



Department of Human Molecular Genetics



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Introduction

The search for genetic factors in common diseases is hampered by their complexity and heterogeneity, which has been widely underestimated, and by the enormous size and variability of the human genome. Indeed, it has become apparent that several dozen genes contribute to the genetic predisposition for diabetes, and the same is true for a wide variety of other common disorders. Each of these genes increase or lower the individual disease risk only marginally, and because of the massive effects of lifestyle and other environmental factors, it is easy to see that the identification of these genetic risk factors will have little or no diagnostic consequences. The involvement of so many genes and non-genetic factors in the aetiology of common diseases must also greatly complicate the search for commercially attractive 'block-buster' drugs that are effective in the majority of the patients. Above all, the identification of specific base changes in the human DNA sequence conferring specific disease risks in a sea of functionally neutral sequence variants is a truly Herculean task.

The crucial step for such attempts is the mapping of these risk factors to specific chromosomes and chromosomal regions. Unfortunately, the resolution of the population-based methods that are commonly employed in this context (such as the affected sib pair method) is relatively low, which explains the slow progress in this field. The identification of conserved haplotypes encompassing one to hundred thousand base pairs will not solve this problem, but it may reduce its size by an order of magnitude. Once the haplotype structure of all human populations is known, typing five hundred thousand instead of all five million individual single nucleotide polymorphisms may suffice to extract most of the relevant information. However, linking these haplotypes to disease and identifying the relevant genes will remain a major challenge.

For almost all common disorders, including cardiovascular diseases, diabetes, dementia or cancer, there are varieties which are transmitted as monogenic traits. This is particularly striking for mental retardation, a common disorder with a prevalence (in Western societies) of about 2,5 percent, since most of the severe cases, with an IQ of below 50, are due

to defects of single genes or to chromosomal abnormalities. To date, about 5,500 single gene disorders are known, and only a quarter of these have been elucidated. However, with the human genome comprising about 30,000 genes, this cannot be more than the proverbial tip of the iceberg, and it is safe to predict that many more disorders will be identified that are due to defects of single genes.

The recent completion of the human genome sequence has greatly facilitated the identification of the molecular defects underlying monogenic disorders. If one and a half decades ago, it took several years to map and identify the gene defects underlying Duchenne muscular dystrophy and cystic fibrosis by positional cloning, today this task would be completed within weeks if not days, and at a tiny fraction of the previous costs. Indeed, in the Eighties, the central argument for sequencing the human genome has been the prediction that it would lead to the diagnosis and prevention of all genetic disorders, and eventually, to therapy. It is somewhat ironic, therefore, that since several years, government-funded genome research in developed countries has concentrated almost exclusively on common complex disorders. On the other hand, no systematic attempts have been made so far anywhere in the world to accomplish the original mission of the human genome programme, i.e. to elucidate the molecular basis of the genetic diseases that are due to defects of single genes.

Focusing initially on eye disorders and deafness, our group has been one of the first in Europe to use positional cloning strategies for the identification of the underlying genetic defects (Cremers et al, 1990). We were also the first to perform large-scale characterization of disease-associated balanced chromosome rearrangements (DBCRs) as a way to clone disease genes in a systematic fashion (see below). Roughly 50 percent of these DBCRs were found to be associated with (syndromic or non-syndromic) forms of mental retardation (MR). These findings, and the fact that mental retardation is one of the biggest unsolved problems in Clinical Genetics, have convinced us that MR should become the central research theme of our department.

Recruitment and clinical characterization of patients and families

The most important factor limiting large-scale research into monogenic disorders is the availability of clinically well-characterized patients and families with Mendelian phenotypes. Since almost all of these families are seen by the Clinical Geneticist, we had proposed to generate a network linking all major Clinical Genetic Centres in Germany for the recruitment and clinical characterization of such families. Despite unanimously positive recommendations by international reviewers, this proposal was eventually rejected because it 'did not fit into the framework of the NGFN'. Therefore, we have established formalized bilateral and multilateral collaborations with suitable partners in Europe and elsewhere for collaborative, systematic research into mental retardation and other disorders.

In 1996, together with groups from France, Belgium and the Netherlands, we have founded the European MRX Consortium to systematically study X-linked MR (XLMR), which is believed to account for at least 25 percent of all genetic forms of MR. To date, more than 450 XLMR families have been collected by this Consortium and associated partners, and this unique resource was instrumental in the identification of many novel XLMR genes. X-linked MR is also the central research theme of a Polish 'Partner Group' of our department founded in 2002 at the Medical University in Poznan with the support of the Max-Planck Society (collaboration with A. Latos-Bielenska). Since early 2003, we are jointly funded by an EU grant as part of the 5th Framework.

Also in 1996, in close collaboration with N. Tommerup (Copenhagen), the world-wide Mendelian Cytogenetic Network (MCN) was founded which comprises >300 cytogenetic laboratories. The aim of this network with its two Reference Centres in Copenhagen and Berlin is the recruitment and systematic clinical, cytogenetic and molecular characterization of patients with DBCRs. The Max Planck Society supports the ascertainment and clinical (re-) examination of patients with DBCRs through a Tandem Project grant, while the bulk of the support for this project has come from the National Genome Research



Network (NGFN). Recently, DNA from > 200 clinically and cytogenetically well characterized patients with conspicuous, 'chromosomal-looking' phenotypes but apparently normal karyotypes have been obtained for CGH array-based high-resolution deletion and duplication screening (collaboration with C. Lundsteen, Copenhagen; supported by the NGFN). Most of the defects detected by studying patients with balanced and unbalanced chromosome rearrangements will be due to haplo-insufficiency and thus behave as autosomal dominant traits.

In 2003, we have also concluded far-reaching, formalized collaboration agreements with potent partners in India and Iran, respectively, to study X-linked and autosomal recessive forms of MR (ARMR) in a systematic fashion. ARMOR may account for up to 60 percent of all mentally retarded patients in our population, but due to small family sizes, most of them appear as 'idiopathic' sporadic cases, and almost nothing is known so far about the underlying gene defects. Therefore, an important aim of our collaborations (with J.R. Singh, Amritsar; B.T. Thelma, New Delhi; S.Hasnain, Hyderabad, India; and H. Najmabadi, Tehran, Iran) is the recruitment and systematic autozygosity mapping in large consanguineous families with ARMOR and related monogenic disorders as a prerequisite for mutation screening and gene finding. Wherever possible, these collaborations will be put under the umbrella of already existing or future bilateral research agreements between Germany and India or Iran.

Systematic search for gene defects underlying monogenic disorders

Thus, we employ several complementary strategies to identify the molecular defects underlying MR, including the characterization of balanced and unbalanced chromosome rearrangements in patients (with autosomal dominant and X-linked MR), autozygosity mapping in large consanguineous families (with ARMOR) as well as linkage mapping and large-scale mutation screening (in XLMR families). It goes without saying that these strategies can and will also be employed for elucidating other monogenic forms of disease.

The systematic characterization of DBCRs, performed in the group of V. Kalscheuer, has turned out to be a particularly successful strategy to identify disease genes that play a role in MR and related disorders. In total, more than 30 candidate genes have been identified in this way, and the identity of several X-linked ones could be confirmed by mutation screening in unrelated families. For several years, our department has also been engaged in the development and implementation of methods allowing rapid detection of *unbalanced* submicroscopic rearrangements in the human genome. Subtelomeric deletions are a frequent cause of idiopathic mental retardation, but there is compelling evidence that small unbalanced genome rearrangements also occur in other regions of the genome and form a major cause of disease. As one of few laboratories worldwide, we have recently introduced DNA array-based Comparative Genomic Hybridization (array CGH, U. Nuber and co-workers, collaboration with R. Ullmann, Graz), a novel technique allowing high-resolution detection of unbalanced chromosome rearrangements which does not depend on the availability of metaphase chromosomes. With the support of the NGFN, a CGH array with a resolution of 1 megabase has been generated. This array is being validated by serial investigation of DNA from 200 clinically and cytogenetically well-characterized patients, and its BAC density will be further improved, subject to continued funding (application pending). Thus, the analysis of balanced or unbalanced chromosome rearrangements in mentally retarded patients is a suitable strategy for finding gene defects underlying autosomal dominant and X-linked forms of MR.

In parallel, by analysing linkage intervals from a large number of XLMR families, regions on the human X-chromosome with a high density of causative gene defects could be identified. Subsequent high-throughput mutation screening of genes in a pericentromeric X-chromosome segment has led to the identification of several novel candidate genes for XLMR (Ropers, Lenzner and co-workers). A prerequisite for these studies was the implementation of DHPLC-based high-throughput mutation detection. Presently we are imple-

menting a novel endonuclease-based method for mismatch detection which may allow us to scale up mutation screening even further (S. Lenzner, collaboration with R.Plasterk et al, Utrecht, R. Reinhardt et al. and Transgenomic Inc.). Recent studies of this group have also included the search for low-copy repeats on the X-chromosome, which may predispose to genomic disorders or form hotspots of mutation due to gene conversion.

Linkage or rather, autozygosity mapping in large consanguineous families followed by mutation screening in genes of the relevant interval is the strategy of choice for elucidating autosomal recessive forms of MR, as outlined above. Through our formalized collaborations with groups in India and particularly, Iran, we are in an excellent position for making rapid progress in this field, which is still largely unexplored. To map the respective gene defects, several different options for high-throughput/low cost genome scanning are being considered (collaboration with P. Nürnberg).

Elucidating the function of MR genes in health and disease

Compared to gene finding, unraveling the function of these genes in the normal brain and elucidating their role in the pathogenesis of MR is a much bigger challenge, which requires various different approaches. These include *in silico* sequence comparisons, analysis of intracellular and tissue-specific expression patterns, protein-protein and protein-DNA interactions, as well as the study of cellular and animal models at various levels. Complementary expertise in this area is available in most groups, and this is where many research lines of our department converge.

Protein-protein interaction and biochemical studies are the domain of S. Schweiger, who focuses on defects of the ventral midline which frequently involve the brain, and on the role of ubiquitin-mediated protein degradation. Her expertise is the mainstay in present and future attempts of our department to unravel the function of novel proteins which are mutated in MR.

Chip-based chromatin immuno-precipitation (ChIP on chip) is being introduced by the group of U. Nuber to study protein-DNA interactions. This group has also long-standing experience with cDNA array-based gene expression profiling, employing different human, mouse, tissue and chromosome-specific arrays. Recent work on the transdifferentiation of murine bone marrow stromal cells and the differentiation of neurospheres *in vitro* is beginning to shed light on the molecular basis of neuronal differentiation and may even have implications for the molecular diagnosis of MR.

siRNA-mediated knock-down experiments are being performed in the group of C. Scharff, an experienced neurobiologist with profound knowledge of neuroanatomy and histology, who is involved in the generation and characterization of cellular models for MR. Moreover, her work on zebra finches suggests that birds may be suitable model organisms for studying genetic defects of speech development in humans.

H. Scherthan, an experienced cytologist and cytogeneticist with a focus on telomere topology and function, has expertise in yeast genetics and is involved in complementation tests performed to unravel the function of novel human genes.

D. Walther has a central role in the generation of transgenic, knock-out or knock-down mouse models for human disease, employing classical targeting procedures as well as a novel method allowing to introduce specific point mutations in a single step (collaboration with H. te Riele, Amsterdam). His recent work on the serotonin modification of regulatory proteins provides exciting links to mental retardation and related disorders given the important role of Rho and Rab GTPases in neurite outgrowth and synaptic vesicle transport.

The experience of M. Hoeltzenbein as Clinical Geneticist is indispensable for recruiting patients and families, and for characterizing the clinical phenotype of patients with chromosomal rearrangements. Together with her, A. Tzschach plays a pivotal role in ongoing activities to include patients with late-onset and complex diseases in these studies in the context of the NGFN.



Finally, several of these groups are actively involved in a DFG-funded Collaborative Research Program (SFB 577) entitled ‘Molecular Basis of Clinical Variability in Mendelian Disorders’ which was founded almost three years ago. The aim of this program is the identification of modifier genes and regulatory pathways. This will enable more reliable prognoses about the severity and course of monogenic disorders and shed more light on their pathogenesis.

Conclusion and outlook

Despite substantial ‘brain drain’ due to the appointment of group leaders as department heads in Düsseldorf (B. Royer-Pokora), Mainz (T. Haaf), Zürich (W. Berger) and Uppsala (R. Fundele), the Department of Human Molecular Genetics has established itself as one of the major players in its field. Its long-term investments into the systematic investigation of Mendelian disorders, and mental retardation in particular, are already beginning to pay off. This holds for the establishment of formalized collaborations with research institutions in Denmark, Poland, Iran and India, but also for the implementation of pivotal concepts and methods for gene finding and functional studies. Given the increasing awareness of the difficulties inherent in the search for risk factors for common diseases and their limited relevance for health care, our activities promise to bear even more fruit in the years to come - provided continued funding can be obtained, through the NGFN or from other sources.

General information

External funding

BMBF-DHGP, 01KW9908/7: *Systematic clinical, cytogenetic and molecular characterization of balanced chromosome rearrangements that are associated with disease*

BMBF-NGFN, Plattform 6.4: *Translokationsbruchpunkte*

EFRE, 01GR0203: *Zentrale Einrichtung für die systematische Suche nach submikroskopischen Deletionen und Duplikationen bei monogenen und komplexen Krankheiten*

DFG, Ro389/17-3: *Schwerpunktprogramm Dysmorphie*

DFG, BE 1559/2-1: *Molekulare Aufklärung X-chromosomaler Netzhaut-Degeneration und Funktionsstörungen*

British Retinitis Pigmentosa Society: *Molecular and functional characterization of the gene for retinitis pigmentosa 3 and cloning of the genes underlying other X-linked forms of RP and related disorders*, jointly with F. Cremers, Nijmegen, until 2002

5th EU Framework (QLRT-2001-01810): *X-linked Mental Retardation*, 2003-2005

Tandem Project MPG: *Ascertainment of patients with disease-associated balanced chromosome rearrangements for the systematic, large-scale elucidation of genetic disorders*, 2001-2005

MPG-Partner Group in Poznan, collaboration with Prof. A. Latos-Bielenska, 2002-2006

Appointments, scientific honors & memberships

Thomas Haaf, C4 Professorship at University Mains, 2001

Wolfgang Berger, C4 Professorship at University Zürich, 2002

Reinald Fundele, C4 Professorship at University Uppsala, 2002

H.-H. Ropers, Elected Member Berlin Brandenburg Academy of Sciences (2002)

H.-H. Ropers, member of Royal Netherlands Academy of Arts and Sciences (2002)

H.-H. Ropers, member of HUGO Council (2003)

State doctorate (Habilitation)

Berger, W.: *Aufklärung von Gendefekten hereditärer Augenerkrankungen*. Habilitationsschrift, Humboldt Universität Berlin, 1999

Theses

- Meunier, D.: *Functional analysis of the mouse G90 gene*. Albert-Ludwigs-Universität Freiburg, 2003
- Zend-Ajusich, E.: *Die Evolution des menschlichen Chromosoms 3 in Primaten*. PhD Thesis, Technische Universität Berlin, 2002
- Grützner, F.: *Vergleichende Gen- und Genomkartierung in Vertebraten unter besonderer Berücksichtigung der Geschlechtschromosomen*. PhD Thesis, Freie Universität Berlin, 2001
- Hardt, T.: *Neue Fluoreszenzmethoden zur strukturellen und funktionellen Genomanalyse (Fish and Chips)*. PhD Thesis, Justus-Liebig-Universität Gießen, 2001
- Raderschall, E.: *Zytochemische und funktionelle Analyse des Rekombinase/DNA Reparatur-Proteins Rad51*. PhD Thesis, Freie Universität Berlin, 2001
- Voigt, R.M.: *Mikrodeletionen bei 100 Patienten mit konotrunkalen Herzfehlern auf Chromosom 22q11 und Chromosom 10p13/14*. PhD Thesis, Humboldt Universität Berlin, 2001
- Hamel, B.: *X-linked mental retardation: A clinical and molecular study*. PhD Thesis, Katholieke Universiteit Nijmegen, 1999
- Hemberger, M.: *Genetische und molekulare Ansätze zur Identifizierung von Plazentagenen*. PhD Thesis, Albert-Ludwigs-Universität, Freiburg, 1999
- Hol, F.A.: *Genetic factors in human neural tube defects*. PhD Thesis, Katholieke Universiteit Nijmegen, 1999
- Schröder, A.: *Genetische Ursachen der unspezifischen geistigen Behinderung – zytogenetische und molekulargenetische Untersuchungen an einer geistig behinderten Patientin mit einer t(X;20)-Translokation*. PhD Thesis, Humboldt Universität Berlin, 1999
- Grandy, I.: *Häufigkeit und Kernorganisation von strahleninduzierten Translokationen in Mikro- und Makrochromosomen des Hühnchens (Gallus gallus)*. Diploma Thesis, Ludwig-Maximilians-Universität München, 2002
- Hägebarth, A.: *Analyse einer möglichen Funktion des G90 Gens in der Regulation des Zellzyklus*. Diploma Thesis, Humboldt Universität Berlin, 2002
- Pantchechnikova, E.: *Zytogenetische Charakterisierung von krankheitsassoziierten balancierten Chromosomenbruchpunkten bei Patienten mit autosomalen Translokationen mittels FISH-Analyse*. Diploma Thesis, Freie Universität Berlin, 2001
- Zwintscher, A.: *Molekulare Charakterisierung von Kandidatgenen für familiäre Netzhautdystrophien*. Diploma Thesis, Technische Universität Berlin, 2001
- Münscher, S.: *Identifizierung und Charakterisierung neuer, augenspezifischer Gene*. Diploma Thesis, Technische Fachhochschule Berlin, 2000
- Scheer, M.: *Funktionelle Studien zu DXS6673E, einem Kandidatengen für X-gekoppelte geistige Behinderung, und seinem Mausortholog*. Diploma Thesis, Freie Universität Berlin, 1999
- Schlösser, T.: *Charakterisierung differenziell exprimierter Gene in einem Tiermodell für congenitale Blindheit (Norrie-Krankheit)*. Diploma Thesis, Freie Universität Berlin, 1999
- Techritz, S.: *Retinopathia pigmentosa 2 (RP2): Untersuchungen zur intrazellulären Lokalisation des Genproduktes*. Diploma Thesis, Fachhochschule Lausitz, 1999
- Vester, A.: *Cytogenetische und molekulare Charakterisierung von krankheitsassoziierten chromosomalen Rearrangements zur Identifizierung und Isolierung von Krankheitsgenen*. Diploma Thesis, Technische Universität Berlin, 1999
- Weber, A.: *Zytogenetische und molekulare Charakterisierung von krankheitsassoziierten chromosomalen Rearrangements zur Identifizierung und Isolierung von Krankheitsgenen*. Diploma Thesis, Rheinisch-Westfälische Technische Hochschule Aachen, 1999
- Wuttke, R.: *Molekulare Charakterisierung des X-chromosomalen Bruchpunktes einer mit geistiger Behinderung assoziierten balancierten X;17-Translokation*. Diploma Thesis, Freie Universität Berlin, 1999
- Zeitze, C.: *Mutationsanalysen bei Patienten mit X-chromosomaler Retinopathia pigmentosa und Untersuchungen zur Transkription des RPGR Gens*. Diploma Thesis, Freie Universität Berlin, 1999

Collaboration agreements

- Prof. Jai Rup Singh, Guru Nanak Dev University, Amritsar, India
- Dr. Sayed Hasnain, Institute for DNA Fingerprinting and Diagnosis, Hyderabad, India
- Dr. Hossein Najmabadi, Genetics Research Center, University of Social Welfare and Rehabilitation, Teheran, Iran
- Prof. Thelma B.K., Department of Genetics, University of Delhi South Campus, New Delhi, India



Neurochemistry Group & Mouse Lab

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Introduction

Diego J. Walther leads the „Neurochemistry Group and Mouse Lab“ at the MPIMG since February 2003. After finishing his scholar education in Guatemala, and his studies in chemistry, biochemistry and molecular biology at the Technical University in Berlin, the German scientist, born in Buenos Aires Argentina, finished his Diploma thesis in chemistry (biochemistry) in 1996. Then he worked on the establishment and histologic and physiologic characterization of different transgenic animal models especially focused on models with defined dysfunction of the serotonergic system, and finished his PhD thesis at the Max-Delbrück-Center (MDC) for Molecular Medicine and the Free University Berlin in 2000. During his postdoc time and as stipendiate of the „Verbund Klinische Pharmakologie Berlin-Brandenburg“ at the MDC, he expanded his research interests onto the interdisciplinary analysis of serotonin-producing tumors and the development of tryptophan hydroxylase-dependent procytostatic agents, the mechanistical elucidation of the role of serotonin in primary hemostasis and the immune system, as well as on the characterization of a novel, receptor-independent signaling mechanism of serotonin and other biogenic monoamines. He moved to the MPIMG and now co-operates with other groups of the institute and with groups at the MDC, the Charité and the Free University of Berlin on the field of neurochemistry and neurobiology and the establishment of further animal models for neuroendocrine disorders, vesicular trafficking defects, and mental retardation.

Scientific overview

The use of transgenic mice is one of the most straightforward tools to study gene function. Our facility offers the generation of transgenic and knockout mice to all groups in the Department, the Max-Planck-Institute and other interested laboratories. Our group is focused on the elucidation of molecular causes of human diseases by the generation, analysis, and rescue experiments of transgenic and knockout mouse models. We generate transgenic and knockout mice using classical methods and novel gene targeting procedures that allow the specific integration of

point mutations and frame shifts into genes of interest. Currently we work on animals with defined genetic dysfunctions in the serotonergic system and related biochemical pathways aiming the elucidation of the numerous hormonal and neurotransmitter effects of serotonin. Furthermore, we study the uptake and release mechanisms from vesicles and the involved signal transduction, using platelets as model system for neuronal vesicular processes. Moreover, we are establishing a mouse model for choroideremia to evaluate possible genetherapeutic approaches in the treatment of this X-linked eye-disease.

Another field is the use of harmless prodrugs that are enzyme-specifically toxified in tissues expressing either endogeneous tryptophan hydroxylase or transgenic nitroreductase of *E. coli* to induce defined lesions in tissues of interest.

Characterization of tryptophan hydroxylase 1 (TPH1)

Tryptophan hydroxylase 1 catalyzes the rate-limiting step of serotonin biosynthesis in extraneuronal tissues. Extraneuronal serotonin is involved in:

- primary hemostasis
- T cell-mediated immune responses
- gastrointestinal function, water and electrolyte homeostasis

Our collaborators and we are working on these items and also on the analysis of tissue-specific expression of splicing isoforms of TPH1.

Characterization of tryptophan hydroxylase 2 (TPH2)

Tryptophan hydroxylase 2 catalyzes the rate-limiting step of serotonin biosynthesis in neurons. The neurotransmitter serotonin is involved in multiple facets of mood control and the regulation of sleep, anxiety, alcoholism, drug abuse, food intake, and sexual behavior. Our collaborators and we are working on the elucidation of TPH2-dependent human psychiatric disorders.

Tryptophan hydroxylase-based cytotoxic prodrug development

Tryptophan hydroxylase metabolizes 7-hydroxytryptophan to 5,7-dihydroxytryptophan, which is rapidly decarboxylated to 5,7-dihydroxytryptamine (5,7-DHT) only in tryptophan hydroxylase-expressing cells. 5,7-DHT is a potent cytotoxic agent in the cytoplasm but not in the extracellular milieu. Therefore, its synthesis *in situ* can be used to specifically target serotonin-producing tumors, such as small cell lung carcinomas and carcinoids. Moreover, this prodrug can be used as an experimental tool for the induction of specific lesions of tryptophan hydroxylase-expressing tissues, when applied at high dosage.

NTR-based transgenic lesion models

Ecstasy abuse leads to the degeneration and death of serotonergic neurons, thereby causing anxiety and depressive syndromes. Using transgenics expressing *E. coli* nitroreductase under control of the *Tph2* promoter and the prodrug CB 1954 we are able to analyze the effect of the loss of serotonergic neurons in the adult brain. This model allows testing therapeutical approaches after the loss of serotonergic neurons. Moreover, we can use our NTR-cassette also to target other neuroendocrine systems, thereby obtaining models for parkinsonism and other neurodegenerative diseases.

Ribozyme-based transgenics

Ribozymes cut target mRNAs with high sequence-specificity. Together with the group of M. Bader at the Max-Delbrück-Center for Molecular Medicine we are working on animal models with reduced expression of genes of interest, based on tRNA-fused ribozymes.

CHM knockout

A mouse knockout model for the X-linked human eye disease choroideremia will be established. At first instance male chimeras and heterozygous Chm⁺/Chm⁻ females are viable, but hemizygous Chm⁻/Y males and heterozygous Chm⁻/Chm⁺ females are not. Our rescue strategy focuses in the correction of the placental defect to obtain viable animals. These rescued-mutant mice will then be used for gene therapy and gene expression



studies. Furthermore, these mice will be useful for the identification of genetic modifiers, which in the human disease are responsible for a pronounced clinical variability.

IDO and TDO animal models

Indoleamine-2,3- (IDO) and tryptophan-2,3- dioxygenases (TDO) compete with tryptophan hydroxylase for their substrate: tryptophan. However, little knowledge has been accumulated for the influence of the dioxygenase pathways on the serotonin biosynthesis. We hope to elucidate the biochemical interaction of these three enzymes using transgenic and knockout animal models.

Platelets as models for synaptic processes

Platelets can be easily obtained from peripheral blood. Washed platelets are an accepted model for synaptic vesicle metabolism mechanisms. Therefore, the platelets of our tryptophan hydroxylase 1 knockout mice deliver the first opportunity to study transmitter-devoid vesicles. G. Ahnert-Hilger at the Institute for Anatomy, Charite, and we are cooperating to elucidate the vesicular trafficking mechanisms.

General information

Selected Publications 2003

Walther DJ, Peter JU, Bashammakh S, Hörtnagl H, Voits M, Fink H & Bader M (2003). *Synthesis of serotonin by a second tryptophan hydroxylase isoform.* Science 299 :76

Höltje M, Winter S, **Walther D**, Pahnner I, Hörtnagl H, Ottersen OP, Bader M & Ahnert-Hilger G (2003). *The vesicular monoamine content regulates VMAT2 activity through Gaq in mouse platelets: Evidence for autoregulation of vesicular transmitter uptake.* J Biol Chem 278:15850-15858

Walther DJ (2003). *Die Serotonin-Biosynthese wird im zentralen Nervensystem von einem neuronenspezifischen Tryptophan-Hydroxylase-Isoenzym geschwindigkeitsbestimmend katalysiert.* BIOSpektrum 9:184-186

Walther DJ & Bader M (2003). *A unique central tryptophan hydroxylase isoform.* Biochem Pharmacol 66:1673-1680 (review)

External funding

DFG, SFB 577: *Analysis of clinical variability in Mendelian disorders*, subproject *Establishing a mouse model for the degenerative human eye disease choroideremia*, 1 PhD student and 1 technician funded

Patents

Walther DJ & Bader M. *Verwendung von Tryptophan-Derivaten zur zytostatischen Behandlung von Serotonin-produzierenden Tumoren.* Amtliches Aktenzeichen 101 12 882.7, März 2001. Internationale Anmeldung PCT/DE Verfahren läuft.

Walther D & Bader M. *Neuronal exprimierte Tryptophan-Hydroxylase und ihre Verwendung.* Amtliches Aktenzeichen 102 32 151.5, Juli 2002. Internationale Anmeldung PCT/DE Verfahren läuft.

Clinical Genetics



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Scientific overview

The clinical genetics group was set up in October 2001 to improve clinical characterisation and recruitment of patients with disease-associated balanced chromosomal rearrangements.

Disease-associated balanced chromosomal rearrangements

The systematic study of disease-associated balanced chromosomal rearrangements (DBCRs) is a powerful tool for identifying genetic changes underlying human disease. We have re-examined and characterized patients with DBCRs analysed in V. Kalscheuer's group. For example, patients with balanced translocations had varying degrees of mental retardation with or without epilepsy due to truncation of *STK9*, *KIAA1202* or *ZNF41* (further details are given under "Chromosome rearrangements and disease"). Detailed knowledge about the phenotype is also a prerequisite for the identification of further patients suitable for mutation analysis of those genes identified by breakpoint analysis. Whereas initially we concentrated on characterization of patients with DBCRs already under investigation at our department, we are now focussing on recruiting new patients with DBCRs and interesting phenotypes.

To gain more insight into the role of balanced chromosomal rearrangements (BCRs) in complex late-onset diseases, we have started a survey among previously healthy carriers of balanced chromosomal rearrangements in Germany, Denmark (in close collaboration with N. Tommerup), and Poland (collaboration with A. Latos-Bielenska). Questionnaires are being sent to adult carriers of BCRs covering numerous health-related aspects, with a focus on neurodegenerative, cardiovascular and metabolic disorders as well as cancer and infertility. Sixteen potential disease-associated breakpoints have already been identified, including a patient with psoriasis carrying a translocation involving chromosome 4q31.1, which has been shown to harbour a psoriasis susceptibility locus.

X-linked mental retardation

We are continuing to collect families with X-linked mental retardation (XLMR) in collaboration with clinical geneticists from Germany and Poland (A. Latos-Bielenska). Phenotypes of those families investigated in the familial mental retardation group are further characterized clinically. Among the syndromic forms with XLMR are families with Lujan-Fryns syndrome, Renpenning syndrome, a family with mental retardation and retinitis pigmentosa and a family with primary ciliary dyskinesia.



Noonan syndrome and related disorders

We have collected more than 100 patients with Noonan syndrome and similar phenotypes for mutation analysis in the *PTPN11*-gene (close collaboration with V. Kalscheuer's group) and genotype-phenotype correlation. As only about 30% of these patients have mutations in the *PTPN11*-gene and the remaining families are too small for linkage analysis, breakpoint mapping in a patient with Noonan syndrome and another patient with a Noonan-like phenotype both with balanced translocations are currently performed (collaboration with V. Kalscheuer's group). Within the group of patients referred for investigation of Noonan syndrome a 5 generation family with isolated Pterygium colli was identified and linkage analysis is planned. In 7 clinically well characterized patients with the rare LEOPARD syndrome 3 different missense mutations were found (Hoeltzenbein et. al in preparation).

Genetic counseling and clinical genetics

We offer genetic counseling for patients and their families at the Institute of Medical Genetics, Charité, Berlin. Ongoing projects comprise further clinical investigations of large families with autosomal dominant inheritance of Emery-Dreifuss muscular dystrophy (collaboration with M. Wehnert, Greifswald and P. Nürnberg, Berlin), spastic paraplegia and paroxysmal kinesigenic choreoathetosis, all without mutations in known genes. In addition we are trying to establish genotype-phenotype correlations in patients with small chromosomal deletions or duplications, i.e. in a large family with mental retardation and peripheral neuropathy with an unusually large 17p11.2-12 duplication (collaboration with B. Rautenstrauss, Erlangen), and a patient with dystrophy and mental retardation with a deletion of 5q23-31 (Tzschach et. al in preparation).

General information

Publications 2002-2003

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Huehne K, Benes V, Thiel C, Kraus C, Kress W, **Hoeltzenbein M**, Ploner CJ, Kotzian J, Reis A, Rott HD & Rautenstrauss BW (2003). *Novel mutations in the Charcot-Marie-Tooth disease genes PMP22, MPZ, and GJB1*. Hum Mutat 21(1):100

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Kalscheuer VM, Tao J, Donnelly A, Hollway G, Schwinger E, Kubart S, Menzel C, **Hoeltzenbein M**, Tommerup N, Eyre H, Harbord M, Haas E, Sutherland GR, **Ropers HH** & Geçz J (2003). *Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation*. Am J Hum Genet 72(6):1401-11

Musante L, Kehl HG, Majewski F, Meinecke P, Schweiger S, Gillissen-Kaesbach G, Wiczorek D, Hinkel GK, Tinschert S, **Hoeltzenbein M**, **Ropers HH** & Kalscheuer VM (2003). *Spectrum of mutations in PTPN11 and genotype-phenotype correlation in 96 patients with Noonan syndrome and five patients with cardio-facio-cutaneous syndrome*. Eur J Hum Genet 11(2):201-6

Ropers HH, **Hoeltzenbein M**, Kalscheuer V, Yntema H, Hamel B, Fryns JP, Chelly J, Partington M, Geçz J & Moraine C (2003). *Nonsyndromic X-linked mental retardation: where are the missing mutations?* Trends Genet 19(6):316-20

Shoichet SA, Hoffmann K, Menzel C, Trautmann U, Moser B, **Hoeltzenbein M**, Echenne B, Partington M, van Bokhoven H, Moraine C, Fryns JP, Chelly J, Rott HD, **Ropers HH** & Kalscheuer VM (2003). *Mutations in the ZNF41 gene are associated with cognitive deficits: identification of a new candidate for X-linked mental retardation*. Am J Hum Genet (in press)

Horvath R, Scharfe C, **Hoeltzenbein M**, Do BH, Schroder C, Warzok R, Vogelgesang S, Lochmuller H, Muller-Hocker J, Gerbitz KD, Oefner PJ & Jaksch M (2002). *Childhood onset mitochondrial myopathy and lactic acidosis caused by a stop mutation in the mitochondrial cytochrome c oxidase III gene*. J Med Genet 39(11):812-6

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Book contributions

Hoeltzenbein M & Wehnert M (2003). *Emery-Dreifuss Muskeldystrophie*. In: Spuler, Simone, Moers, Arpad, eds., *Muskelkrankheiten*, Schattauer 2003 (in press)

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Teaching

Andreas Tzschach: Seminars and practical courses *Human Genetics*, for 1st and 3rd year medical students at the Humboldt University, summer term 2002- winter term 2003/2004

Awards

Award for the best poster in „Syndromology“ of the German Society of Human Genetics 2003. **Hoeltzenbein M**, Musante L, Neubauer B, Stephani U, Wiegand U, Tzschach A, Kalscheuer V, Ropers HH, Tinschert S & Meinecke P. *Clinical features of 6 patients with LEOPARD-Syndrome and mutation analysis of the PTPN11-gene*. Medgen (2003)15: 309

External funding

Tandem Project MPG: *Ascertainment of patients with disease-associated balanced chromosome rearrangements for the systematic, large-scale elucidation of genetic disorders*, 2001-2005



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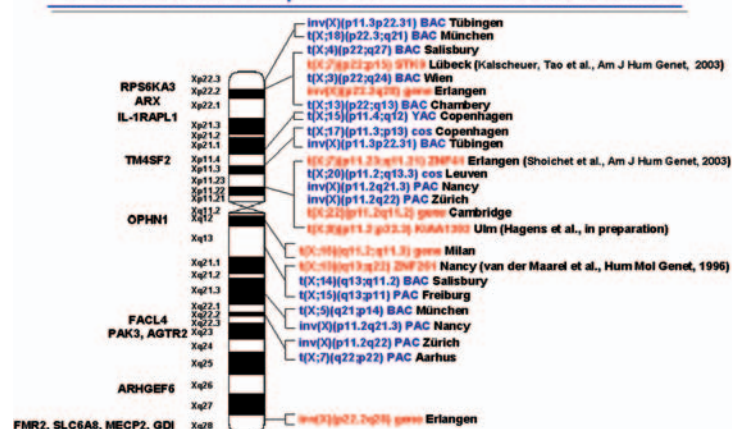
Scientific overview

To identify new genes that play a role in the development and function of the human brain and other organs, we systematically study disease-associated balanced chromosome rearrangements (DBCRs). With this powerful approach, we have found >25 new candidates for MR and related disorders.

One example is *serine/threonine kinase 9* (*STK9*), located on Xp22.2. The gene is disrupted in two female patients with severe X-linked West-syndrome (WS), also called X-linked infantile spasms (ISSX), characterized by early onset generalised seizures, hypsarrhythmia and mental retardation (collaboration with J. Gécz, Adelaide) (Kalscheuer et al, 2003). Functional absence of *STK9* in two unrelated patients with almost identical phenotype suggests its causal role in this disorder. To gain more insight into *STK9* function and the pathomechanism of ISSX, we currently establish a mouse model (in collaboration with D. Walther). A search for *STK9* interacting proteins by yeast-two hybrid screen elucidated three putative partners. One of the candidates has been shown previously to play a role in non-syndromic X-linked mental retardation. The findings in yeast are currently being confirmed in mammalian cells.

Another example is a female patient with severe non-specific mental retardation and a *de novo* balanced translocation t(X;7)(p11.3;q11.21) with a zinc finger gene truncated by the X-chromosomal breakpoint. Moreover, screening of a panel of MRX patients led to the identification of two other *ZNF41* mutations that were not found in healthy controls (Shoichet et al, in press). The function of *ZNF41* is presently unknown but other zinc finger genes have been implicated in a variety of disorders.

X-chromosomal breakpoints associated with mental retardation



Likewise, in two female patients with breakpoints in Xp11.2, the *KIAA1202* gene was found disrupted (collaboration with A. Hanauer, Strasbourg) (Hagens et al, in preparation). Current studies aim to unravel the role of KIAA1202 protein. In one of the translocation patients cloning of the junction fragment revealed that the autosomal breakpoint also truncated a gene, *FBX25*, which is a member of the F-box protein family. Detailed characterisation of the mouse orthologue showed that in adult brain transcription is confined to the hippocampus and the cerebral cortex, suggesting a major role for mouse Fbx25, and most likely also for human FBX25, in neuronal development and hippocampal function during adulthood (Hagens et al, in preparation).

Regarding other autosomal breakpoints, we recently found the gene defect in a family with non-progressive and mild cerebellar ataxia co-segregating with a familial balanced translocation t(8;20)(p22;q13) (Hertz et al, in press, Silahatoglu et al, in preparation). Likewise, breakpoint mapping in two unrelated patients with mild MR, respectively severe MR and dysmorphic features, revealed disruption of the same large gene. The function of its product is presently unknown. Remarkably another group found this gene truncated in a twin pair with MR and autism. Truncation of the same gene in three unrelated patients suggests that MR likely resulted from the chromosomal rearrangement event.

In another project we investigated three patients displayed with limb malformations, and additional MR in one of the patients. All rearrangements included the chromosomal region 2q31. Fine-mapping of the breakpoints revealed that they map proximal, respectively distal in the vicinity of the *HOXD* cluster (collaboration with N. Tommerup, Copenhagen, S. Mundlos, and H. Neitzel, Berlin) (Dlugaszewska et al, in preparation).

During the characterization of the chromosome 12 breakpoint region in a patient with a balanced t(2;12)(q37;24) translocation and a clinical diagnosis of Noonan syndrome (NS), we identified a novel gene, *thyroid hormone receptor-associated protein 2 (THRAP2)*. Interestingly this gene is expressed at a lower level in a patient cell line than in control cell lines, although the breakpoint maps 28 kb to its 5' end. We therefore characterized *THRAP2* and its mouse counterpart in more detail (Musante et al, submitted). In addition, we have screened the first known NS gene, *PTPN11*, which maps to chromosome 12q24 in a distance of about 3.4 Mb proximal to *THRAP2*, for mutations in >160 clinically well characterised NS patients (collaboration with M. Hoeltzenbein). *PTPN11* encodes the non-receptor protein tyrosine phosphatase SHP-2, which is an important molecule in several intracellular signal transduction pathways that control diverse developmental processes. Most mutations identified were clustered in the SH2 domain at the N-terminus of the SHP-2 proteins which acts as a molecular switch between the inactive and active protein form (Musante et al, 2003).

Additionally, in a male patient with a balanced t(Y;4)(q11.2;q21) and a severe neurodegenerative disorder, accompanied by MR and seizures, the breakpoint on chromosome 4 disrupts the *JNK3* gene. *JNK3* is predominantly expressed in the central nervous system and the protein has been implicated in both apoptosis and neuronal differentiation. Interestingly a truncated *JNK3* protein is present in a patient cell line, suggesting that the phenotype may result from a dominant effect, rather than a loss of function of the normal protein. Current studies aim to understand the mechanism by which this truncated protein may result in neurodegeneration and MR (collaboration with S. Schweiger, T. Herdegen, Kiel).

Past activities included the search for novel imprinted genes of human chromosome 7 and their possible involvement in Silver-Russell syndrome (SRS) (Mergenthaler et al, 2001, Blagitko et al, 2000, 1999, Riesewijk et al, 1998).



General information

Selected Publications

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- Kalscheuer VM**, Freude K, Musante L, Jensen LR, Yntema HG, Gécz J, Sefiani A, Hoffmann K, Moser B, Haas S, Gurok U, Haesler S, Aranda B, Nshedjan A, Tzschach A, Hartmann N, Roloff TC, Shoichet S, Hagens O, Tao J, van Bokhoven H, Turner G, Chelly J, Moraine C, Fryns JP, Nuber U, Hoeltzenbein M, Scharff C, Scherthan H, Lenzner S, Hamel BCJ, Schweiger S & Ropers HH (2003). *Mutations in the polyglutamine-binding protein 1 gene cause X-linked mental retardation*. *Nature Genetics* (in press)
- Kalscheuer VM**, Tao J, Donnelly A, Hollway G, Schwinger E, Kübart S, Menzel C, Hoeltzenbein M, Tommerup N, Eyre H, Harbord M, Haan E, Sutherland GR, Ropers HH & Gécz J (2003). *Disruption of the Serine/Threonine Kinase 9 gene causes severe X-linked infantile spasms and mental retardation*. *Am J Hum Genetics* 72:1401-11
- Musante L, Kehl HG, Majewski F, Meinecke P, Schweiger S, Gillissen-Kaesbach G, Wiczorek D, Hinkel GK, Tinschert S, Hoeltzenbein M, Ropers HH, **Kalscheuer VM**. (2003). *Spectrum of mutations in PTPN11 and genotype-phenotype correlation in 96 patients with Noonan syndrome and 5 patients with cardio-facio-cutaneous syndrome*. *Eur J Hum Genetics* 11:201-6
- Prudlo J, Alber B, **Kalscheuer VM**, Roemer K, Martin T, Dullinger J, Sittlinger H, Niemann S, Heutink P, Ludolph AC, Ropers HH, Zang K & Meyer T (2003). *Chromosomal translocation t(18;21)(q23;q22) indicates novel susceptibility loci for frontotemporal dementia in ALS*. *Annals Neurology* (in press)
- Ropers HH, Hoeltzenbein M, **Kalscheuer V**, Yntema H, Hamel B, Fryns JP, Chelly J, Partington M, Gécz J & Moraine C (2003). *Non-syndromic X-linked mental retardation: where are the missing mutations in Xp11*. *Trends Genetics* 6:316-20
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External funding

DFG, SFB 577: *Analysis of Clinical Variability in Mendelian Disorders*, subproject *Molecular Pathology and Embryology of HOXD-realted Limb Malformations*, 1 position funded DAAD-DST (D0209618): *Identification and characterization of new genes for X-linked mental retardation*. Joint with Prof. Dr. B.K. Thelma, University of Delhi South Campus, New Delhi, India

Teaching

Seminar and practical course *Biologie für Mediziner*, WS 1998, SS 1999, Freie Universität Berlin

Theses

Luciana Musante, *Molecular Characterization of Noonan Syndrome*. PhD Thesis, Università Degli Studi Di Torino, Italy, 2003

Nadja Blagitko-Dorfs, *Novel imprinted genes from human chromosome 7 and study of their possible involvement in Silver-Russell syndrome*. PhD Thesis, Humboldt-Universität Berlin, 2001

Bodo Brunner, *Der Kugelfisch als Modellorganismus für die Identifizierung funktionell relevanter Genomabschnitte in höheren Vertebraten*. PhD Thesis, Humboldt Universität Berlin, 2001

Jens Ruschmann: *Funktionelle Untersuchungen zum KIAA1202 Protein, einem neuen Kandidaten für X-chromosomal vererbte geistige Behinderung*. Diploma Thesis, Freie Universität Berlin, 2003

Kirsten Lenzen, *Zytogenetische und molekulargenetische Charakterisierung von krankheitsassoziierten Chromosomenbruchpunkten*. Diploma Thesis, Technische Fachhochschule Berlin, 2002

Rainer Kalamajka, *Isolierung und Charakterisierung von neuen, human Genen der chromosomalen Abschnitte Xp22 und 7q32*. Diploma Thesis, Märkische Fachhochschule Iserlohn, 2000

Michael Lang, *Isolierung und molekulargenetische Charakterisierung des 2-COP-Gens der Maus*. Diploma Thesis, Freie Universität Berlin, 1999



DNA Microarrays

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Scientific overview

Adult stem cells

We make use of the cDNA array technology to determine molecular profiles of adult stem cells and to monitor gene expression patterns during their differentiation. In the adult organism, stem cells are mainly present in regenerative organs and give rise to differentiated, specialized cell types of the respective tissue (e.g. bone marrow, liver, skin). One of the most fascinating and highly specialized types of cells are those of the nervous system: neurons and glial cells. In contrast to regenerative tissues, it was a long-held dogma in neuroscience that in the mature brain no new neurons can be generated. However, stem cells are now also known in the adult brain. We isolate neural stem cells from the subventricular zone and study dynamic gene expression changes during their *in vitro* differentiation. These studies revealed genes known or suggested to play a role in neurogenesis, but in addition many new interesting genes with potential relevance for the maintenance and differentiation of neural progenitor cells.

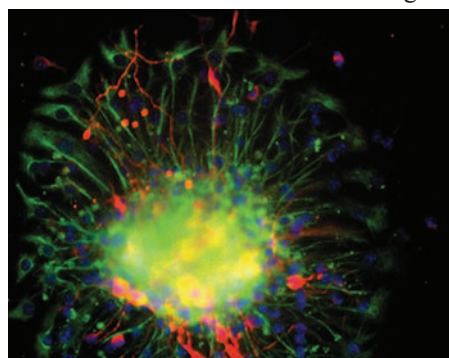


Figure: *In vitro* differentiation of neural progenitor cells into neurons (β III tubulin staining, red) and astrocytes (GFAP staining, green).

Recent findings indicate that neuron-like cells can also be generated from other types of adult stem cells that are derived from bone marrow. The unexpected plasticity of bone marrow stromal cells (BMSC) has gained increased attention, yet major gaps of knowledge concerning the exact identity of these cells and their potential remain. We have established and characterized mouse BMSC cultures and analyzed three indepen-

dent samples by cDNA microarrays. As a probabilistic model for the expression of genes in these cells, the fitting of a mixture of normal densities was applied to the dataset (Steinhoff et al., 2003). To gain clues about the positional context and biology of the isolated cells within the bone marrow stroma, we searched our data for genes which encode proteins of the extracellular matrix, cell adhesion proteins, cytoskeletal proteins and cytokines / cytokine receptors. This analysis revealed a close association of BMSC with vascular cells and indicated that BMSC are highly similar to pericytes (Wieczorek et al., 2003).

Rett syndrome

Among the different means of gene expression control, epigenetic mechanisms play an important role. A fundamental epigenetic mechanism is methylation at CpG dinucleotides in genomic DNA. Effects of DNA methylation are mediated through proteins which bind to symmetrically methylated CpGs. Many of these proteins contain a specific domain, the methyl-CpG-binding domain (MBD). So far, five vertebrate MBD proteins have been identified as members of the MBD protein family: MBD1, MBD2, MBD3, MBD4 and MECP2. Loss of MECP2 function in the nervous system is implicated in a human neurological disorder called Rett syndrome. Symptoms of this syndrome are mental retardation, loss of speech and purposeful hand use, autism, ataxia, and stereotypic hand movements. It remains unknown, why these patients present with a neurological phenotype, although *MECP2* is ubiquitously expressed. A possible explanation is the complementation of MECP2 by other MBD proteins in non-neural tissues. We have found two new polypeptide sequences with an MBD as well as four MBD proteins in man and mouse that had not been mentioned as MBD protein family members up to date. Analysis of their amino acid sequence revealed additional domains associated with chromatin and point to a function in transcription control (Roloff et al., 2003).

Array CGH

Unbalanced chromosomal aberrations (deletions, amplifications) can lead to abnormal gene expression through the disruption or abolishment of coding sequences or regulatory sequences, increased gene or regulatory element dosage, or an altered chromatin environment. Many diseases are caused by unbalanced chromosomal aberrations. In a pilot array CGH study that involved the investigation of two X-chromosomal deletions at Xq21, we found *TBX22* to be deleted in one male patient that had been earlier described with a cleft lip and palate. *TBX22* codes for a transcription factor of the T-box gene family, mutated in patients with X-linked cleft palate and ankyloglossia (CPX), but the underlying pathogenetic mechanism remained unknown so far. We have identified mouse *Tbx22* and analyzed its expression during embryogenesis by RT-PCR and *in situ* hybridization. In mouse embryos, it is expressed in distinct areas of the head, namely the mesenchyme of the inferior nasal septum, the posterior palatal shelf before fusion, the attachment of the tongue, and mesenchymal cells surrounding the eye anlage. The localization in the tongue frenulum perfectly correlates with the ankyloglossia phenotype in CPX. We furthermore identified positionally conserved binding sites for transcription factors, two of which (*MSX1*, *PRX2*) have been previously implicated in palatogenesis (Herr et al., 2003).

Mouse placentopathies

Together with Reinald Fundele (previously at the MPIMG, now University of Uppsala, Sweden), we performed gene expression studies of three different models of placental hypoplasia to identify genes and gene networks involved in this disorder (Singh et al., submitted 2003).

BRCA1-mediated repression of select X chromosome genes

The influence of BRCA1 on the expression of X-chromosomal genes was investigated in a collaboration with scientists from the NCI and the Memorial Sloan-Kettering Cancer Center, USA (Jazaeri et al., submitted 2003).



General information

Publications

Herr A, Meunier D, Muller I, Rump A, Fundele R, Ropers HH & **Nuber UA** (2003). *Expression of mouse Tbx22 supports its role in palatogenesis and glossogenesis*. Dev Dyn 226(4):579-86

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Steinhoff C, Müller T, **Nuber UA** & Vingron M (2003). *Gaussian Mixture Density Estimation applied to Microarray Data*. LNCS (Lecture Notes in Computer Sciences) 2810:418-429

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Erdogan F, Kirchner R, Mann W, Ropers HH & **Nuber UA** (2001). *Detection of mitochondrial single nucleotide polymorphisms using a primer elongation reaction on oligonucleotide microarrays*. Nucleic Acids Res 29(7):E36

Sudbrak R, Wieczorek G, **Nuber UA**, Mann W, Kirchner R, Erdogan F, Brown CJ, Wohrle D, Sterk P, Kalscheuer VM, Berger W, Lehrach H & Ropers HH (2001). *X chromosome-specific cDNA arrays: identification of genes that escape from X-inactivation and other applications*. Hum Mol Genet 10(1):77-83

Teaching

Seminar and practical course *Humangenetik für Medizinstudenten*, Charité, Humboldt University Berlin, WS 2001/2002

Seminar *Humangenetik für Medizinstudenten*, Charité, Humboldt University Berlin, SS 2002, WS 2002/03, SS 2003

Lecture *Genetik für Bioinformatiker*, Free University, Berlin, SS 2003, 2 SWS

Theses

Fikret Erdogan: *Typisierung biallelischer Marker (SNPs) mit DNS-Mikrorastern*. PhD Thesis, Freie Universität Berlin, 2003

Wieczorek, G.: *Untersuchung der X-Inaktivierung beim Menschen mit X-Chromosom-spezifischen cDNS Chips*. Diploma Thesis, Rheinische Friedrich-Wilhelms-Universität Bonn, 2000

External funding

SFB 577: *Analysis of clinical variability in Mendelian Disorders*, Project C3 *Rett syndrome*, Z1 project *DNA Microarrays*

BMBF: *Fördermaßnahme Verbesserung der Leistungsfähigkeit der klinischen Forschung an den medizinischen Fakultäten der neuen Bundesländer*

NGFN, Plattform 6.7: *Matrix-CGH*

EFRE, 01GR0203: *Zentrale Einrichtung für die systematische Suche nach submikroskopischen Deletionen und Duplikationen bei monogenen und komplexen Erkrankungen*

EU FP6: *Integrated Project Genostem*

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Scientific overview

Since the inception of our group in the fall of 2001 our efforts have been directed towards two main goals: we are aiming to complement the MR gene identification efforts in the department by establishing *in vitro* methods to functionally characterize candidate MR genes. In addition, we are investigating the 'human speech gene' FoxP2 in songbirds.

Establishing in vitro methods to functionally characterize candidate MR genes

In collaboration with Dr. Schweiger's group, we are applying siRNA technology in cell culture to investigate the Opitz BBB/G syndrome (OS). OS is a congenital disorder, affecting ventral midline development. It is often accompanied by mental retardation. OS can be caused by mutations in the MID1 gene whose product is a microtubule-associated protein with E3 Ubiquitin ligase activity. We are targeting the mRNA of MID1 by RNA interference (RNAi) to assess effects of reduced MID1 levels on other pathway components and on cell behavior. Knockdown of MID1 in HeLa cells induced cell death as shown by the presence of a prominent SubG1 peak in FACS. This indicates fragmentation of genomic DNA, which is a late sign of apoptosis. Apoptosis was confirmed using TUNEL staining as well as Annexin 5 labeling. In a complementary approach, we also targeted ALPHA4 mRNA, because ALPHA4 protein connects Protein Phosphatase 2A (PP2A) to MID1. Binding of PP2A to MID1 *via* ALPHA4 leads to ubiquitinylation of PP2A and subsequent degradation. In Opitz patients, the MID1-ALPHA4 interaction is compromised leading to elevated levels of PP2A. In HeLa cells we found that the subcellular localization of ALPHA4 is cell cycle dependent. Interestingly, the siRNA induced knockdown of ALPHA4 leads to dramatically attenuated cell growth. This attenuated cell growth is due to reduced cell division rate, as shown by an approximately 50% reduction of BrdU labeled S-phase cells. The absence of a subG1 peak in ALPHA4 knockdown excludes the possibility

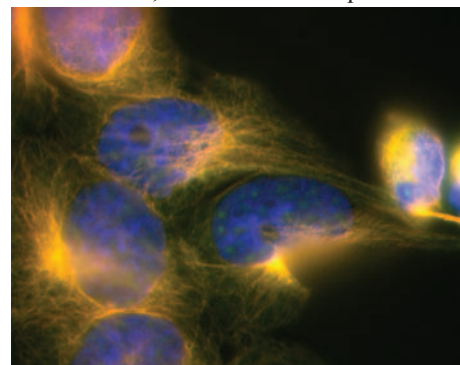


Figure 1: Microtubules of HeLa cells revealed by antibodies against tubulin and PP2Ac resulting in yellow color. Nuclei are blue.



that in addition to reduced proliferation there was increased apoptosis. Our working hypothesis is that RNAi mediated reduction of ALPHA4 prevents PP2A from binding to MID1 which recapitulates the situation in OS patients. To test this hypothesis we are quantifying the amount of ubiquitinated PP2A by immunoprecipitation. One of the candidate developmental processes affected in OS patients is the epithelial-mesenchymal transition (EMT) of premigratory cells from the neural crest. Therefore we will analyze the reported cell cycle effect also in these cells. In addition, in collaboration with Dr. Schweiger's and Dr. Nuber's group we are extending the use of RNAi to other cell types (primary neurons, neurospheres, human melanoma cell line, bone marrow stromal cells) to screen for function of additional candidate MR genes.

Using songbirds as a model to study the FoxP2 gene, which is implicated in a human speech and language deficit

Human speech and birdsong share behavioral and neural similarities. Both are learned during a critical period *via* the interaction of auditory and motor centers and require a set of specialized cerebral structures. While innate dispositions to learn and produce species-appropriate sounds are present in both humans and birds, until recently no genes had been linked to learned vocalizations. Mutations of the FOXP2 gene (of the winged-helix/forkhead box (Fox) transcription factor gene family) were identified in related individuals with severe difficulty articulating speech (Lai et al., 2001, Nat 413; 519-23). Structural and functional brain anomalies of affected individuals implicate the basal ganglia as one of the key affected brain regions. Since vocal learning in songbirds depends in part on a specialized pathway through the basal ganglia, we cloned the FoxP2 gene from zebra finch, and compared its expression pattern with eight other species of 'vocal learners' and two species of vocal non-learners. The latter lack specialized telencephalic vocal structures but vocalize innately *via* a set of sub-telencephalic nuclei common to both vocal learners and non-learners. Sequence homology between human, mouse and songbird FoxP2 was >90%. Amino acids previously reported to be unique for the human lineage were not 'human'-like in zebra finch. FoxP2 was expressed by embryonic day 3.5 and was strongest during development, but persisted in attenuated form into adulthood. During the vocal learning phase of zebra finches, FoxP2 was upregulated in Area X, a striatal nucleus characteristic for learners only. In adults, different types of vocal learners showed species specific expression differences limited to Area X. The rest of the striatum and non-telencephalic auditory and visual regions, among them nuclei of the dorsal thalamus, nucleus rotundus, the inferior olive and Purkinje cells of the cerebellum expressed FoxP2 in both vocal learning and non-learning birds. FoxP1, FoxP2's closest homologue, was expressed in a partly overlapping but distinct pattern. Double labeling with FoxP2 and a number of markers for distinct populations of striatal neurons are in progress. These findings are compatible with a role of FoxP2 in shaping the neural circuits that are necessary but not sufficient for vocal learning.

A second project investigates whether the FOXP2 gene has evolved differently in birds that learn their song from those whose song is innate. Analysis of the molecular evolution of human FOXP2 showed a high degree of conservation among all vertebrates tested so far. Furthermore human FOXP2

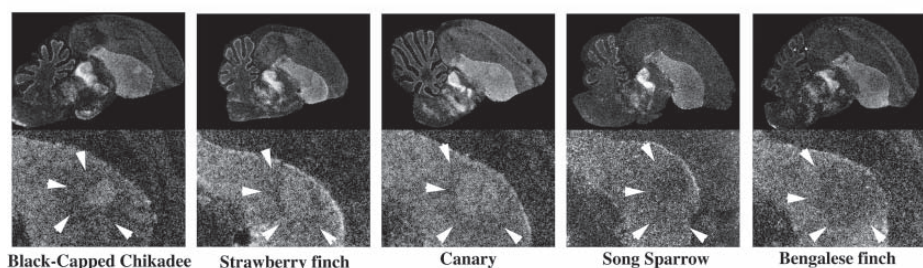


Figure 2: FoxP2 is differentially expressed in a song learning region ('Area X') within the basal ganglia of different avian species.

contains changes in amino-acid coding and a pattern of nucleotide polymorphism that suggest that this gene has been the target of selection during recent human evolution (Enard et al., 2002, Nat 418:869-72; Zhang et al., 2002, Genetics 162:1825-35). This indicates that FOXP2 might have been pivotal for the development of human language. Although language is a uniquely human trait, learned vocalizations are also found in a few other species, among them whales, bats, and most prominently three orders of only distantly related birds. To address the question of whether a selective sweep towards learned vocalization also occurred in FoxP2 during songbird evolution, we have sequenced and are currently analyzing the FOXP2 ORF from 12 species of avian vocal learners, non-learners and evolutionary more distant relatives.

General information

Publications 1998-2003

Doetsch F & Scharff C (2001). *Challenges for brain repair: insights from adult neurogenesis in birds and mice*. Brain Behav Evol 58(5):306-322

Nehrbass N, Jarvis E, **Scharff C**, Nottebohm F & Mello CV (2000). *Site-specific retinoic acid production in the brain of adult songbirds*. Neuron 27:359-370

Scharff C (2000). *Chasing fate and function of new neurons in adult brains*. Curr Opin Neurobiol 10:774-783

Scharff C, Kim J, Macklis J, Nottebohm F (2000). *Targeted neuronal death affects neuronal replacement and vocal behavior in adult songbirds*. Neuron 25:481-492

Jarvis E, **Scharff C**, Ramos J, Grossman M & Nottebohm F (1998). *For whom the bird sings: Context-dependent gene expression*. Neuron 21:775-788

Scharff C, Nottebohm F & Cynx C (1998). *Conspecific and heterospecific song discrimination in male zebra finches with lesions in the anterior forebrain pathway*. J Neurobiol 36:81-90

Constance Scharff: Invited lectures
Insights from bird brains: pathways for learned vocal communication, regulation of adult neurogenesis, and characterization of a "speech" gene. Max-Planck-Institute for Neurobiology, Martinsried, 14/1/2003

On the trail of bird speech: cloning of a "language" gene and its expression patterns in zebra finch brain. SFB 515 symposium, Berlin, 21/2/2003

Insights from bird brains: pathways for learned vocal communication, regulation of adult neurogenesis, and characterization of a 'speech' gene. Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, 13/3/2003

On the Trail of Bird Speech: Cloning of the 'Language Gene' FoxP2 and its Expression in the Avian Brain. Symposium 'Evolution of Institute of Cognitive Neuroscience, Dept. Biopsychology & International Graduate School for Neuroscience, Ruhr-Universität Bochum GAFO 03/252, 20/3/2003

Regulation and function of adult neuronal replacement in songbirds. Philippe Laudat Conference INSERM. Neural stem cells: from development to the clinic. 20/2/2002

Killing me softly with your song: regulation and function of adult neuronal replacement in adult zebra finches. Berlin Neuroscience Forum, Liebenwalde, Brandenburg, 18/4/2002

On the trail of bird speech: cloning of a "language" gene and its expression patterns in zebra finch brain. Behavioral Neurobiology of Birdsong 16th Annual Symposium of the Center for Study of Gene Structure & Function, Hunter College, CUNY, New York, 12/12/2002

Teaching

Lecture *Behavioral plasticity in songbirds*. Biology Department, Free University Berlin, Seminar for High-School Teachers, 12/10/2001

Lecture *Neuronal control of song*. Free University Berlin, Ornithology lecture series, 11/6/2002

Lecture *Hormones and neurogenesis in songbirds*. Free University Berlin, Ornithology lecture series, 18/6/2002

Lecture *Neurogenesis and behavior: song development in zebra finches*. Humboldt University Berlin, 25/6/2002

Lecture *Acoustic communication, birdsong*. Part of the series: from the brain to behavior – from behavior to the brain. Free University Berlin, 1/7/2003

Lecture *Sexual Dimorphism in communication behavior*. Part of the series: from the brain to behavior – from behavior to the brain. Free University Berlin, 8/7/2003

External funding

DFG, SFB 515, *Developmental and experience-dependent neural plasticity*; Subproject *Behavioral and cellular consequences of targeted cell death in songbirds*, PhD student and technical assistant funded, 2002-2004

NIH, RO1 Mental Health 63132-01 (collaborator), *Functional recovery after induced neuronal death*, one Postdoc funded

Organization of scientific events

Organization of the 1st day of Scientific Exchange at Harnack House, February 28th, 2002

I hosted the following speakers for the Dahlem Colloquia series

Luis Puelles, PhD, University of Murcia, Spain: *A molecular Bauplan of the vertebrate nervous system*



Christoph Redies, Prof. Dr., Institute of Anatomy, University Hospital Essen, Germany: *Cadherins: An adhesive code for brain development*

Jeffrey D. Macklis, M.D., D.HST, Associate Professor of Neurology and Neuroscience, Harvard Medical School, Director, MGH-HMS Center for Nervous System Repair, Massachusetts General Hospital, Boston: *Cellular Repair of Complex Cortical Circuitry by Neural Precursors: Induction of Neurogenesis*

Fiona Doetsch, Ph.D., Dept. of Molecular and Cellular Biology, Biolabs, Harvard University, Cambridge, MA, USA: *Biology of Stem Cells in the Adult Mammalian Brain*

Rogier Versteeg, Department of Human Genetics, Academic Medical Center, University of Amsterdam, The Netherlands: *The Human Transcriptome Map: visualization of absolute gene expression levels by SAGE for cancer and genome research*

Pierre-Marie Lledo, Prof. Dr., Dept. of Neuroscience, Lab of Olfactory Perception and Memory, Institut Pasteur, Paris, France: *Importance of newly formed neurons for adult olfaction*

Public relations work

I presented career options in Molecular Biology in general and at the MPIMG specifically at a career orientation evening at the Sophie-Charlotte-Gymnasium

'Personal Portrait' article appeared in Max Planck Research 3/2002

Cytology Group

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Scientific overview

Aneuploidy represents the leading genetic cause of developmental disabilities and mental retardation among neonates – ~30% of all miscarriages are aneuploid – a major cause of pregnancy loss. Chromosome rearrangements and missegregation that are transmitted to the offspring often occur in the germ line. A major interest and focus of our study is the chromosome behavior in germ cell differentiation. Moreover, we investigate the mechanism of chromosome rearrangements during karyotype evolution.

Meiosis

Because all evolutionarily fixed rearrangements have been disseminated in a population, one line of our research centers on chromosome behavior in germ cell differentiation. The nucleus at meiosis sees a drastic rearrangement of chromosome structure and location which culminates in the formation of bivalents, paired homologous chromosomes, that are requisite for segregation of the homologues in the meiosis I division that lies at the heart of gamete formation. Meiosis reduces the chromosome complement to the haploid, thereby compensating for the genome doubling at fertilization.

Homologous recombination occurs during first meiotic prophase and provides for physical links (chiasmata) between homologues, which are the cytological manifestation of homologous

recombination. Recombination at illegitimate sites is thought to fuel the chromosome rearrangements, also those that are seen in evolution. It is thus imperative to understand the location of break points, fragile sites and recombinogenic sequences in the context of genome architecture and nuclear topology. Since many of the genes involved in meiotic differentiation are required for fertility, we have established tools to fine-stage prophase I progression in the model systems budding yeast and mouse, which allow to detect errors in this complex differentiation progress.

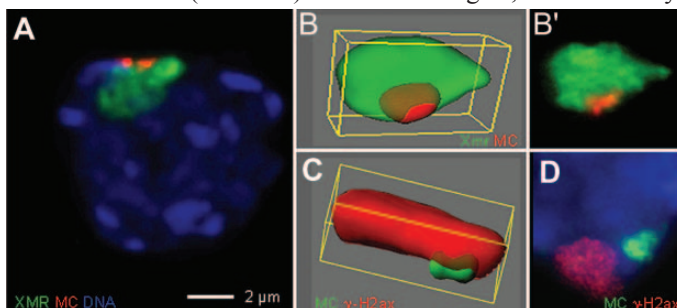


Figure 1: (A) Human microchromosomes (MC, red) escape the pachytene checkpoint by association with the transcriptionally inactive XY body (green, Xmr fluorescence; A,B') in transchromosomal mice. (B,C) 3D reconstructions of a portion of a spermatocyte the XY body chromatin and a MC. The MC is embedded in the XY chromatin (green) but do not adopt the γ -H2ax histone chromatin mark specific for this compartment (C). MCs (green) off the XY-body are transcriptionally active since they lack the γ -H2ax fluorescence (red) (Voet et al. 2003).



Since my move to the MPIMG in 11/2001 we were able to transfer our molecular cytological techniques and could show in budding yeast meiosis that *SIR3*, *KAR3*, *SPO13* gene products are involved in the control of meiotic chromosome dynamics off the telomeres. In a collaboration with Dr. A. Goldman, Sheffield, we were able to show that double strand break (DSB) formation is required for homologue pairing in yeast meiosis and that a single DSB can not rescue defects in chromosome pairing in the presence of a catalytic inactive form of the SPO11 transesterase.

In the mouse we have recently used trans-chromosomal mice (collaboration with P. Marynen and T. Voet, Leuven Univ., Belgium) to investigate checkpoint response and germ line transmission of telomere-less mini-ring chromosomes. We could delineate a novel way of how mini ringchromosomes bypass to the need for telomere clustering for pairing by association with the sex body (Figure 1) and how they escape checkpoints during pachytene. We have also shown that failure of ring-chromosome cohesion during metaphase I was sensed by the spindle checkpoint. These strategies may be applicable to human microchromosomes that are transmitted to the offspring and are occasionally associated with mental retardation.

In an attempt to understand the role of the nuclear envelope for the pairing of meiotic chromosomes and telomere attachment, we are currently investigating the spermatogenesis of *Lmna* and *Sycp3* knockout mice. Furthermore, we test the impact of the DSB repair pathway on meiotic telomere behavior in that we investigate the spermatogenesis of *Spo11*, *Dmc1*, *Gadd45*, and *H2ax* knockout mice.

Altogether our efforts will establish a cytological map of prophase I arrest phenotypes and help to build a circuitry around the meiotic telomere, which may aid analysis of failure of germ cell differentiation in infertile patients. Furthermore, our analysis will help to understand why up to one third of fertilized human eggs are trisomic or monosomic, with aneuploidy representing the leading genetic cause of developmental failure and pregnancy loss.

Karyotype evolution

In the past I have worked with model species like the Indian muntjac deer (*Muntiacus muntjac* *vag.*) which has the lowest chromosome number in all mammals. Its $2n=6$ fusion chromosomes have likely been formed by chromosome fusions and translocations, while it is still a phenocopy of its close relative the Chinese muntjac that harbours $2n=46$ chromosomes. My group was among the pioneers to establish molecular cytogenetic tools that allow for demonstrating regions of conserved synteny among mammalian genomes. We have also provided first evidence on the nature of sequences located at evolutionary breakpoints in the fusion genome of the Indian muntjac. After my arrival at the MPI-MG, Nils Hartman joined the group as Ph.D. student. He has a strong interest in chromosome evolution and he continued research in muntjac chromosome fusions. We were able to amplify sequences from the ancestral chromosome fusion points that contain GC-rich repetitive satellite DNA sequences and telomere repeats (Fig. 2), confirming hypotheses that these sequences were involved in muntjac karyotypic evolution. A manuscript on this topic has recently been submitted for publication.

Currently, Nils is cloning the *Terf1* and *Terf2* genes, which encode telomere-binding proteins and confer karyotype stability, from two muntjac genomes. He is currently analyzing *Terf* expression and could detect Muntjac-specific alternatively spliced transcripts. We are planning to investigate the role of these transcripts for chromosome stability by knocking them down by RNAi in cell culture systems and by over-expressing them in muntjac fibroblasts and HeLa cells.

Finally, I have provided expertise in advanced immunofluorescence and FISH methods as well as microscopy to the department and its students, which is reflected in a shared first author paper with the Berger/Schweiger groups.

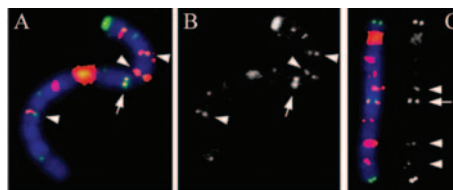


Figure 2: Detection of ancestral chromosome fusion points by FISH of telomere &/ breakpoint satellite PCR products. Interstitial telomere repeats (green in A,C; gray in B and C left) colocalize with remnants of centromeric sequences (red in A,C; arrows) in Indian muntjac chromosomes 1 (A,B) and 2 (C).

General information

Selected Publications 2002 – 2003

Fernandez-Capetillo O, Liebe B, Scherthan H & Nussenzweig A (2003). *H2AX regulates meiotic telomere clustering*. J Cell Biol 163(1): 15–20

Kalscheuer VM, Freude K, Musante L, Jensen LR, Yntema HG, Géczy J, Sefiani A, Hoffmann K, Moser B, Haas S, Gurok U, Haesler S, Aranda B, Nshedjan A, Tzschach A, Hartmann N, Roloff TC, Shoichet S, Hagens O, Tao J, van Bokhoven H, Turner G, Chelly J, Moraine C, Fryns JP, Nuber U, Hoeltzenbein M, Scharff C, **Scherthan H**, Lenzner S, Hamel BCJ, Schweiger S & Ropers HH (2003). *Mutations in the polyglutamine-binding protein 1 gene cause X-linked mental retardation*. Nat Genet (in press)

Pfeifer C, **Scherthan H** & Thomsen PD (2003). *Sex-specific telomere redistribution and synapsis initiation in cattle oogenesis*. Dev Biol 255:206-215

Trelles-Sticken E, Loidl J & **Scherthan H** (2003). *Increased ploidy as well as KAR3 and SIR3 disruption alter meiotic chromosome behavior and bouquet formation*. J Cell Sci 116:2431-2442

Voet T, Liebe B, Labaere C, Marynen P & **Scherthan H** (2003). *Telomere-independent homologous pairing and checkpoint escape of accessory ring chromosomes in male mouse meiosis*. J Cell Biol 162:795-808

Zeitz C*, **Scherthan H***, Freier S, Feil S, Suckow V, Schweiger S & Berger W (2003). *NYX (nyctalopin on chromosome X), the gene mutated in congenital stationary night blindness, encodes a cell surface protein*. Inv Ophthalmol 44:4184-4191

Scherthan H (2003). *Interphase cytogenetics in understanding chromosome and telomere dynamics during prophase I: implications for meiotic telomere movements*. Chromosomes Today 14 (in press)

Lorenz A, Fuchs J, Trelles-Sticken E, **Scherthan H** & Loidl J (2002). *Spatial organisation and behaviour of the parental chromosome sets in the nuclei of Saccharomyces cerevisiae × S. paradoxus hybrids*. J Cell Sci 115:3829-3835

Neale MJ, Ramachandran M, Trelles-Sticken E, **Scherthan H** & Goldman ASH (2002). *Wild-type levels of Spo11-induced DSBs are required for normal single-strand resection during meiosis*. Mol Cell 9:835-846

Selected Invited Plenary Lectures

Gordon Research Conference on Meiosis. Colby Sawyer College, NH, USA, 6/2002

DFG Priority Program *Functional Architecture of the Cell Nucleus*, Heidelberg, 4/2004

Gordon Research Conference on Meiosis; Chair and plenary lecture, NH, USA, 6/2004

Teaching

Special practical course *Molekulare Cytologie/Cytogenetik*; lecture *Grundlagen der molekularen Cytologie/Cytogenetik*, each term; University of Kaiserslautern

External funding

DFG, Sche 350/8-4: *Bukettbildung*, 1 Postdoc & 2 PhD students funded

Co-operations

Chromosome dynamics in S. cerevisiae meiosis, with Prof. Dr. J. Loidl, Botanisches Institut, Abt. Genetik und Cytologie der Universität Wien, Österreich

Nuclear periphery, telomeres and DNA repair state, with Dr. U. Nehrbaas, Institut Pasteur, Paris, France

The role Histone methyltransferases for meiotic progression, with Dr. V. Geli, CRNS, Marseille, France

Interdependence of DSB repair and Telomere clustering in mice, with Dr. S. Keeney, Sloan Kettering Cancer Center, NYC, USA

Germ line transit of accessory chromosome vectors, with Prof. P. Marynen, Human Genetics, University of Leuven, Belgium

Meiotic telomere behavior in histone H2ax-deficient mice, with Dr. A. Nussenzweig, NIH, Bethesda, USA

Meiosis in FancA-mutant mice, with Dr. M. Digweed, Charité Berlin



Biochemistry of Inherited Brain Disorders



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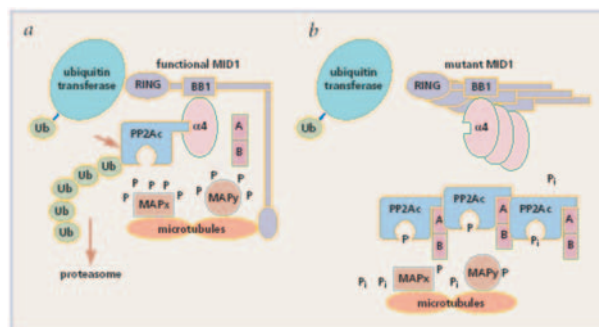
Scientific overview

While transgenic mice are firmly established as an indispensable model for the study of human disease, there are obvious limitations to this approach, the most striking being the modelling of complex traits such as intelligence and behaviour. Nature's own knock-out experiments leading to monogenic disorders can efficiently fill this gap. For the last couple of years my group has been focussing on the elucidation of human pathology starting from the underlying genetic defect of a monogenic disorder characterized by developmental defects of the ventral midline, the so-called Opitz BBB/G-syndrome (OS).

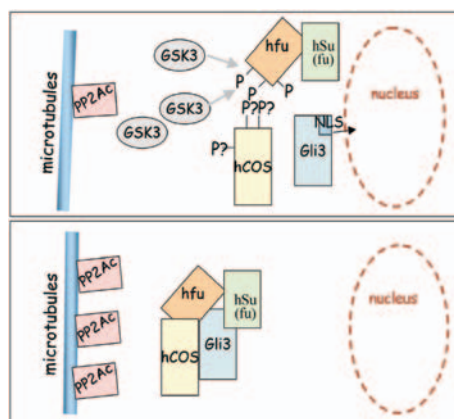
Most important symptoms of OS are hypertelorism and hypospadias. In addition, a variable set of midline defects, such as agenesis of the corpus callosum, cleft lip and palate, laryngo-tracheo-esophageal clefts, congenital heart defects and intestinal malformations have been observed in OS patients. Some (10-30 %) of the patients are mentally retarded. Taking advantage of a balanced translocation associated with the OS phenotype in a three generation pedigree, we identified the causative gene for the X-linked form of the syndrome, *MID1*, in a positional cloning approach (Quaderi et al. 1997).

We could show that the gene product of the *MID1* gene, a member of the RING finger protein family, associates to microtubules. Mutations found in OS patients prohibit microtubules-association and lead to the formation of intracytosolic clumps instead (Schweiger et al. 1999). Further studies showed that, similar to other members of the RING finger protein family, the MID1 protein exhibits ubiquitin ligase activity. *Via* interaction with the $\alpha 4$ protein it triggers microtubules-associated protein phosphatase 2A towards ubiquitin specific degradation (Trockenbacher et al. 2001). Aberrant MID1 protein detached from the microtubules, can no longer fulfill this function which leads to an enrichment of microtubules associated PP2A. In line, we could demonstrate hypophosphorylation of microtubules associated proteins in OS embryonic fibroblasts compared to age-matched controls.

On the basis of the work summarized above, we hypothesized that the MID1 protein might be involved in the regulation of the sonic hedgehog (shh) signalling pathway. This



conclusion was made due to striking phenotypic overlaps of OS and Greig-syndrome, a developmental malformation syndrome caused by mutations in the *Gli3* gene, which codes for a major transcription factor of the shh pathway. In addition, a central regulatory role of microtubule-associated serine/threonine phosphorylation in the hedgehog signaling cascade in drosophila had been demonstrated previously. In extensive experiments, we could indeed show that the MID1/PP2A complex regulates the subcellular localisation of the Gli3 protein. Further studies showed that the activity of PP2A phosphatase in this signaling network is counter-acted by a kinase which we identified as glycogen synthase kinase 3 (GSK3). We went on to show that, depending on the balance between the activities of PP2A and GSK3, yet another protein, humanFused, which binds Gli3, is phosphorylated, thus causing retention of Gli3 in the cytosol (manuscript in preparation). Our data allowed the identification of a previously unknown, and completely unsuspected, signalling network which is illustrated below.



The ubiquitous expression pattern of *MID1* transcripts and the central cellular role of its gene product poses the question as to how mutations in the *MID1* gene lead to the highly specific OS phenotype despite ubiquitous expression of the *MID1* message. This could be mediated through alternative splicing leading to the expression of tissue-specific mRNAs and protein isoforms with specified functions. By *in-silico* analysis of the available genomic sequences of the *MID1* gene in human, mouse and Fugu, we have now identified a complex pattern of alternative splicing of the *MID1* RNA. Taking this as a basis we discovered

several mechanisms mediating a subtle regulation of MID1 protein function that could be observed in all three organisms (manuscript submitted).

The discovery of the MID1/ α 4/PP2A regulatory complex, its participation in the shh pathway and the establishment of GSK3 as antagonistic kinase of PP2A connects the shh pathway with two other major signalling pathways: the wnt-signalling cascade and the TOR pathway. One main purpose of future work will be to further characterize the complex interactions of these pathways in order to define the interdependent network elements in detail, as well as to elaborate their relevance for development, tumorigenesis and the pathogenesis of neurodegenerative disorders. This will also pave the way for the identification of novel drug targets for the treatment of cancer and Alzheimer's dementia.

Closely related to this, in an affinity-chromatographie-approach employing domains of the α 4 protein, we are currently trying to identify the components of the MID1 protein complex. The identified proteins will enhance the complexity of the suggested network. In addition, if one maps to chromosome 22, it would form a candidate for causing the autosomal form of OS.

General information

Selected Publications 1998-2003

Kalscheuer VM, Freude K, Musante L, Jensen LR, Yntema HG, Gécz J, Sefiani A, Hoffmann K, Moser B, Haas S, Gurok U, Haesler S, Aranda B, Nshedjan A, Tzschach A, Hartmann N, Roloff TC, Shoichet S, Hagens O, Tao J, van Bokhoven H, Turner G, Chelly J, Moraine C, Fryns JP, Nuber U, Hoeltzenbein M, Scharff C, Scherthan H, Lenzner S, Hamel BCJ, **Schweiger S** & Ropers HH (2003). *Mutations in the polyglu-tamine-binding protein 1 gene*

cause X-linked mental retardation. Nature Genetics (in press)

Musante L, Kehl HG, Majewski F, Meinecke P, **Schweiger S**, Gillissen-Kaesbach G, Wiczorek D, Hinkel GK, Tinschert S, Hoeltzenbein M, Ropers HH & Kalscheuer VM (2002). *Spectrum of mutations in PTPN11 and genotype-phenotype correlation in 96 patients with Noonan syndrome and five patients with cardio-facio-cutaneous syndrom*. EJM 11:201-206



Schweiger S, Chaoui R, Tennstedt C, Lehmann K, Mundlos S & Tinschert S (2003). *Antenatal onset of cortical hyperostosis (Caffey disease): Case report and review*. Am J Med Genet (in press)

Schweiger S & Schneider R (2003). *The MID1/PP2A complex: a key to the pathogenesis of Opitz BBB/G syndrome*. Bioassays 25: 356-366 (invited review)

Winter J*, Lehmann T*, Suckow V, Kijas Z, Kulozic A, Hamel B, Opitz J, Lenzner S, Ropers HH & **Schweiger S** (2003). *Duplication of exon 1 of the MID1 gene in a patient with Opitz G/BBB syndrome*. Hum Genet 112: 249-254

Raderschall E, Stout K, Freier S, Suckow V, **Schweiger S** & Haaf T (2002). *Elevated levels of Rad51 Recombination Protein in Tumor Cells*. Cancer Res 69:219-225

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Rinderle C, Christensen H-M, **Schweiger S**, Lehrach H & Yaspo M-L (1999). *AIRE encodes a nuclear protein co-localizing with cytoskeletal filaments: altered sub-cellular distribution of mutants lacking the PHD zinc fingers*. Hum Mol Genet 8(2):277-290

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Vonrhein C, Schmidt U, Ziegler GA, **Schweiger S**, Hanukoglu I & Schulz GE (1999). *Chaperone-assisted expression of authentic bovine adrenodoxin reductase in Echerichia coli*. FEBS Lett 443:167-169

Suckow V, Fartmann B, Todt T, van der Maarel S, Foerster J & **Schweiger S** (1998). *A rapid and inexpensive method for large-scale DNA sequencing of regions with large amounts of repetitive elements*. TIGS, TTO 01332

Teaching

Seminar *Klinische Genetik und Humangenetik für Medizinstudenten*, FB Medizin, Charité, SS 2001, 1 SWS

Seminar *Klinische Genetik und Humangenetik für Medizinstudenten*, FB Medizin, Charité, SS 2002, 1 SWS

Lecture (Praktikumsbegleitende Vorlesung) *Biochemie*, FB Chemie, Freie Universität Berlin, SS 2002, WS 2002/03, je 3 SWS

Lecture and practical course *Genetik für Bioinformatiker*, Freie Universität Berlin SS 2003, 3 SWS

Theses

T. Lehmann, *Isolierung und Charakterisierung exprimierter Sequenzen im Bereich des MID1-Gens*. PhD Thesis, Humboldt Universität Berlin, 2003

J. Winter, *Molekulare Charakterisierung des MID1 Gens*. PhD Thesis (submitted), Freie Universität Berlin, 2003

S. Krauss, *Molekulare Charakterisierung des MID1 Gens bei Mensch, Maus und Fugu*. Diploma Thesis, Fachhochschule Berlin, 2002

External funding

SFB 557: *Analysis of clinical variability in Mendelian disorders*, Teilprojekt C04

Patents

US-Patentanmeldung 60/380,590: *Intervention in intracellular PP2A levels via its interaction with the $\alpha 4$ protein: implications for Alzheimer and cancer treatment*

Co-operations

Biochemistry of the MID1 protein, with Prof. Rainer Schneider, University Innsbruck, Austria

Structural analysis of the MID1/ $\alpha 4$ complex, with Prof. Konrat, Biocenter Vienna, Austria

The MID1 gene during development, with Dr. Nandita Quaderi, MRC, London, UK

The MID1/PP2A complex, with Prof. Brian Wadzinski, Nashville, USA

Shh and rapamycin, with Dr. John Foerster, Dr. Uwe Trefzer, Charité, Berlin

Familial Mental Retardation



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Scientific overview

In our population, monogenic forms of mental retardation (MR) appear usually as sporadic, because dominant MR will not be transmitted unless it is mild, and in small families, recessive forms will be rarely observed more than once.

The only exception is X-linked MR (XLMR) where pedigrees with several affected patients are not uncommon. Because of the well established preponderance of mentally retarded males, about 25 percent of the severe forms of MR are thought to be due to X-chromosomal gene defects. One third of these patients have syndromic forms of XLMR, where MR is associated with recognisable clinical signs such as skeletal abnormalities or dysmorphic facial features. So far, the underlying gene defect has been identified in 30 clinically distinguishable forms of syndromic XLMR. In contrast, finding the molecular causes of non-syndromic (NS-) XLMR has turned out to be very difficult because of genetic heterogeneity, which precludes pooling of linkage data from different families. Against this background, we and four other European laboratories (Chelly et al, Paris; Moraine et al, Tours; Frijns et al, Leuven; and Hamel et al, Nijmegen) have founded the European MRX Consortium, which aims to elucidate all frequent forms of X-linked mental retardation, with a focus on non-syndromic XLMR. For several years, our most significant contribution to this consortium was the mapping and cloning of breakpoints in numerous mentally retarded patients with balanced X-chromosome rearrangements (see group Kalscheuer). Only in 2002, at about the same time when we started our collaboration with A. Latos-Bielenska in Poznan (Poland), a separate group was formed at our department to search for XLMR genes by studying families in a systematic fashion.



Until 2002, 13 genes have been implicated in NS-XLMR, and the European XLMR Consortium was involved in the isolation of 8 of these. However, with one possible exception, mutations in these genes turned out to be very rare. Extrapolation of these findings suggested that close to 100 different genes might be involved in non-syndromic XLMR, 5-10 times more than previously thought, and that mutations in the known genes accounted for less than 20 percent of all families with NS-XLMR.

To find out how these missing mutations are distributed on the X-chromosome and whether they are clustered in specific regions, we have recently compiled and analysed linkage data from all published and numerous unpublished families with NS-XLMR. As shown in Figure 1, the causative mutations in non-syndromic XLMR are conspicuously clustered at Xq28, and even more so in the proximal Xp11 region where no single XLMR gene had been detected so far.

This observation has prompted us to screen 30 European and Australian XLMR families with overlapping linkage intervals for mutations in all known and several not well-annotated brain-expressed genes that are located in a 7.7 Mb interval of the Xp11 region that is flanked by ELK1 and ALAS2. Subsequent screening of >200 XLMR families revealed multiple mutations in at least 5 genes (Kalscheuer et al, under revision; Freude et al, in preparation; Lenzner et al, in preparation; Gurok et al, unpublished). Several of these are frame shift, stop codon or splice site mutations which are expected to truncate and inactivate the gene products. Together, mutations in these genes have been found in 12 of the 30 Xp11 families tested. Thus, defects of these genes may be responsible for >30 percent of the missing mutations in this region, and for 10 percent of all defects that give rise to NS-XLMR (reviewed by Jensen, Lenzner et al, in preparation). A list of the genes tested is given in Figure 2.

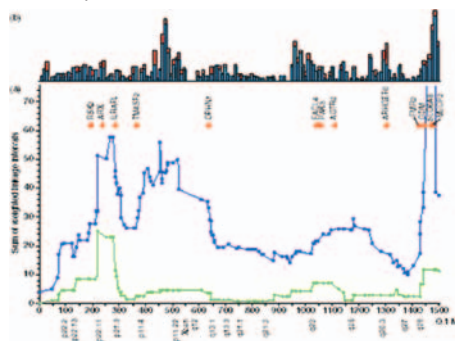


Figure 1: Regional distribution of mutations in families with non-syndromic X-linked mental retardation (NS-XLMR) (a) and gene density on the human X-chromosome (b). (a) Upper curve: all families analysed; lower curve: families with known mutations analysed. The surface under these curves corresponds to the sum of the 'weighted' linkage intervals for individual families, each of which is represented by bars of different height, to compensate for different lengths of these intervals. Triangles indicate the map positions of known NS-XLMR genes (b) Distribution of genes on the human X-chromosome (blue bars: known genes, red bars: other genes) (from: http://www.ensembl.org/Homo_sapiens/mapview?chr=X)

Gene	Amplicons	Coding	Non-coding	Gene	Amplicons	Coding	Non-coding
ABCB7 (Xq13.3)	17	2258	921	LIMO6	10	1848	689
ALAS2	10	1763	710	MAGED2	18	1821	681
APE2	12	1557	718	MAGE-E1, KIAA1859	15	2260	1198
CCNB3	19	4005	753	MGE1 sim, PIMI	18	1727	2026
DTIP1A10	6	576	473	PCSKIN1, SAAS	5	783	478
FGD1	19	2886	1571	PIM2	10	1005	562
FLJ10613	16	1748	644	PLP2	5	459	726
FLJ10628	16	363	1563	PPP1R3F	10	2803	573
FLJ14103	4	624	165	PQBP1	6	797	598
FLJ20344	4	531	297	PRKWNK3	36	3012	1336
FLJ21687	6	828	406	PRO0659	2	525	60
FTSJ1	11	990	1096	RBM3	8	473	614
KIAA1167	25	2412	2583	SLC35A2	9	1191	623
GSPT2	6	1887	40	SLC38A5, JM24, SN2	17	1419	1260
HADH2	7	786	451	SMC1L1	26	3690	1099
HDAC6	31	3647	2315	SMCX	37	1563	2181
I-4 (5 MB distal)	7	608	75	SUV39H	9	1239	557
JM1	19	1884	1866	T54, fibulin1	12	1431	1109
JM4	6	539	678	TFE3	23	1728	6356
JM5	12	1213	1833	TIMP1	5	623	247
JM11	13	1892	4441	TRO, KIAA1114	28	4052	1033
KIAA0522	24	4485	1457	UREB1	92	13745	7565
KIAA1111	22	3182	819	UXT	8	474	1032
KIAA1202	34	4765	2118	WDR13	14	1457	1473

Figure 2: Survey of brain-expressed genes from a 7.7 Mb interval of the Xp11 region flanked by ELK1 and ALAS2 screened for mutations in 30 European and Australian XLMR families with overlapping linkage intervals. Altogether, 769 amplicons from 46 different genes comprising 94.390 coding and 61.904 non-coding bases were analysed.

Presently, 30 additional brain-expressed genes from the pericentric region of the X-chromosome are being screened, which had not been fully annotated at the outset of this project or had been omitted for other reasons. Since there are numerous examples for MR being due to mutations in genes which are not highly expressed in the brain (such as phenylalanine hydroxylase in PKU), our next step will be to include such genes in this study, again with a focus on genes in regions of the X-chromosome with a high mutation density. The identification of novel inborn errors of metabolism that give rise to MR would not only open up possibilities

for the development of universal biochemical tests, suitable for the diagnosis of all kinds of mutations in these genes, but might also have therapeutic consequences.

Another promising approach is the search for low-copy repeats (LCRs) on the X-chromosome, which may give rise to disease through unequal pairing and recombination, or through gene conversion between duplicated (pseudo) genes. Recent *in silico* studies (of Chen Wei et al., see Figure 3) have revealed >400 LCRs with a minimum length of 5 kb and a sequence identity of >98 percent, which are present on the X-chromosome in at least two copies. Many of these LCRs could be confirmed in 'wet' experiments. It is tempting to speculate that such LCRs and their interaction play a role in the aetiology of several of the mutations which cannot be detected by conventional mutation screening. Given our unique collection of XLMR families, we are in an excellent position to test this hypothesis.

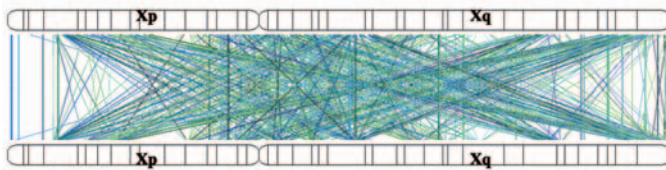


Figure 3: Distribution of 437 low-copy repeats (LCRs) on the human X chromosome. LCRs in non-identical positions are connected by straight lines.

Finally, clinically relevant mutations may lead to reduced mRNA concentrations in cells of patients, e.g. due to nonsense-mediated RNA decay if they result in a frame shift or stop codon causing early truncation of the gene product. To identify these mutations by cDNA array-based gene expression profiling in lymphoblastoid cell lines from patients with XLMR, a

cDNA array representing most of the genes encoded by the human X-chromosome (Sudbrak et al, Hum. Mol. Genet 10:77, 2001) was employed (by U.Nuber and co-workers). However, due to widely varying gene expression patterns in cultured cells and to experimental variation, results of these studies were largely disappointing. Ongoing studies in our laboratory utilize semi-quantitative RT-PCR methods to search for mutations altering the expression of positional or functional candidate genes, as well as Northern blotting to search for aberrant splicing.

Recent, as yet unpublished, observations from several laboratories indicate that none of the known genes for XLMR, not even the gene for the fragile X-syndrome, accounts for more than 2 percent of all mutations in large series of unselected patients with idiopathic MR. One explanation for this unexpected finding could be that X-linked MR is less frequent than commonly thought, and that there are also other causes for the higher prevalence of MR in males. If so, this would probably mean that autosomal recessive defects, generally thought to account for 60 percent of severe MR, are even more important in the aetiology of this disorder.

So far, next to nothing is known about the genes that play a role in autosomal recessive MR (ARMR), because in Western civilizations, small family sizes preclude the mapping and identification of these genes. In contrast, very large families are common in Iran where 70 percent of the population is below 30 years, and mapping is greatly facilitated by the fact that in the Western provinces, 60 percent of the children are born to parents that are first cousins. In many parts of India, the situation is similar. Because of our formalized collaborations with three different institutes in India and a far-ranging agreement with a particularly potent partner in Iran, prospects are excellent for making rapid progress in this field. Apart from the recruitment and detailed clinical examination of large families (some with a theoretical lod score of >8), genome scanning will be performed to localize the respective genes by autozygosity mapping. Initially, commercial (Affymetrix) SNP chips will be employed for this purpose, but other cost-effective options are also being considered (collaboration with P. Nürnberg).

In collaboration with E. Cuppen and R. Plasterk (Utrecht), R. Reinhardt and Transgenomic Inc., experiments are in progress to replace the existing DHPLC (=WAVE) technology by an endonuclease-based mutation screening procedure that lends itself to automation and promises to be both faster and less expensive than the established method. Since screening numerous genes in a given linkage interval for a single mutation can be quite demanding and costly, high-throughput/high resolution/low cost mutation detection is of crucial importance for gene finding in ARMAR and related disorders.

Functional studies will be performed in collaboration with other groups of the department, the institute, from the EURO-XLMR Consortium and elsewhere third parties. E.g., close collaborations exist (with G. Eichele, Hannover) to study the expression of these genes in early developmental stages of the mouse. Through collaborations within an ongoing EU project on XLMR, neurobiological and behavioural studies in mice will be possible if required.



General information

Selected publications 1998-2003

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Teaching

H.-Hilger Ropers: Lecture *Genetics for Bioinformaticians*, Free University Berlin, SS 2002, 2SWS

Theses

S. Prietz: *Expressionsanalysen mit cDNA-Mikroarrays – Aufklärung der Pathogenesemechanismen einer seltenen Augenkrankheit*. PhD Thesis, Freie Universität Berlin, 2002

R. Kirschner: *Zur Struktur und Funktion des Gens für die X-chromosomale Retinitis pigmentosa 3*. PhD Thesis, Humboldt-Universität Berlin, 2002

U. Schwahn, *Positionsklonierung des Gens für Retinopathia Pigmentosa 2 (RP2) und molekulare Analyse der Pathogenesemechanismen*. PhD Thesis, Freie Universität Berlin, 2001

M.R. Toliat, *Molekulargenetische Charakterisierung von neuroendokrinen Tumoren des gastroenteropankreatischen Systems*. PhD Thesis, Freie Universität Berlin, 2001

B. Meyer, *Identifizierung und Charakterisierung früher Genomveränderungen in benignen Hirntumoren*. PhD Thesis, Humboldt-Universität Berlin, 2001

Collaboration with other groups of the department and the MPIMG

Balanced chromosome rearrangements with MR, with V. Kalscheuer and co-workers

Gene expression profiling, with U. Nuber and co-workers

Protein interactions, biochemical studies, with S. Schweiger and co-workers

Cell and animal models, RNAi, with D. Walther and co-workers, C.Scharff and co-workers

Recruitment of patients, clinical characterization, with M. Hoeltzenbein, A. Tzschach

Technical support, with R. Reinhardt and co-workers

Bioinformatics, with M. Vingron, S. Haas and co-workers, A. Beck

External co-operations

EURO-MRX Consortium

A. Latos-Bielenska, Poznan

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E. Hasnain, Hyderabad

A. Gal and K. Kutsche, Hamburg

G Rappold, Heidelberg

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