material".

Mit der Doktorprüfung am 24. September 2013 schloss **Sabrina Di Fulvio** von der Forschungsgruppe Neuromuscular Research (Departement Biomedizin Klingelbergstrasse) erfolgreich ihre Dissertationszeit ab. Das Thema ihrer Doktorarbeit lautete: "Characterization of the dysferlin protein and its binding partners reveals rational design for therapeutic strategies for the treatment of dysferlinopathies".

Am 22. Oktober 2013 stellte sich **Massimiliano Donzelli** von der Forschungsgruppe Clinical Pharmacology (Departement Biomedizin Hebelstrasse) dem Dissertationskomitee. Der Titel seiner Dissertation hiess: "Development, Validation and Applications of the Basel Phenotyping cocktail".

Auszeichnungen

Rolf Zeller in den Nationalen Forschungsrat gewählt

Rolf Zeller von der Forschungsgruppe Developmental Genetics (Departement Biomedizin Mattenstrasse) ist zum Mitglied des Nationalen Forschungsrats (Abteilung III: Biologie und Medizin) gewählt worden. Er tritt sein neues Amt zum 1. April 2014 an und wird für den Bereich Entwicklungsbiologie und Evolution zuständig sein.

Preise der Universität Basel an Michael Dill und Anne-Valerie Burgener

Am diesjährigen Dies Academicus hat **Michael Dill** von der Forschungsgruppe Hepatology (Departement Biomedizin Hebelstrasse) den Preis der Medizinischen Fakultät erhalten. **Anne-Valerie Burgener** von der Forschungsgruppe Immunobiology (Departement Biomedizin Hebelstrasse) durfte den Nachwuchsförderpreis der studentischen Verbindung "Schwizerhüsli" entgegen nehmen.

Herzliche Gratulation an alle!

Selected publications by DBM members

Below you can find the abstracts of recent articles published by members of the DBM. The abstracts are grouped according to the impact factor of the journal where the work appeared. To be included, the papers must meet the following criteria:

1. The first author, last author or corresponding author (at least one of them) is a member of the DBM.
2. The DBM affiliation must be mentioned in the authors list as it appeared in the journal.
3. The final version of the article must be available (online pre-publications will be included when the correct volume, page numbers etc. becomes available).

We are primarily concentrating on original articles. Due to page constraints, abstracts of publications that appeared in lower ranked journals may not be able to be included. Review articles are generally not considered, unless they appeared in the very top journals (e.g. Cell, Science, Nature, NEJM, etc.). The final decision concerning inclusion of an abstract will be made by the chair of the Department of Biomedicine.

If you wish that your article will appear in the next issue of DBM Facts please submit a pdf file to the Departmental Assistant, Manuela Bernasconi: manuela.bernasconi@unibas.ch

Deadline for the next issue is January 31, 2014.

Butyrophilin 3A1 binds phosphorylated antigens and stimulates human $\gamma\delta$ T cells

Stefano Vavassori^{1,*}, Anil Kumar^{2,*}, Gan Siok Wan², Gundimeda S Ramanjaneyulu¹, Marco Cavallari¹, Sary El Daker², Travis Beddoe³, Alex Theodossis³, Neal K Williams³, Emma Gostick⁴, David A Price⁴, Dinish U Soudamini⁵, Kong Kien Voon⁵, Malini Olivo⁵, Jamie Rossjohn^{3,4}, Lucia Mori^{1,2} & Gennaro De Libero^{1,2}

Human T cells that express a T cell antigen receptor (TCR) containing γ -chain variable region 9 and δ -chain variable region 2 (V γ 9V δ 2) recognize phosphorylated prenyl metabolites as antigens in the presence of antigen-presenting cells but independently of major histocompatibility complex (MHC), the MHC class I-related molecule MR1 and antigen-presenting CD1 molecules. Here we used genetic approaches to identify the molecule that binds and presents phosphorylated antigens. We found that the butyrophilin BTN3A1 bound phosphorylated antigens with low

affinity, at a stoichiometry of 1:1, and stimulated mouse T cells with transgenic expression of a human V γ 9V δ 2 TCR. The structures of the BTN3A1 distal domain in complex with host- or microbe-derived phosphorylated antigens had an immunoglobulin-like fold in which the antigens bound in a shallow pocket. Soluble V γ 9V δ 2 TCR interacted specifically with BTN3A1-antigen complexes. Accordingly, BTN3A1 represents an antigen-presenting molecule required for the activation of V γ 9V δ 2 T cells.

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* These authors contributed equally to this work.

Rapid effector function of memory CD8⁺ T cells requires an immediate-early glycolytic switch

Patrick M Gubser^{1,*}, Glenn R Bantug^{1,*}, Leyla Razik¹, Marco Fischer¹, Sarah Dimeloe¹, Gideon Hoenger¹, Bojana Durovic¹, Annaïse Jauch¹ & Christoph Hess¹

Antigen-experienced memory T cells acquire effector function with innate-like kinetics; however, the metabolic requirements of these cells are unknown. Here we show that rapid interferon- γ (IFN- γ) production of effector memory (EM) CD8⁺ T cells, activated through stimulation mediated by the T cell antigen receptor (TCR) and the costimulatory receptor CD28 or through cognate interactions, was linked to increased glycolytic flux. EM CD8⁺ T cells exhibited more glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity at early time points, before proliferation com-

menced, than did naive cells activated under similar conditions. CD28 signaling via the serine-threonine kinase Akt and the metabolic-checkpoint kinase mTORC2 was needed to sustain TCR-mediated immediate-early glycolysis. Unlike glycolysis in proliferating cells, immediate-early glycolysis in memory CD8⁺ T cells was rapamycin insensitive. Thus, CD8⁺ memory T cells have an Akt-dependent 'imprinted' glycolytic potential that is required for efficient immediate-early IFN- γ recall responses.

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* These authors contributed equally to this work.

Sox4 Is a Master Regulator of Epithelial-Mesenchymal Transition by Controlling Ezh2 Expression and Epigenetic Reprogramming

Neha Tiwari^{1,4}, Vijay K. Tiwari^{2,5}, Lorenz Waldmeier¹, Piotr J. Balwiercz³, Phil Arnold³, Mikhail Pachkov³, Nathalie Meyer-Schaller¹, Dirk Schübeler², Erik van Nimwegen³ and Gerhard Christofori¹

SUMMARY

Gene expression profiling has uncovered the transcription factor Sox4 with upregulated activity during TGF- β -induced epithelial-mesenchymal transition (EMT) in normal and cancerous breast epithelial cells. Sox4 is indispensable for EMT and cell survival in vitro and for primary tumor growth and metastasis in vivo. Among several EMT-relevant genes, Sox4 directly regulates the expression of *Ezh2*, encoding the Polycomb group histone methyltransferase that trimethylates histone 3 lysine 27 (H3K-

27me3) for gene repression. Ablation of *Ezh2* expression prevents EMT, whereas forced expression of *Ezh2* restores EMT in Sox4-deficient cells. *Ezh2*-mediated H3K27me3 marks associate with key EMT genes, representing an epigenetic EMT signature that predicts patient survival. Our results identify Sox4 as a master regulator of EMT by governing the expression of the epigenetic modifier *Ezh2*.

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PKC β Phosphorylates PI3K γ to Activate It and Release It from GPCR Control

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Abstract

All class I phosphoinositide 3-kinases (PI3Ks) associate tightly with regulatory subunits through interactions that have been thought to be constitutive. PI3K γ is key to the regulation of immune cell responses activated by G protein-coupled receptors (GPCRs). Remarkably we find that PKC β phosphorylates Ser582 in the helical domain of the PI3K γ catalytic subunit p110 γ in response to clustering of the high-affinity IgE receptor (Fc ϵ RI) and/or store-operated Ca²⁺-influx in mast cells. Phosphorylation of p110 γ correlates with the release of the p84 PI3K γ adapter subunit from the p84-p110 γ complex. Ser582 phospho-mimicking mutants show

increased p110 γ activity and a reduced binding to the p84 adapter subunit. As functional p84-p110 γ is key to GPCR-mediated p110 γ signaling, this suggests that PKC β -mediated p110 γ phosphorylation disconnects PI3K γ from its canonical inputs from trimeric G proteins, and enables p110 γ to operate downstream of Ca²⁺ and PKC β . Hydrogen deuterium exchange mass spectrometry shows that the p84 adaptor subunit interacts with the p110 γ helical domain, and reveals an unexpected mechanism of PI3K γ regulation. Our data show that the interaction of p110 γ with its adapter subunit is vulnerable to phosphorylation, and outline a novel level of PI3K control.

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Transient targeting of phosphoinositide 3-kinase acts as a roadblock in mast cells' route to allergy

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Background: Tissue mast cell numbers are dynamically regulated by recruitment of progenitors from the vasculature. It is unclear whether progenitors are recruited during allergic sensitization and whether recruitment promotes allergic responses.

Objective: We sought to (1) determine the effect of mast cell recruitment on acute allergic responses and (2) to define the role of phosphoinositide 3-kinase (PI3K) isoforms in sequential steps to allergic responses.

Methods: Gene-targeted mice for PI3K γ or PI3K δ or mice treated with isoform-specific PI3K inhibitors (a novel PI3K γ -specific inhibitor [NVS-PI3-4] and the PI3K δ inhibitor IC87114) were used to monitor IgE-mediated mast cell recruitment, migration, adhesion by means of intravital microscopy, degranulation, TNF- α release, and subsequent endothelial cell activation *in vivo* or in bone marrow-derived mast cells.

Results: Functional PI3K γ , but not PI3K δ , was crucial for mast cell accumulation in IgE-challenged skin, TNF- α release from IgE/antigen-stimulated mast cells, and mast cell/endothelial interactions and chemotaxis. PI3K γ -deficient bone marrow-derived mast cells did not adhere to the endothelium in TNF- α -treated cremaster muscle, whereas PI3K δ was not required. Depletion of TNF- α blocked IgE-induced mast cell recruitment,

which links tissue mast cell-derived cytokine release to endothelial activation and mast cell recruitment. Interference with mast cell recruitment protected against anaphylaxis and was superior to blockage of tissue mast cell degranulation.

Conclusions: Interference with mast cell recruitment to exacerbated tissues provides a novel strategy to alleviate allergic reactions and surpassed attenuation of tissue mast cell degranulation. This results in prolonged drug action and allows for reduction of drug doses required to block anaphylaxis, an important feature for drugs targeting inflammatory disease in general. (J Allergy Clin Immunol 2013.)

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Homing frequency of human T cells inferred from peripheral blood depletion kinetics after sphingosine-1-phosphate receptor blockade

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Naive and central memory (CM) T cells home through lymph nodes (LNs), whereas T cells with an effector memory (EM) phenotype preferentially screen peripheral tissues in search of cognate antigen.¹ LN entry and egress are distinct and highly regulated processes mediated by an orchestrated interplay of chemokines/chemokine receptors and adhesion molecules.² Interaction of peripheral node addressins with L-selectin on T cells allows tethering/rolling along high endothelial venules (HEVs).² Interaction of the chemokine receptor CCR7 with its ligands CCL19/CCL21 and CXCR4 with CXCL12 then mediates firm adhesion to HEVs through high-affinity interactions of lymphocyte function-associated antigen 1

and intercellular adhesion molecule 1, permitting transmigration of T cells across the HEV cell layer.² Within the LNs, T-cell migration is directed through T-cell zones toward the cortical sinuses.³ Sphingosine-1-phosphate (S1P) gradient established across the endothelial cells of the cortical sinuses is directing LN egress of T cells through efferent lymph back to the peripheral blood circulation.⁴ Acting as a functional antagonist on the S1P receptor, the pharmacologic compound fingolimod, which has shown efficacy in the treatment of multiple sclerosis (MS), blocks this egress.^{4,5} As a consequence, in fingolimod-treated subjects naive and CM T cells are trapped in LNs and reduced in the blood circulation.⁶

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Impairing MLL-fusion gene-mediated transformation by dissecting critical interactions with the lens epithelium-derived growth factor (LEDGF/p75)

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The lens epithelium-derived growth factor (LEDGF/p75) tethers the mixed-lineage leukemia (MLL1) protein complex to chromatin. Likewise, LEDGF/p75 tethers the HIV-1 pre-integration complex to chromatin. We previously demonstrated that expression of the C-terminal fragment fused to enhanced green fluorescent protein (eGFP) (eGFP-LEDGF_{325–530}) impaired HIV-1 replication. Here, we explored this strategy to selectively interfere with the leukemogenic activity of MLL-fusion proteins. We found that expression of LEDGF_{325–530} impaired the clonogenic growth of MLL-fusion gene transformed human and mouse hematopoietic cells, without affecting the growth of control cells immortalized by the FLT3-ITD mutant or normal lineage-marker-depleted murine bone marrow cells.

Expression of LEDGF_{325–530} was associated with downregulation of the MLL target Hoxa9 and impaired cell cycle progression. Structure-function analysis revealed two small eGFP-fused LEDGF/p75 peptides, LEDGF_{424–435} and LEDGF_{375–386} phenocopying these effects. Both LEDGF_{325–530} and the smaller active peptides were able to disrupt the LEDGF/p75–MLL interaction. Expression of LEDGF_{325–530} or LEDGF_{375–386} fragments increased the latency period to disease development *in vivo* in a mouse bone marrow transplant model of MLL–AF9-induced AML. We conclude that small peptides disrupting the LEDGF/p75–MLL interface have selective anti-leukemic activity providing a direct rationale for the design of small molecule inhibitors targeting this interaction.

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CXCR4-SERINE339 regulates cellular adhesion, retention and mobilization, and is a marker for poor prognosis in acute myeloid leukemia

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The CXCR4 receptor is a major regulator of hematopoietic cell migration. Overexpression of CXCR4 has been associated with poor prognosis in acute myelogenous leukemia (AML). We have previously shown that ligand-mediated phosphorylation of the Serine339 (CXCR4-S339) residue of the intracellular domain by PIM1 is implicated in surface re-expression of this receptor. Here, we report that phosphorylation of CXCR4-S339 in bone marrow (BM) biopsies correlated with poor prognosis in a cohort of AML patients. To functionally address the impact of CXCR4-S339 phosphorylation, we generated cell lines-expressing CXCR4 mutants that mimic constitutive phosphorylation (S339E) or abrogate phosphorylation (S339A). Whereas the expression of CXCR4 significantly increased, both

CXCR4-S339E and the CXCR4-S339A mutants significantly reduced the BM homing and engraftment of Kasumi-1 AML cells in immunodeficient mice. In contrast, only expression of the CXCR4-S339E mutant increased the BM retention of the cells and resistance to cytarabine treatment, and impaired detachment capacity and AMD3100-induced mobilization of engrafted leukemic cells. These observations suggest that the poor prognosis in AML patients displaying CXCR4-S339 phosphorylation can be the consequence of an increased retention to the BM associated with an enhanced chemoresistance of leukemic cells. Therefore, CXCR4-S339 phosphorylation could serve as a novel prognostic marker in human AML.

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A Versatile Toolkit to Produce Sensitive FRET Biosensors to Visualize Signaling in Time and Space

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Genetically encoded, ratiometric biosensors based on fluorescence resonance energy transfer (FRET) are powerful tools to study the spatiotemporal dynamics of cell signaling. However, many biosensors lack sensitivity. We present a biosensor library that contains circularly permuted mutants for both the donor and acceptor fluorophores, which alter the orientation of the dipoles and thus better accommodate structural constraints imposed by different signaling molecules while maintaining FRET efficiency. Our strategy improved the brightness and dynamic range of preexisting RhoA and extracellular signal-regulated protein kinase (ERK) biosensors. Using the improved RhoA biosensor, we found micrometer-

sized zones of RhoA activity at the tip of F-actin bundles in growth cone filopodia during neurite extension, whereas RhoA was globally activated throughout collapsing growth cones. RhoA was also activated in filopodia and protruding membranes at the leading edge of motile fibroblasts. Using the improved ERK biosensor, we simultaneously measured ERK activation dynamics in multiple cells using low-magnification microscopy and performed in vivo FRET imaging in zebrafish. Thus, we provide a construction toolkit consisting of a vector set, which enables facile generation of sensitive biosensors.

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Tissue decellularization by activation of programmed cell death

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Abstract

Decellularized tissues, native or engineered, are receiving increasing interest in the field of regenerative medicine as scaffolds or implants for tissue and organ repair. The approach, which offers the opportunity to deliver off-the-shelf bioactive materials without immuno-matching requirements, is based on the rationale that extracellular matrix (ECM)-presented cues can be potentially instructive towards regeneration. However, existing decellularization protocols typically result in damage to the source ECM and do not allow the controlled preservation of its structural, biochemical and/or biomechanical features. Here we propose the deliber-

ate activation of programmed cell death as a method to selectively target the cellular component of a tissue and thereby to preserve the integrity of the decellularized ECM. In the case of engineered tissues, the approach could be complemented by the use of (i) an immortalized cell line, engineered to undergo apoptosis upon exposure to a chemical inducer, and (ii) a perfusion bioreactor system, supporting efficient removal of cellular material. The combination of these tools may lead to the streamlined development of more appropriate materials, based on engineered and decellularized ECM and including a customized set of signals specifically designed to activate endogenous regenerative processes.

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Effects of Chenodeoxycholic Acid on the Secretion of Gut Peptides and Fibroblast Growth Factors in Healthy Humans

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Context: Recent evidence suggests bile acids (BAs) are involved in the glycemic control via TGR5 activation with the subsequent release of gut peptides and farnesoid X receptor activation with ensuing release of fibroblast growth factors (FGFs).

Objective: We hypothesized that intraduodenal infusions of chenodeoxycholic acid (CDCA) would stimulate FGF and gut peptide secretion, thereby positively influencing glucose homeostasis.

Design, Setting, Participants, and Intervention: This randomized, double-blind, placebo-controlled, crossover trial included 12 healthy volunteers who received intraduodenal infusions (2.0 mL/min for 180 minutes) of saline, CDCA (5 or 15 mmol/L), and a fatty acid (sodium oleate), either alone or with 5 mmol/L CDCA. After 60 minutes, an oral glucose tolerance test (oGTT) was performed.

Main Outcome Measures: Plasma levels of glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine, cholecystokinin (CCK), total BAs, FGF19, FGF21, C-peptide, insulin, glucose, and glucagon were measured.

Results: Within the first 60 minutes, high-concentration CDCA induced a small but significant increase in GLP-1 and CCK secretion ($P = .016$ and $P = .011$), whereas plasma C-peptide, insulin, and glucose were not affected. Attenuated C-peptide and insulin release was observed after the oGTT with 15 mmol/L CDCA ($P = .013$ and $P = .011$). Plasma BA and FGF19 levels significantly increased after CDCA administration ($P = .001$ and $P < .001$).

Conclusions: CDCA modulates GLP-1 and CCK secretion; the effect is small and does not influence glucose levels. The marked increase in plasma BAs and the attenuated insulin release after the oGTT indicate the role of BAs in glycemic control, independent of the incretin axis, and suggest involvement of farnesoid X receptor activation pathways.

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Molecular Imaging Reveals Rapid Reduction of Endothelial Activation in Early Atherosclerosis With Apocynin Independent of Antioxidative Properties

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Objective—Antioxidative drugs continue to be developed for the treatment of atherosclerosis. Apocynin is a nicotinamide adenine dinucleotide phosphate oxidase inhibitor with anti-inflammatory properties. We used contrast-enhanced ultrasound molecular imaging to assess whether short-term apocynin therapy in atherosclerosis reduces vascular oxidative stress and endothelial activation

Approach and Results—Genetically modified mice with early atherosclerosis were studied at baseline and after 7 days of therapy with apocynin (4 mg/kg per day IP) or saline. Contrast-enhanced ultrasound molecular imaging of the aorta was performed with microbubbles targeted to vascular cell adhesion molecule 1 (VCAM-1; MB_V), to platelet glycoprotein Iba₁ (MB_{PI}), and control microbubbles (MB_{Ctrl}). Aortic vascular cell adhesion molecule 1 was measured using Western blot. Aortic reactive oxygen species generation was measured using a lucigenin assay. Hydroethidine oxidation was used to assess aortic superoxide generation. Baseline signal for MB_V (1.3±0.3 AU) and MB_{PI} (1.5±0.5 AU) was higher than for MB_{Ctrl} (0.5±0.2 AU; $P<0.01$). In saline-treated animals, signal did not significantly change for any microbubble agent, whereas short-term apocynin significantly

($P<0.05$) reduced vascular cell adhesion molecule 1 and platelet signal (MB_V: 0.3±0.1; MB_{PI}: 0.4±0.1; MB_{Ctrl}: 0.3±0.2 AU; $P=0.6$ between agents). Apocynin reduced aortic vascular cell adhesion molecule 1 expression by 50% ($P<0.05$). However, apocynin therapy did not reduce reactive oxygen species content, superoxide generation, or macrophage content.

Conclusions—Short-term treatment with apocynin in atherosclerosis reduces endothelial cell adhesion molecule expression. This change in endothelial phenotype can be detected by molecular imaging before any measurable decrease in macrophage content and is not associated with a detectable change in oxidative burden.

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Pharmacokinetics and Pharmacodynamics of Recombinant Human Angiotensin-Converting Enzyme 2 in Healthy Human Subjects

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Abstract

Background and Objectives Angiotensin-converting enzyme 2 (ACE2) converts angiotensin II (Ang1-8) to angiotensin 1-7 (Ang1-7), a functional antagonist of Ang1-8, with vasodilatory, antiproliferative, antiangiogenic, and anti-inflammatory properties. In conditions with an unbalanced renin-angiotensin-aldosterone system with elevated Ang1-8, administration of ACE2 has shown promising effects in a variety of animal models. Enhancing ACE2 activity by exogenous administration of ACE2 might also be beneficial in human diseases with pathologically elevated Ang1-8. As a first step we performed a first-in-man study to determine pharmacokinetics, pharmacodynamics, safety, and tolerability of recombinant ACE2 in healthy volunteers. **Methods** Recombinant human ACE2 (rhACE2) was administered intravenously to healthy human subjects in a randomized, double-blind, placebo-controlled, single-dose, dose-escalation study followed by an open-label multiple-dose study. ACE2 concentrations were determined by quantifying ACE2 activity and ACE2 content in plasma samples. Concentrations of the angiotensin system effector peptides Ang1-8, Ang1-7, and Ang1-5 were determined using a liquid chromatography–tandem mass spectrometry method. **Results** Single rhACE2 doses of 100–1,200 µg/kg caused a dose-dependent

increase of systemic exposure with biphasic elimination and adose-independent terminal half-life of 10 h. In all single-dose cohorts, Ang1-8 decreased within 30 min postinfusion, angiotensin 1-7 (Ang1-7) either increased (100 and 200 µg/kg doses), decreased, or remained unchanged (400–1,200 µg/kg doses), whereas angiotensin1-5 (Ang1-5) transiently increased for all doses investigated. With the exception of the lowest rhACE2 dose, the decrease in Ang1-8 levels lasted for at least 24 h. Repeated dosing (400 µg/kg for 3 or 6 days) caused only minimal accumulation of ACE2, and Ang1-8 levels were suppressed over the whole application period.

Conclusions Administration of rhACE2 was well tolerated by healthy human subjects. Exposure was dose dependent with a dose-independent terminal elimination half-life in the range of 10 h. Despite marked changes in angiotensin system peptide concentrations, cardiovascular effects were absent, suggesting the presence of effective compensatory mechanisms in healthy volunteers.

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Gain of function in the immune system caused by a ryanodine receptor 1 mutation

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Summary

Mutations in *RYR1*, the gene encoding ryanodine receptor 1, are linked to a variety of neuromuscular disorders including malignant hyperthermia (MH), a pharmacogenetic hypermetabolic disease caused by dysregulation of Ca^{2+} in skeletal muscle. *RYR1* encodes a Ca^{2+} channel that is predominantly expressed in skeletal muscle sarcoplasmic reticulum, where it is involved in releasing the Ca^{2+} necessary for muscle contraction. Other tissues, however, including cells of the immune system, have been shown to express ryanodine receptor 1; in dendritic cells its activation leads to increased surface expression of major histocompatibility complex II molecules and provides synergistic signals leading to cell maturation. In the present study, we investigated the impact of an MH mutation

on the immune system by studying the *RYR1*_{Y522S} knock-in mouse. Our results show that there are subtle but significant differences both in resting 'non-challenged' mice as well as in mice treated with antigenic stimuli, in particular the knock-in mice: (i) have dendritic cells that are more efficient at stimulating T cell proliferation, (ii) have higher levels of natural IgG₁ and IgE antibodies, and (iii) are faster and more efficient at mounting a specific immune response in the early phases of immunization. We suggest that some gain-of-function MH-linked *RYR1* mutations might offer selective immune advantages to their carriers. Furthermore, our results raise the intriguing possibility that pharmacological activation of RyR1 might be exploited for the development of new classes of vaccines and adjuvants.

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Non-invasive neural stem cells become invasive *in vitro* by combined FGF2 and BMP4 signaling

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Summary

Neural stem cells (NSCs) typically show efficient self-renewal and selective differentiation. Their invasion potential, however, is not well studied. In this study, Sox2-positive NSCs from the E14.5 rat cortex were found to be non-invasive and showed only limited migration *in vitro*. By contrast, FGF2-expanded NSCs showed a strong migratory and invasive phenotype in response to the combination of FGF2 and BMP4. Invasive NSCs expressed Podoplanin (PDPN) and p75NGFR (Ngfr) at the plasma membrane after exposure to FGF2 and BMP4. FGF2 and BMP4 together upregulated the expression of *Msx1*, *Snail1*, *Snail2*, *Ngfr*, which are all found in neural crest (NC) cells during or after epithelial–mesenchymal transition (EMT), but not in forebrain stem cells. Invasive cells downregulated the expression of *Olig2*, *Sox10*, *Egfr*, *Pdgfra*, *Gsh1/Gsx1* and *Gsh2/Gsx2*. Migrating and

invasive NSCs had elevated expression of mRNA encoding Pax6, Tenascin C (TNC), PDPN, Hey1, SPARC, p75NGFR and Gli3. On the basis of the strongest upregulation in invasion-induced NSCs, we defined a group of five key invasion-related genes: *Ngfr*, *Sparc*, *Snail1*, *Pdpn* and *Tnc*. These genes were co-expressed and upregulated in seven samples of glioblastoma multiforme (GBM) compared with normal human brain controls. Induction of invasion and migration led to low expression of differentiation markers and repressed proliferation in NSCs. Our results indicate that normal forebrain stem cells have the inherent ability to adopt a glioma-like invasiveness. The results provide a novel *in vitro* system to study stem cell invasion and a novel glioma invasion model: tumoral abuse of the developmental dorsoventral identity regulation.

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Hepatocellular toxicity of clopidogrel: Mechanisms and risk factors

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Abstract

Clopidogrel is a prodrug used widely as a platelet aggregation inhibitor. After intestinal absorption, approximately 90% is converted to inactive clopidogrel carboxylate and 10% via a two-step procedure to the active metabolite containing a mercapto group. Hepatotoxicity is a rare but potentially serious adverse reaction associated with clopidogrel. The aim of this study was to find out the mechanisms and susceptibility factors for clopidogrel-associated hepatotoxicity. In primary human hepatocytes, clopidogrel (10 and 100 μ M) was cytotoxic only after cytochrome P450 (CYP) induction by rifampicin. Clopidogrel (10 and 100 μ M) was also toxic for HepG2 cells expressing human CYP3A4 (HepG2/CYP3A4) and HepG2 cells co-incubated with CYP3A4 supersomes (HepG2/CYP3A4 supersome), but not for wildtype HepG2 cells (HepG2/wt). Clopidogrel (100 μ M) decreased the cellular glutathione content in HepG2/ CYP3A4 su-

persome and triggered an oxidative stress reaction (10 and 100 mM) in HepG2/CYP3A4, but not in HepG2/wt. Glutathione depletion significantly increased the cytotoxicity of clopidogrel (10 and 100 μ M) in HepG2/ CYP3A4 supersome. Co-incubation with 1 μ M ketoconazole or 10 mM glutathione almost completely prevented the cytotoxic effect of clopidogrel in HepG2/CYP3A4 and HepG2/CYP3A4 supersome. HepG2/CYP3A4 incubated with 100 μ M clopidogrel showed mitochondrial damage and cytochrome c release, eventually promoting apoptosis and/or necrosis. In contrast to clopidogrel, clopidogrel carboxylate was not toxic for HepG2/wt or HepG2/CYP3A4 up to 100 μ M. In conclusion, clopidogrel incubated with CYP3A4 is associated with the formation of metabolites that are toxic for hepatocytes and can be trapped by glutathione. High CYP3A4 activity and low cellular glutathione stores may be risk factors for clopidogrel-associated hepatocellular toxicity.

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Epstein-Barr Virus Negativity among Individuals Older than 60 Years Is Associated with HLA-C and HLA-Bw4 Variants and Tonsillectomy

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Epstein-Barr virus (EBV) infects ~95% of the adult population. The factors that confer protection in the remaining ~5% remain unknown. In an exploratory study, we assessed immunogenetic factors and tonsillectomy in a cohort of 17 EBV-negative and 39 EBV-positive healthy individuals aged >60 years. Analyses of HLA genotypes revealed an association between EBV negativity and the presence of HLA-C-35T/T and/or HLA-Bw4 alleles. In addition, EBV-negative donors presented with a history of tonsillectomy more often than EBV-positive donors.

For most, primary Epstein-Barr virus (EBV) infection occurs during childhood and is asymptomatic or causes an acute self-limiting lymphoprolif-

erative disease (infectious mononucleosis). After acute infection, EBV enters life-long latency, which leaves the infected individual at risk for viral reactivation and, in rare cases, the development of EBV-associated malignancy (1). Why ~5% of the adult population remain EBV-seronegative throughout their lives is not known, yet understanding natural resistance to EBV infection might provide fundamental insight into the host-pathogen interaction and pinpoint targets for novel preventive and/or therapeutic strategies.

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Priming 3D Cultures of Human Mesenchymal Stromal Cells Toward Cartilage Formation Via Developmental Pathways

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The field of regenerative medicine has increasingly recognized the importance to be inspired by developmental processes to identify signaling pathways crucial for 3D organogenesis and tissue regeneration. Here, we aimed at recapitulating the first events occurring during limb development (ie, cell condensation and expansion of an undifferentiated mesenchymal cell population) to prime 3D cultures of human bone marrow-derived mesenchymal stromal/stem cells (hBM-MSC) toward the chondrogenic route. Based on embryonic development studies, we hypothesized that Wnt3a and fibroblast growth factor 2 (FGF2) induce hBM-MSC to proliferate in 3D culture as an undifferentiated pool of progenitors (defined by clonogenic capacity and expression of typical markers), retaining chondrogenic potential upon induction by suitable morphogens. hBM-MSC were responsive to Wnt signaling in 3D pellet culture, as

assessed by significant upregulation of main target genes and increase of unphosphorylated β -catenin levels. Wnt3a was able to induce a five-fold increase in the number of proliferating hBM-MSC (6.4% vs. 1.3% in the vehicle condition), although total DNA content of the 3D construct was decreasing over time. Preconditioning with Wnt3a improved transforming growth factor- β 1 mediated chondrogenesis (30% more glycosaminoglycans/cell in average). In contrast to developmental and 2D MSC culture models, FGF2 antagonized the Wnt-mediated effects. Interestingly, the CD146⁺ subpopulation was found to be more responsive to Wnt3a. The presented data indicate a possible strategy to prime 3D cultures of hBM-MSC by invoking a “developmental engineering” approach. The study also identifies some opportunities and challenges to cross-fertilize skeletal development models and 3D hBM-MSC culture systems.

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Establishment of a human skeletal muscle-derived cell line: biochemical, cellular and electrophysiological characterization

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Excitation–contraction coupling is the physiological mechanism occurring in muscle cells whereby an electrical signal sensed by the dihydropyridine receptor located on the transverse tubules is transformed into a chemical gradient (Ca^{2+} increase) by activation of the ryanodine receptor located on the sarcoplasmic reticulum membrane. In the present study, we characterized for the first time the excitation–contraction coupling machinery of an immortalized human skeletal muscle cell line. Intracellular Ca^{2+} measurements showed a normal response to pharmacological activation of the ryanodine receptor, whereas 3D-SIM (super-resolution structured illumination microscopy) revealed a low level of structural or-

ganization of ryanodine receptors and dihydropyridine receptors. Interestingly, the expression levels of several transcripts of proteins involved in Ca^{2+} homeostasis and differentiation indicate that the cell line has a phenotype closer to that of slow-twitch than fast-twitch muscles. These results point to the potential application of such human muscle-derived cell lines to the study of neuromuscular disorders; in addition, they may serve as a platform for the development of therapeutic strategies aimed at correcting defects in Ca^{2+} homeostasis due to mutations in genes involved in Ca^{2+} regulation.

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Up-regulation of GABA_B Receptor Signaling by Constitutive Assembly with the K⁺ Channel Tetramerization Domain-containing Protein 12 (KCTD12)

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GABA_B receptors are the G-protein coupled receptors (GPCRs) for GABA, the main inhibitory neurotransmitter in the central nervous system. Native GABA_B receptors comprise principle and auxiliary subunits that regulate receptor properties in distinct ways. The principle subunits GABA_{B1a}, GABA_{B1b}, and GABA_{B2} form fully functional heteromeric GABA_{B(1a,2)} and GABA_{B(1b,2)} receptors. Principal subunits regulate forward trafficking of the receptors from the endoplasmic reticulum to the plasma membrane and control receptor distribution to axons and dendrites. The auxiliary subunits KCTD8, -12, -12b, and -16 are cytosolic proteins that influence agonist potency and G-protein signaling of GABA_{B(1a,2)} and GABA_{B(1b,2)} receptors. Here, we used transfected cells to study assembly, surface trafficking, and internalization of GABA_B receptors in the presence of the KCTD12 subunit. Using bimolecular fluorescence complementation and metabolic labeling, we show that GABA_B receptors associate with KCTD12 while they

reside in the endoplasmic reticulum. Glycosylation experiments support that association with KCTD12 does not influence maturation of the receptor complex. Immunoprecipitation and bioluminescence resonance energy transfer experiments demonstrate that KCTD12 remains associated with the receptor during receptor activity and receptor internalization from the cell surface. We further show that KCTD12 reduces constitutive receptor internalization and thereby increases the magnitude of receptor signaling at the cell surface. Accordingly, knock-out or knockdown of KCTD12 in cultured hippocampal neurons reduces the magnitude of the GABA_B receptor-mediated K⁺ current response. In summary, our experiments support that the up-regulation of functional GABA_B receptors at the neuronal plasma membrane is an additional physiological role of the auxiliary subunit KCTD12.

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Susceptibility of Podocytes to Palmitic Acid Is Regulated by Stearoyl-CoA Desaturases 1 and 2

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Type 2 diabetes mellitus is characterized by dyslipidemia with elevated free fatty acids (FFAs). Loss of podocytes is a hallmark of diabetic nephropathy, and podocytes are highly susceptible to saturated FFAs but not to protective, monounsaturated FFAs. We report that patients with diabetic nephropathy develop alterations in glomerular gene expression of enzymes involved in fatty acid metabolism, including induction of stearoyl-CoA desaturase (SCD)-1, which converts saturated to monounsaturated FFAs. By IHC of human renal biopsy specimens, glomerular SCD-1 induction was observed in podocytes of patients with diabetic nephropathy. Functionally, the liver X receptor agonists T0901317 and GW3965, two known inducers of SCD, increased Scd-1 and Scd-2 expres-

sion in cultured podocytes and reduced palmitic acid-induced cell death. Similarly, overexpression of Scd-1 attenuated palmitic acid-induced cell death. The protective effect of T0901317 was associated with a reduction of endoplasmic reticulum stress. It was lost after gene silencing of Scd-1/-2, thereby confirming that the protective effect of T0901317 is mediated by Scd-1/-2. T0901317 also shifted palmitic acid-derived FFAs into biologically inactive triglycerides. In summary, SCD-1 up-regulation in diabetic nephropathy may be part of a protective mechanism against saturated FFA-derived toxic metabolites that drive endoplasmic reticulum stress and podocyte death.

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The Editorial Team of DBM Facts
wishes all its readers a Merry
Christmas and a Happy New Year!

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„Yes, Virginia, there is a Santa Claus“



Newsman Francis
Pharcellus Church
wrote The Sun's
response to Virginia.

Eight-year-old Virginia O'Hanlon wrote a letter to the editor of New York's Sun, and the quick response was printed as an unsigned editorial Sept. 21, 1897. The work of veteran newsman Francis Pharcellus Church has since become history's most reprinted newspaper editorial, appearing in part or whole in dozens of languages in books, movies, and other editorials, and on posters and stamps.

"DEAR EDITOR: I am 8 years old.
Some of my little friends say there is no Santa Claus.
Papa says, 'If you see it in THE SUN it's so.'
Please tell me the truth; is there a Santa Claus?"

VIRGINIA O'HANLON.
115 WEST NINETY-FIFTH STREET."

VIRGINIA, your little friends are wrong. They have been affected by the skepticism of a skeptical age. They do not believe except they see. They think that nothing can be which is not comprehensible by their little minds. All minds, Virginia, whether they be men's or children's, are little. In this great universe of ours man is a mere insect, an ant, in his intellect, as compared with the boundless world about him, as measured by the intelligence capable of grasping the whole of truth and knowledge.

Yes, VIRGINIA, there is a Santa Claus. He exists as certainly as love and generosity and devotion exist, and you know that they abound and give to your life its highest beauty and joy. Alas! how dreary would be the world if there were no Santa Claus. It would be as dreary as if there were no VIRGINIAS. There would be no childlike faith then, no poetry, no romance to make tolerable this existence. We should have no enjoyment, except in sense and sight. The eternal light with which childhood fills the world would be extinguished.

Not believe in Santa Claus! You might as well not believe in fairies! You might get your papa to hire men to watch in all the chimneys on Christmas Eve to catch Santa Claus, but even if they did not see Santa Claus coming down, what would that prove? Nobody sees Santa Claus, but that is no sign that there is no Santa Claus. The most real things in the world are those that neither children nor men can see. Did you ever see fairies dancing on the lawn? Of course not, but that's no proof that they are not there. Nobody can conceive or imagine all the wonders there are unseen and unseeable in the world.

You may tear apart the baby's rattle and see what makes the noise inside, but there is a veil covering the unseen world which not the strongest man, nor even the united strength of all the strongest men that ever lived, could tear apart. Only faith, fancy, poetry, love, romance, can push aside that curtain and view and picture the supernal beauty and glory beyond. Is it all real? Ah, VIRGINIA, in all this world there is nothing else real and abiding.

No Santa Claus! Thank God! he lives, and he lives forever. A thousand years from now, Virginia, nay, ten times ten thousand years from now, he will continue to make glad the heart of childhood.



Christmas in Poland



Cathedral Poznan

In contrast to Western Europe and the United States the Polish Christmas Season starts later, but it also lasts longer. This is due to the All Saint's Day (1st of November), which, in contrast to Halloween, is rather melancholic and in traditional Polish catholic culture it clashes with the cheery mood of anticipating Christmas. Advent officially initiates the awaiting for Jesus's Birth. However, in some stores, press and television the commercial Christmas time starts much earlier – even in October, which, in my opinion, disturbs the particular atmosphere of that period. The celebration itself

starts on Christmas Eve afternoon and lasts until Candlemass (2nd of February). In some places the Christmas decorations are present to the end of the Carnival.

Advent – a particular anticipation

That four-week period preceding Christmas is, in Poland, considered a time of spiritual preparation for the Birth of Jesus. There is the tradition of the special masses (or communion services), called "roraty", which take place at dawn every



Two goats (symbol of the city) made from the ice, in the old city square of Poznań during the International Ice Sculptures Festival.

day of the week except Sundays and which are dedicated to Mary for receiving the good news from Archangel Gabriel. In the central-west region of the country, where I come from, Greater Poland (Wielkopolska), children go to church with colourful lanterns alight. These are usually self-made and symbolize the Hail Mary. Meanwhile, the nativity scenes or cribs (descriptions of the birth of Jesus) called 'Szopka' are being prepared. Sometimes they are just paintings or movies, but usually they are three-dimensional constructions that are located either indoors or outdoors. They contain waxy or woody figures depicting the infant Jesus placed in a manger, Mary and Joseph, but also shepherds, angels and animals. There are also living nativity scenes where children play roles.

In general, Advent is a time when people try to be peaceful and remember the real reason for Christmas, which is not always easy in the Advent rush. Some will even fast, giving up their favourite foods or drinks and also avoiding parties and loud music. Many visit Church quite frequently. However, there are few exceptions from the fasting rule. The first is St. Andrew's Day (Andrzejki) which takes place on the 30th of November. This is also known as magic day due to future predic-

tions, which people perform as well as partying. The second one on 4th of December, named St. Barbara's Day (Barbórka or Miner's Day) after the patron saint of miners, is always celebrated with accompanying parades, especially in southern Poland, where most of the mines are located. Another break in the fasting of Advent is St. Nicholas Day (Mikołajki) on the 6th of December, when the saint visits children on the evening and through the night donating some small gifts. The children are told to clean their shoes and put them outside the

house or on a balcony. In the morning they find sweets or small games inside.

Similarly to western European countries, in bigger cities Christmas markets with food, porcelain or wood products and toys are constructed. Lights beautifully decorate the city centres. In the old centre of the capitol of Greater Poland, Poznań, there is a new accompanying tradition: the International Ice Sculptures Festival. Artists are invited to show their ice-work in the old town square using chainsaws, drills and chisels.

During Advent, Poles also prepare their houses for Christmas. A lot of outside decorations such as illuminated figures or light chains are becoming more popular. People wash windows and clean their carpets very thoroughly. Everything must be perfectly neat to welcome the God coming to the World. Moreover, an ancient belief is that forces of evil would dwell in all things left dirty on that day.

Christmas Eve – the day that counts from dusk

In Poland, which is a mostly catholic country, Christmas is considered to be the most important celebration of the year. Christmas Eve, which takes

place on the 24th of December, can be divided in two parts: before and after dusk. Traditionally, till dusk is still Advent time with abstinence and fasting – meat is not normally allowed to be eaten in any form. People prepare the Christmas Eve dinner, do last minute shopping and cleaning. This is a working day, but most of the shops and offices close in the early afternoon. This is also the last moment to bring home a Christmas tree, real or artificial, and decorate it. Children usually use lights, apples, nuts, candies, hand-blown glass, crystal and paper ornaments for that purpose. Recently, one-colour decorations have become popular in the bigger cities, usually red or gold. Mistletoe is also very common Christmas requisite in Poland – it is hung under the ceiling and believed to attract luck and wealth to the house.

The real Christmas celebration, called 'Wigilia', starts at dusk. People wear their festive clothes and they sit together at the prepared tables when, at around 4:30 pm, a special sign appears: the first star on the night sky, symbolizing the Bethlehem Star. The table is usually covered with a white tablecloth, symbolizing Mary's veil, which became the Babe's swaddling cloth. Some straw is placed underneath it, to remind people that Jesus was born in a stable and also to bring wealth in the upcoming year. At the beginning of the meal, a blessed, large, white wafer biscuit called 'Opłatek', is passed around and everyone is saying wishes to each other, while breaking off a piece and eating it. It can be a very emotional time as grudges are forgotten and deceased family members, not being present at the table anymore, are remembered. Sometimes a small piece of the wafer biscuit is given to any farm animals or pets that the family



The typical Polish Christmas Eve dinner table.

might have. A place is often left empty at the table. It is prepared for an unexpected guest, who may knock at the door, in the same way Joseph wandered from home to home looking for a place for Mary to give birth. That special evening, no one should be left alone.

The typical Polish Christmas Eve dinner should include twelve dishes symbolizing the number of Apostles or months in the year. In general, food represents the four corners of the earth: mushrooms from the forest, grain from the fields, fruit from the orchards, and fish from the lakes and sea. It is strictly forbidden to eat meat. Hence, typical dishes include: Christmas Eve borscht (barszcz wigilijny) with mushrooms dumplings (uszka), carp in aspic, herring (śledzie), dumplings filled with either cheese and potato or cabbage and mushroom (pierogi,) breaded whitefish, meatless cabbage rolls chopped with rice and onions (gołąbki) and noodles with poppy seed (makielki). Desserts might include nuts, tangerines, chocolates, sweet poppy seed roll (makowiec), a jam-filled flat pastry (mazurek), honey-spice cake (piernik), gingerbread cookies (pierniczki), fruit compote, cognac, liqueurs, mead and a honey-spiced vodka (krup-

nik). 'Kutia', a kind of gruel with cracked wheat and honey, is also eaten in some parts of Poland on Christmas Eve. After the dinner the Christmas Carols (kolędy) are sung or played. Poland has very rich repertoire of them and they are also sung in Churches from Christmas Eve till the 6th of January.

The next point in the celebration is the gift giving. In the region of Greater Poland the Starman (gwiazdor) gives the gifts to the children, not Santa Claus. The Starman is not as jovial and kind as Santa Claus. He first threatens the children with a wooden birch, but then relents and opens a sack of presents to be passed around. The tradition of the Starman is very old indeed. It is speculated it came to Poland from Germany through the so-called 'Weihnachtsmann', which could have been secularized from St. Joseph. Another theory says that the name came from the Communist epoch in order to substitute the catholic name with a secularized one. The role of Starman is usually taken by the grandfather or father in the family, who silently disappears while the other family member sing the Christmas Carols and changes clothes to a red coat with white collar and cuffs, red trousers, black leather belt, boots and carries a bag full of gifts. Frequently an artificial beard is also added to improve the characterization. That makes small children very surprised and gives them a lot of fun. In the region of Lesser Poland (Małopolska, Kraków) and in Silesia (Śląsk), it is the baby Jesus or his messenger, a small angel, that brings the presents and, since they are invisible, their presence is signalled by the ringing of a bell. Children are supposed to remain silent during the Christmas Eve dinner. After the dinner the family prepares for Midnight Mass, known as 'Pasterka' or 'Shepherds Mass', because they were the first to greet the Baby Jesus. During the night animals are said to talk in a human voice and have the power to tell the future. Nowadays, it is taken with sense of humour and people ask each other afterwards what did they hear from their animals.

Christmas Days and New Year's time

In Poland both Christmas days begin by going to Church for Christmas Mass. The Churches are crowded, the Christmas Carols are sung and in general, despite the cold winter weather, there is very cheerful and pleasant atmosphere inside. That is a great chance to meet neighbours, wish them Merry Christmas (Wesołych Świąt!) and visit a nativity crib. Many people travel to meet family and friends. Frequently, they also visit graveyards to put some lights on graves of deceased family members. Since there is no fasting anymore, the dinner typically contains ham, Polish sausage (kielbasa), roast duck or goose, or the Polish National Dish – a savoury stew of cabbage and meat (bigos). Luckily, most of the shops are closed and therefore there is no rush and race to catch some sale discounts.

The celebration of New Year's time is similar to that in western European countries. Young people spend it partying in clubs or in flats; some prefer more quiet places in mountain huts or attending New Year's Balls. Others just spend it at home with family.

On the 6th of January Poles take small boxes to churches to be blessed. They contain chalk, a gold ring, incense and a piece of amber in memory of the gifts of the Magi. Once home, they inscribe "K+M+B" with the blessed chalk above every door in the house. The letters stand for Three Kings: Caspar, Melchior and Balthasar. They remain above the doors all year, until they are inadvertently dusted off or replaced by new markings the next year.

Wishing all DBM Facts readers cheerful Christmas Time, Merry Christmas and Happy New Year 2014!

Karol Czaja

Better keep your fingers off

I want to use this opportunity to present one of my hobbies that could be of interest to some of you. I really like carnivorous or insectivorous plants. How I came to love insectivorous plants is quickly answered. As a child, insectivores already fascinated me. I got a Venus flytrap but after a short time it died and I sadly wondered why. Today I know that the darkest place in my room maybe wasn't a good environment for that sun hungry plant. One year ago I found some nice exemplars in the supermarket. That sight rewoke my curiosity and fascination for these plants and I immediately bought two of them. This time, I researched these plants online and in books, something wasn't possible previously. I saw that there are a lot of different types of insectivorous plants. Because they need specific culture requirements I will start with the basics. If you follow them, you will be able to successfully culture most of the less complicated species. These plants don't catch and enzymatically digest insects without a reason. They do it to compensate for a nutrient deficiency as most of them grow on nutrient-poor soil. The substratum should therefore also be nutrient-poor, meaning without fertilizer, to avoid damage to their roots. It is therefore best to avoid the normal potting soil found in the supermarket. Unfor-

tunately, the best results will often only be obtained with mixtures of peat soil and to get it moors have to be destroyed. Another important point is the water, and as you can imagine that should also be nutrient poor. The best options are to use de-ionized water or pure rain water. Most of these plants love it wet, very wet and high air humidity. But there are also other factors like sunshine duration, temperature, dormancy and of course additional feeding that have to be optimized for each individual plant. But now I want to present some of my plants that are also suitable for beginners.

The first insectivorous plants are the butterworts (german: Fettkraut), *Pinguicula sp.* Most of them grow in the northern hemisphere but some can also be found in South America. Today there are more than 100 known species. One very interesting thing about these plants is their leaves. When you look at them through a magnifying lens you can see that there are thousands of hairs. At the end of each hair are glands that produce sticky drops. When a victim, such as a house or fruit fly, lands it will be caught by the sticky hairs. After the insect is caught sessile glands on the surface of the leaf produce acid and enzymes for digestion. The nutrient rich digestive fluid will be absorbed from the sessile glands later.



Butterwort *Pinguicula sp.*



Venus flytrap, *Dionaea muscipula*



Sundew, *Drosera capensis*

Northern Europeans have known about the antibacterial property of this digestive juice for a long time and used to use it to heal sores on cattle.

Maybe one of the most popular insectivorous plants is the Venus flytrap (german: Venusfliegenfalle) and it is also the sun hungriest carnivorous plant I know. The only place native to this plant is a small area in the US states of North and South Carolina. It is interesting that there is only one species, *Dionaea muscipula*, meaning that it is a monotypic genus, but there are a few variations. You can see on the picture that the trap has some trigger hairs; only when an insect stimulates at least two of the trigger hairs at the same time or one hair twice within 20 seconds will the trap close in less than a second. At first the plant doesn't fully close the trap to be



Tropical pitcher plant, *Nepenthes sp.*



American pitcher plant, *Sarracenia sp.*

sure that the victim is big enough because for the plant it costs a lot of energy to start the digestive process. When after seven to ten days the trap opens you can only see the exoskeleton of the insect. Each trap can only close around seven times and can only digest one to three times before the leaf with the trap dies.

The next plants are the sundews (german: Sonnentau). Amongst the carnivorous plants the sundews have the most species and you can find them on all continents. Their leaves can look very different. The picture shows my specimen, *Drosera capensis*, that originally comes from the cape of South Africa. This sundew is an absolute beginner's plant; it can be grown under a wide range of culture conditions and can even survive outside in winter with brief periods of freezing.



American pitcher plant, *Sarracenia sp.* (2)

In addition to the plants above I also have a tropical pitcher plant, *Nepenthes sp.* (german: Kannenpflanze) and different American pitcher plants, *Sarracenia sp.* (german: Schlauchpflanze). I hope this article has awoken your interest and that the next time you see such a plant in the supermarket you might take it home and make it grow.

Toni Krebs

The Art of Fencing

(or how to play chess while running the 100 m dash)

Fencing is a sport based on speed, flexibility, co-ordination, good reflexes and tactics. To be a good fencer one has to have additional qualities such as strength, concentration, perseverance and self-control. It requires continuous training both mental and physical but anyone of any age can practice fencing as a hobby sport and it is a great distraction for the mind and spirit. In addition it has been calculated that a person of 70 kg burns 329 Kcal for an hour of normal fencing and 653 during a competition, so it's a great way to stay fit.

History of fencing:

Fencing is one of the oldest sports, practiced by ancient Chinese warriors more than 4000 years ago; a relief found in the temple of Medinat Habu built by Ramses III near Luxor is one of the first historical documentations showing that fencing was also practiced by ancient Egyptians about 1200 BC. The history of fencing then follows the evolution of human civilization as it was practiced by warriors of ancient civilizations including Persians, Babylonians, Greeks and Romans. The latter implemented specialized schools called *ludi* where specialized legions of gladiators (from the latin *gladium* = sword) were trained by professional instructors called *doctores*. At the time, fencing was entertaining but rather dangerous, since each match was concluded by the death of one of the contenders. The Romans refined the practice of fencing and laid down some of the basic principles of successful fencing: hit your opponent as fast as possible without uncovering your body while attempting your hit.

After the fall of the Roman Empire and during the Dark Ages, fencing regressed and was practiced with much heavier weapons requiring two hands to hold a sword and heavy protective armours. In the late Middle Ages after the discovery of gun powder, the art of fencing changed once again and soldiers were equipped with light swords and their heavy armour was substituted with light protective clothing which facilitated the development of tactics and favoured speed.

In the 1500s an Italian fencing Master, Mr Agrippa, invented the four basic fencing positions (prime, seconde, tierce and quarte) while Masters Grassi

and Vigiani invented the lunge position. Fencing became once again increasingly popular particularly amongst noblemen who would challenge each other to honour duels. The French took over the monopoly of this art, laid down the first set of rules which transformed the art of fencing into a series of alternating attacks and defense; they also introduced the use of new weapons, first the *épée* to replace the rapier, and subsequently the *fleuret* or foil. A new weapon was introduced a Century later in Hungary, the *sabre*, which originated from the Oriental scimitar and at the end of the 1700 the French fencing Master La Boessiere invented the fencing mask, making this sport much safer and *combats non fatal*.

The Japanese also had developed their own fencing sword fighting that goes by the name of kendo. This sport that is practice worldwide combines martial arts and fencing and has its own set of rules and weapons.

By the end of the 1800s fencing was not considered a harmful sport and after World War I fencers were no longer considered warriors. When the modern Olympic games were re-introduced in 1896, men's sabre and foil competitions were present, followed by men's *épée* in the 1900 Olympic games. Women's foil joined the Olympics in 1924, but it was not until 1996 that Women's *épée* joined, followed by Women's sabre in 2004.

Modern fencing equipment: A fencer requires a jacket and vest, a mask, a glove, trousers, white socks, flat-soled shoes and a weapon of choice (which these days have blunted tips). Fencing clothes are made of special material and must resist strikes up to 800 Newtons. One can chose whether to fence with a foil, an *épée* or a sabre (see figure illustrating modern fencing weapons) and each weapon is governed by a different set of rules. At foil, hits must be made with the point of the weapon and are valid only when they land on the prescribed target area (see cartoon illustrating fencing target areas). At *épée*, hits are made with the point and, as its rules are based on the conditions of a duel, are valid wherever they arrive anywhere on the opponent's body. Hits

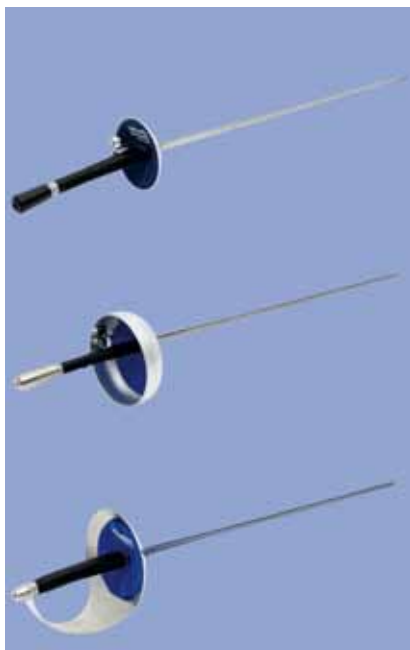


by the sabre are made with the point, with the cutting edge, or with the upper third (the area nearest the point of the sword) of the back edge, on the opponent's body from the waist up. Hits are assessed by an electrical system: fencers wear clothing made of lamé interlaced with copper threads; the lamé is sensitive to the electrical weapon. For foil only the vest worn by the fencer is sensitive, for sabre the vest and mask are sensitive. In épée the fencers do not wear a lame cover and their entire suit is sensitive. Cords are connected to the fencer's clothing, to the weapon, and to the scoring box. When a weapon touches the fencer with a small amount of pressure, a circuit is created and the scoring box reflects a hit.

Fencing is performed in a fencing hall that is equipped with *pistes*, or mats, that can be made of linoleum, a copper strip, rubber, or composition; the strip is 1.5 meters wide and 14 meters long, with an extension, or runback, of 1.5 meters at either end. The *piste* has a centre line, *en garde* lines, warning lines, and rear limit lines. A match starts with the fencers in the *en garde* position so far apart as to require a lunge to reach the opposing fencer.

Fencing conventions: fencing is a discipline and is governed by a set of conventions and rules specific for each weapon and designed to teach fencers to fence as if their blades were sharp. Fencing regulations are updated and posted by the Fédération Internationale de l'Escrime (or FIE); the rules instill in a fencer a specific response to an opponent's move, as opposed to an instinctive reaction. While conventions establish right-of-way, or who scores a point within an exchange of actions, the rules guide the fencer, helping create an advantage in distance and timing over the opponent and allowing the fencer to hit an opponent and not be hit. Gentleman behavior is expected by everyone in a fencing hall and unsporty behavior such as mask throwing, intentional hitting, and abusive language are not tolerated and can be punished by disqualification from a hall, or worse from a competition.

Fencing matches: modern fencing is very different from its initial days, while no longer trying to kill your opponent, your aim is to make the greatest number of points possible within the allocated time. Each round against an opponent is called a match. A *Juge*



Modern fencing weapons:
top foil,
centre épée and
bottom sabre.

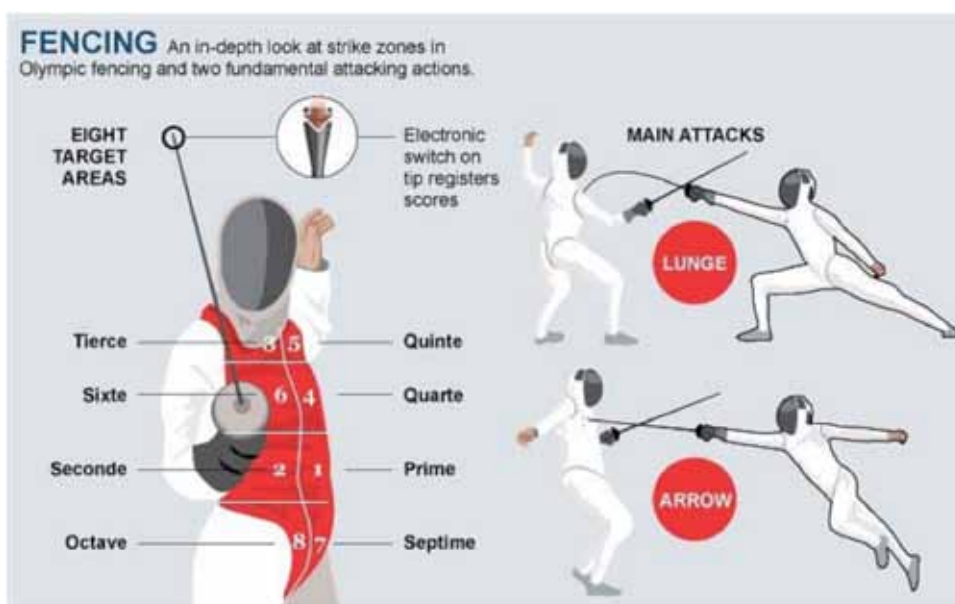
times the matches and has absolute ruling, his word can not be discussed and he can “show” the fencers yellow, red or black warning cards if a fencer faults; a fencer who receives a black card is disqualified for bad behavior; a yellow card is a warning while a red card is a harsher warning; after a red card, the offending fencer receives a point against him. At a tournament, irrespective of which weapon is being used, the first matches are fenced within “pools”, which are small groups of 4–7 fencers possibly from different clubs or nations. Each fencer has to fence against each member of the pool and the first to reach 5 points within 3 minutes wins the match. The number of victories, hits conceded and hits received are marked on a special score sheet by the *Juge* and a rank list is made at the end of the pools. After the pools the tournament proceeds by direct elimination, during which the first fencer to reach 15 points wins and moves on to the next round of direct elimination until the finals. For épée and foil direct eliminations last a total of 9 fencing minutes subdivided in 3 time slots of 3 minutes each and interrupted by a 1 minute break (to drink and recover from the anaerobic and mental stress); sabre which is much faster, is interrupted when a fencer scores 8 points at which point the *Juge* gives a

1 minute break. Medals are usually awarded to the first four fencers, gold to the first, silver to the second and bronze to the two third fencers.

Wheelchair fencing: One of fencing’s most recent developments is wheelchair fencing, introduced by the neurosurgeon Sir Ludwig Guttmann at Stoke Mandeville Hospital in England as a therapy for WWII veterans suffering from spinal cord injuries. In 1948 Guttmann inaugurated Olympic-type competitions for disabled athletes and soon after wheelchair fencing became a regular fencing event in Europe. Wheelchair fencing has been a part of the Paralympic Games since 1960. In this variant, fencing takes place in special frames designed to keep the wheelchairs stable. Wheelchair fencers go for five touches, cannot advance or retreat and must make their points while sitting in their chair; fencers can duck, turn, lean forward and backwards in order to avoid touches and make points. All three weapons are included in wheelchair fencing.

Where to fence in Basel: Basel has three fencing clubs, one run by French Maître d’Armes (Basler Fechtclub), one by a German Maître d’Armes (Basler Fechtgesellschaft) and one run by the ex-olympian Gianna Burki. Because of the small number of fencers, Switzerland has decided to promote only épée at the elite level and a number of Olympians and world class fencers including Marcel Fischer and Max Heinzer have come out of Basel fencing clubs. For more information on fencing you can follow the link “[www. Swiss-fencing.ch](http://www.Swiss-fencing.ch)”.

Susan Treves



Fencing target area, position and main attacks

Heute: Christine Büchi, Reinigungsdienst

Es war im Sommer 1965, nahe Besançon. Der Doubs schob sich gemächlich nordwärts, so wie er es stets zu tun pflegte. Die Sonne brannte mir auf die Stirn, Vögel zwitscherten ihre Lieder und am Ufer flirrten Libellen durchs Dickicht. Grossvater hatte sein altes Holzboot unweit des verwilderten Ufers vertäut. Er mochte das Wasser, solange er damit nicht in Kontakt kam. Die Ruhe, die Einsamkeit, die Besinnlichkeit. Ich beobachtete ihn, wie er mit den knorrigen Fingern seiner rechten Hand seine abgebrannte Gitane zusammendrückte, ein letztes Mal daran zog und sie dann, ohne hinab zu sehen, auf den Holzplanken zwischen seinen Füßen ausdrückte. Dann schnaubte er kurz und zupfte mit der Linken an der Angel, die er unter seiner Bank arretiert hatte.

Vor mir stand ein kleiner Eimer, zur Hälfte gefüllt mit Flusswasser. Darin schwammen winzige Fische, nicht grösser als ein Mittelfinger. Grossvater fing sie mit einem kleinen Netz. Später würde er sie in Mehl wälzen und anschliessend im heissen Öl frittieren. Jedes Mal, wenn er einen seiner grossen Fische gefangen hatte, legte er ihn in einen zweiten Eimer der neben jenem mit den kleinen Fischen stand. Es waren merkwürdige Fische mit breiten Gesichtern und französischen Schnäuzern; sie starrten mich an, durch ihre



schwarzen Augen und mit geöffnetem Mund. Kaum widmete sich mein Grossvater wieder seiner Angel und dem sanft dahin fliessenden Doubs, nahm ich den Eimer, leerte ihn über Bord und gab dem Fluss zurück, was mein Grossvater ihm gestohlen hatte. Grossvater schnaubte und zerrte seine Angel aus ihrer Halterung. Er drehte sich kurz um und warf mir einen missbilligenden Blick zu, der mir bedeutete, still zu sitzen. Ungelenk erhob er sich, um die Seite zu wechseln, dabei begann das Boot bedrohlich zu schaukeln und er verlor das Gleichgewicht. Mit grossen Augen starrte ich ihm hinterher, wie er mit breitem Platschen in den Doubs stürzte. In-

stinktiv beugte ich mich über das Bord des Bootes und griff nach meines Grossvaters Kragen. Der fuchtelte und strampelte mit Armen und Beinen als zöge ihn etwas am Hosenbund unter Wasser. Ich wusste, dass mein Grossvater nicht schwimmen konnte. Das war der Grund, weshalb er sich mit seinem Boot nie mehr als zwanzig Meter vom Ufer entfernte. Mit all meiner Kraft zerrte ich ihn ans Boot heran bis er prustend das Bord zu fassen bekam. Ausser Atem und mit rotem Antlitz befahl er mir, das Boot ans Ufer zu manövrieren. Fisch gab es an diesem Abend keinen.

Es sind Momente wie diese, die mich gerne an meine Kindheit und



Jugend zurückdenken lassen. Den Alltag verbrachte ich mit meinen Eltern und meinem Bruder in Mulhouse in einem tristen Betonbau.

Viele Jahre später machte ich eine Lehre als Schneiderin bei Spengler in Basel und heiratete einen Franzosen. Mein Arbeitsverhältnis bei Spengler dauerte mehr als 11 Jahre; immerhin länger als meine erste Ehe. Danach suchte ich nach mehr Aufregung und fand, nach einem Aufenthalt in einer Laufener Diskothek, einen Job als Serviceangestellte im Restaurant *Old Sailor* in Basel; einfache Küche, illustre Kundschaft. Dort lernte ich Markus kennen. Wir heirateten und bald war unsere erste Tochter unterwegs. Wir kauften uns ein Haus in Bättwil und ich frönte als Hausfrau meiner Leidenschaft: dem Kochen. Im Dorf engagierte ich mich für die Gemeinschaft, was mir nach ein paar Jahren den Posten der Präsidentin der Kindergartenkommission einbrachte. Zuweilen veranstal-

tete ich auch Festivitäten im Dorf. Zwei Jahre nach unserer ersten Tochter, kam das zweite Mädchen zur Welt.

2002 erlag Markus im Schlaf einem Herzversagen. Um der Stille im Haus zu entgehen, entdeckte ich das Reisen für mich. In der Folge besann ich mich auf meine alten Zeiten und fand im *Bahnhöfli* in Rodersdorf eine Stelle als Kellnerin. Das Geschäft lief und ich spürte wieder meine Berufung: Menschen zu bewirten, mich mit

ihnen auszutauschen und sie zu unterhalten. Jahre später kam ich zu meinem heutigen Job im Universitätsspital.

Hier fühle ich mich seither wohl. Im ständigen Kontakt mit Menschen, die unterschiedlichsten Arbeiten nachgehen, denen ich über die Schulter schauen darf und denen ich gerne immer wieder mit einem Lächeln auf den Lippen begegne.

Und nun eines meiner Lieblingsrezepte:

Elsässer Bäckerofen

Dieses saftige Gericht trug man früher in einem grossen Topf, mit einem Deckel aus Schwarzbrotteig hermetisch abgeschlossen, zum Dorfbäcker, um es in dessen grossen Backofen langsam garen zu lassen. Zuweilen geschieht dies auch heute noch im Elsass. Das folgende Rezept lässt sich aber auch gut zu Hause nachkochen.

Zutaten für 8 Personen: 500 g Schweineschulter, 500 g Hammelschulter, 500 g Rinderbrust ohne Knochen, 4 Zwiebeln, 2 Knoblauchzehen, 1 Bund Petersilie, 1 kg Kartoffeln, 50 g Schweineschmalz, Salz, schwarzer Pfeffer, 1/2 bis 3/4 l trockener Weisswein, 1 Lorbeerblatt (pro Person ca. 700 Kalorien)

Zubereitungszeit: 45 Minuten

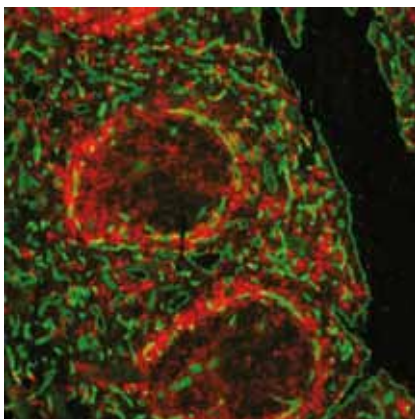
Garzeit: 2 1/2 Stunden

So wird's gemacht: Alles Fleisch in grobe Würfel schneiden. Die Zwiebeln schälen und feinhacken. Die Petersilie waschen, trockentupfen und kleinschneiden. Die Kartoffeln waschen, schälen und in Scheiben schneiden. Das Schmalz in einer grossen Kasserolle erhitzen und die Zwiebeln darin hellgelb braten, dann aus dem Topf nehmen. Die Kartoffeln, das Fleisch und die Zwiebeln lageweise in die Kasserolle schichten. Jede Lage mit Salz und Pfeffer würzen und mit etwas Knoblauch und Petersilie bestreuen. Den Backofen auf 175 Grad vorheizen. Den Wein sorgfältig über die eingeschichteten Zutaten giessen. Das Lorbeerblatt oben auflegen. Die Kasserolle gut verschliessen, in den Backofen schieben und den Bäckerofen geruhsam in 2 1/2 Stunden gar werden lassen.

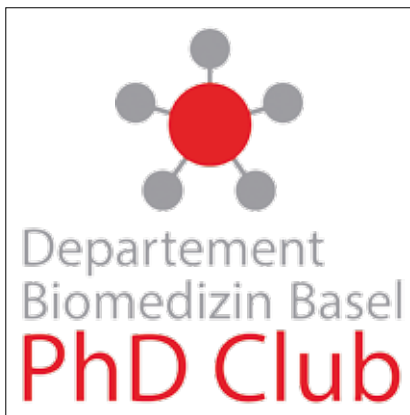
Bon appétit!

VORSCHAU PREVIEW

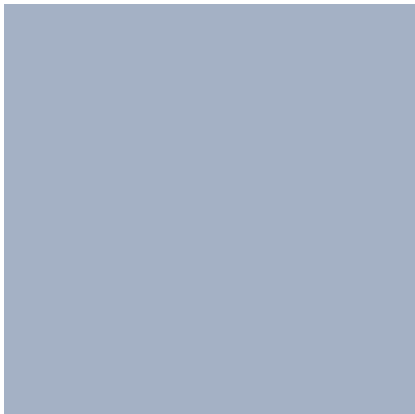
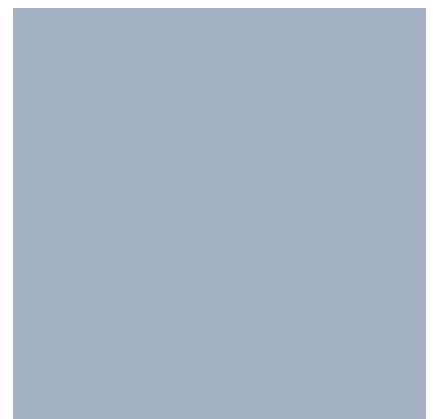
In der nächsten Ausgabe ...



... erfahren wir von Daniela Finke mehr über den aktuellen Stand der Forschung in der Developmental Immunology



... gibt uns der DBM PhD Club einen Einblick in seine vielfältigen Aktivitäten



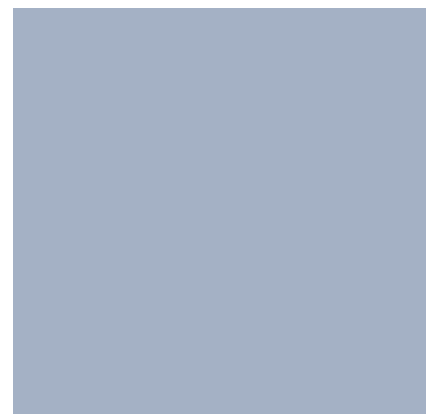
... reisen wir mit Tatiana Pochechueva in ihre Heimatstadt Moskau



... bereiten wir mit Takafumi und Yuki-ko Shimizu echte japanische Sushi zu



... stellt uns Niklaus Vogt seine Musiktruppe «The Wildbunch Drum & Fife Corps» vor



Christkindchen

Wo die Zweige am dichtesten hangen,
die Wege am tiefsten verschneit,
da ist um die Dämmerzeit
im Walde das Christkind gegangen.

Es musste sich wacker plagen,
denn einen riesigen Sack
hat's meilenweit huckepack
auf den schwächtigen Schultern getragen.

Zwei spielende Häschen saßen
geduckt am schneeigen Rain.
Die traf solch blendender Schein,
dass sie das Spielen vergaßen.

Doch das Eichhorn hob schnuppernd die Ohren
und suchte die halbe Nacht,
ob das Christkind von all seiner Pracht
nicht ein einziges Nüsschen verloren.

Anna Ritter (1865-1921)

