The German Mouse Clinic: A platform for systemic phenotype analysis of mouse models

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Abstract

The German Mouse Clinic (GMC) is a large scale phenotyping center where mouse mutant lines are analyzed in a standardized and comprehensive way. The result is an almost complete picture of the phenotype of a mouse mutant line – a systemic view.

At the GMC, expert scientists from various fields of mouse research work in close cooperation with clinicians side by side at one location. The phenotype screens comprise the following areas: allergy, behavior, clinical chemistry, cardiovascular analyses, dysmorphology, bone and cartilage**,** energy metabolism, eye and vision, host-pathogen interactions, immunology, lung function, molecular phenotyping, neurology, nociception, steroid metabolism, and pathology. The German Mouse Clinic is an open access platform that offers a collaboration-based phenotyping to the scientific community (www.mouseclinic.de).

More than 80 mutant lines have been analyzed in a primary screen for 320 parameters, and for 95% of the mutant lines we have found new or additional phenotypes that were not associated with the mouse line before. Our data contributed to the association of mutant mouse lines to the corresponding human disease. In addition, the systemic phenotype analysis accounts for pleiotropic gene functions and refines previous phenotypic characterizations. This is an important basis for the analysis of underlying disease mechanisms.

We are currently setting up a platform that will include environmental challenge tests to decipher genome–environmental interactions in the areas nutrition, exercise, air, stress and infection with different standardized experiments. This will help us to identify genetic predispositions as susceptibility factors for environmental influences.

Introduction

Model organisms, especially the mouse, have proven to be important tools to learn more about gene functions. There are many mouse mutant lines (MMLs) available, however majority of them lacks comprehensive phenotypic characterization (Auwerx et al. 2004, Austin et al. 2004). Due to pleiotropic effects, one gene may have different functions in different organ systems. Thus, MMLs have to be systematically and comprehensively phenotyped in a standardized way to exploit the knowledge, which otherwise might remain undiscovered. To overcome this "phenotyping gap", the German Mouse Clinic (GMC, Gailus-Durner, Fuchs et al. 2005, www.mouseclinic.de) works as an open access phenotyping platform. The goals of the GMC are:

- Systemic phenotype analysis of mouse mutants on the basis of a scientific collaboration
- A primary screening of more than 320 parameters in 14 different areas
- A capacity of up to 50 mouse lines per year in a primary screening
- Detailed analysis in secondary and tertiary screening

The German Mouse Clinic Consortium

The German Mouse Clinic is located in Munich at the Helmholtz Zentrum München. It is operated by the Institute of Experimental Genetics (IEG) that provides a core facility, the core scientists as coordinators, IT specialists for data management, a scientific editor for writing phenotype reports and two project administrators. In addition to the core facility the IEG provides expertise in the dysmorphology, bone and cartilage, the molecular phenotyping, and the steroid metabolism screens. A screen for diabetes research will be implemented. Specialized mouse phenotyping know-how for behavior, eye development and vision, lung function and pathology is contributed by the Institutes of Developmental Genetics, Human Genetics, Inhalation Biology and Pathology from the Helmholtz Zentrum München. Furthermore, the universities of Munich (Technische Universität and Ludwig-Maximilians Universität) closely collaborate with the GMC and provide the screens for allergy, clinical chemistry, immunology and neurology. The fields of cardiovascular function, metabolism and nociception are covered by collaborating groups from the University of Heidelberg, University of Marburg, and University of Bonn, respectively that run "satellite-labs" within the GMC. A secondary screen for host-pathogen-interactions is contributed by the HZI in Braunschweig. Additional partners from the University of Kiel and the TU München joined the consortium within our challenge platforms.

Housing conditions and hygiene management

Mice are kept in individually ventilated cages (IVCs) under standardized housing conditions at a temperature of 22–24°C, humidity of 50%–60% with 20 air exchanges per hour and a 12/12-hour light/dark cycle. Wood shavings serve as bedding. Mice are fed a standardized mouse diet (1314, Altromin) and are provided with drinking water (0.2 µl filtered tab water) *ad libitum*. At the GMC every week mice from a different collaboration partner arrive for

phenotypic characterization, all of which might represent a different health status. To meet this hygienic challenge a hygiene management concept has been developed to prevent horizontal pathogen transfer (Brielmeier *et al.,* 2002). Prior to the transportation into the GMC-facility, a health certificate must be delivered and be evaluated by the veterinarians from the Helmholtz Zentrum München. The GMC is fully equipped with IVC cages and Class II changing stations. The hygiene concept of the GMC comprises standard operating procedures (SOPs) for all operations. Regular health monitoring is carried out by on-site examination of sentinel mice by certified laboratories following FELASA recommendations (http://www.felasa.eu/recommendations.htm).

Primary phenotypic characterization (primary screen)

An innovative workflow was established, allowing a series of non-invasive tests on the same cohort of animals without major interferences between individual examinations. In a first version of the workflow the mice were phenotyped by a single passage of a cohort of 30 mutant mice that were compared to the same number of age- and sex-matched littermate control animals.

Since the start of the European Mouse Disease Clinic (EUMODIC, www.eumodic.org) a new workflow was set up in collaboration with other European mouse clinics. This new European workflow (EMPReSSslim) aims at the improvement of comparability of phenotyping data all across Europe. We adapted our phenotyping procedures to the jointly approved European standard, and complemented this program with additional tests from the former workflow that are not represented by EMPReSSslim. The change of the workflow also enabled us to double our capacity from 25 to 50 MMLs per year.

For a complete primary screen 80 animals (20 mutant males, 20 mutant females, 20 male controls and 20 female controls) are required. The mice are analyzed comprehensively for key

parameters by a passage in two pipelines through the modules of the GMC. Using only non-invasive tests approximately 320 parameters can be measured.

A cohort of ten animals per sex and genotype is analyzed in the first pipeline of experiments that comprises dysmorphology, blood pressure, calorimetry, intraperitoneal glucose tolerance test, X-ray analysis, bone densitometry, axial eye length measurement, lung function and expression profiling. In the second pipeline of experiments (also ten animals per sex and genotype), a series of behavioral and neurological tests (open field, modified SHIRPA, grip strength, rotarod, acoustic startle and pre-pulse inhibition as well as hot plate test), examination of the eyes and vision (ophthalmoscope, slit lamp analysis) are carried out and blood based parameters from the immunology, allergy, clinical-chemical, cardiovascular and steroid screens are determined. This group of animals is tested on request for ECG or echo-cardiography. Finally, the mice from pipeline two are analyzed in the pathology screen (figure 1).

A detailed overview on the analyzed parameter-sets and the used techniques in the primary screen is shown in table 1a. The group sizes and the resulting statistical power of the analysis have been calculated for the different experiments (e.g. Meyer et al. 2007).

After the completion of the primary screen, the data is evaluated and analyzed. The interpretation of the results is done by expert scientists derived from the different research areas covered in the screens based on a comparison with reference data obtained from literature and own baseline data. The complete result is discussed together with the mouse providers in a presentation session. In many cases the cross-view of the data from different screens allowed insights into subtle phenotypic alterations of scientific relevance that otherwise might remain unknown. Further analysis in secondary and tertiary screens as well as possibilities of publishing the data is discussed during the data presentation with providers of the MML.

Table 1a: Screens and technology used in the primary screen

Screen	Parameter set	Technique	Remarks
Dysmorphology, Bone and Cartilage	analysis of body, skeleton, bone and cartilage	morphological observation, bone densitometry, X-ray	for more detailed information see also Fuchs et al. 2000, Fuchs et al. 2006
Behavior	exploratory and anxiety-related behavior, acoustic sensorimotor gating	open field, acoustic startle & PPI	for more detailed information see also Brown et al. 2005
Neurology	assessment of muscle, spinocerebellar, sensory, and autonomic function	modified SHIRPA protocol, grip strength, rotarod	for more detailed information see also Schneider et al. 2006
Eye	assessment of morphological alterations of the eye and vision	funduscopy laser interference biometry slit lamp biomicroscopy, optokinetic drum	for more detailed information see also Favor 1983, Puk et al. 2006, 2008
Clinical Chemistry and Hematology	determination of clinical-chemical and hematological parameters in blood and assessment of glucose tolerance	blood autoanalyzer, ABC-animal blood counter, intraperitoneal glucose tolerance test (IpGTT) using the Accu-Chek Aviva glucose analyzer	for more detailed information see also Klempt et al. 2006
Immunology	analysis of peripheral blood samples for immunological parameters	flow cytometry, Multiplex Bead Array	for more detailed information see also Kalaydjiew et al. 2006
Allergy	analysis of total plasma IgE	ELISA	for more detailed information see also Allessandrini et al. 2001
Steroid Metabolism	plasma testosterone and DHEA	ELISA	
Nociception	pain response	hot plate assay	for more detailed information see also Racz and Zimmer 2006
Cardiovascular	assessment of functional and blood based cardio-vascular parameters	non-invasive tail-cuff blood pressure measurement, ANP, on demand: surface limb ECG, echocardiography	for more detailed information see also Schoensiegel et al. 2007, Hoelter et al. 2008
Lung Function	assessment of alterations in spontaneous breathing patterns	whole body plethysmography	for more detailed information see also Barco Barrantes et al.

			2006
Molecular Phenotyping	RNA expression profiling	DNA-chip technology	for more detailed information see also Horsch et al. 2008
Energy Metabolism	measurement of altered body weight regulation, body temperature and energy balance	indirect calorimetry	for more detailed information see also e.g. Meyer et al. 2007
Pathology	macroscopic and microscopic analysis of more than 30 organs	standard stains (e.g. H&E, PAS, Luxol fast blue	for more detailed information see also Kunder et al. 2007

Secondary and tertiary analysis

For more detailed characterization, screens of the GMC offer additional experiments in secondary and tertiary analyses. For these tests additional cohort(s) of animals are required. In table 1b the established secondary and tertiary screens are listed.

Table 1b: Screens and techniques established for secondary and tertiary analysis

Screen	Technique
Dysmorphology, Bone and Cartilage	micro-CT, peripheral quantitative computer tomography, three-point-bend-test, blood based bone markers, in vitro analysis of osteoblasts
Behavior	modified hole board, light/dark avoidance, elevated plus-maze, social interaction, tail suspension, forced swimming, y-maze, social discrimination, object recognition, fear-potentiated startle, food-rewarded hole board, five-choice serial reaction time task
Neurology	stair case test, footprint analysis, electroencephalography (EEG)
Eye	electroretinography (ERG), histology (Dalke et al. 2004)
Clinical Chemistry and Hematology	Plasma analysis for a variety of clinical-chemical parameter sets, including additional parameters which had not been measured in the primary screen to complement the evaluation of organ function; blood gas analysis, differential blood cell count; reticulocyte count, total blood clotting time; serum Insulin-like Growth Factor I; western ligand blot for Insulin-like Growth Factor Binding Proteins (IGFBPs); estimation of 24h water uptake and urine production; clinical-chemical analysis of 24h and spot urine samples for electrolyte, urea, uric acid, creatinine, glucose and protein concentrations; urinary protein electrophoresis;
Immunology	detailed flow cytometry analysis of the cellular composition of spleen, lymph nodes, bone marrow and thymus
Allergy	sensitisation with model allergens (ovalbumin, recombinant grass pollen allergen Phl p5), total IgE and allergen-specific antibody responses measurement, evaluation of allergen-specific T-cell responses
Host-Pathogen Interaction	Infection challenge with *Listeria monocytogenes* EGD, *Streptococcus pyogenes* A20, *Yersinia enterocolitica* E40, *Trichuris muris*, Influenza virus A (H1N1), survival, pathogen load and dissemination, flow cytometry, analysis of serum immunoglobulins, cytokine and chemokine response, histopathology, mucosal immunity, macrophage mediated pathogen killing and cellular activation, see also Schippers et al. 2008, Pasche et al. 2005
Nociception	von Frey filament test, plantar test, tail-flick test, formalin test, neuropathic pain models, inflammatory hyperalgesia models
Cardiovascular	surface limb ECG, echocardiography, additional cardiovascular biomarkers as osteopontin, cardiac troponin T
Lung Function	lung function unit: lung volumes, mechanics, gas exchange, bronchial responsiveness, ventilatory response to hypercapnia/hypoxia, see also Reinhard et al. 2002, 2005
Molecular Phenotyping	expression profiling of tissues not included in the routine set of organs of the primary screen (e.g. eye, bone, intestine, blood, spinal cord, adrenal glands,

	pancreas, salivary gland, bulbourethral glands, activated T-cells, yolk sac, seminal vesicles, white adipose tissue, brown adipose tissue, thyroid gland, skin and cartilage (auricle)
Energy Metabolism	ad libitum food consumption, energy content of feces (bomb calorimetry), energy assimilation, food assimilation coefficient, body weight, rectal body temperature, parameter recording under food restriction, determination of the thermoneutral zone (TNZ), basal- and resting metabolic rate (BMR and RMR respectively), heat and cold exposure, substrate utilization and energy expenditure during food restriction, telemetrical recording of body temperature and activity, chronobiological analysis: activity, body temperature and metabolism
Pathology	special stainings (histochemistry and immunohistochemistry), electron microscopy, laser-assisted microdissection, X-ray, moleculargenetic analysis (e.g. array-CGH, western blot, LOH-analysis)

Data management

To run a large scale phenotyping project, a professional, tailor-made database system is required. For the German Mouse Clinic a web application called MausDB (Maier et al. 2008) has been developed in-house. The main features of MausDB are:

- Central cohort and facility management
- Scheduling of phenotyping assays
- Data import and export
- Upload of phenotyping results including metadata and files
- User-friendly graphical user interface
- Open source system (free download of manuals and source code as well as a demo system are available at http://jupiter.helmholtz-muenchen.de)

In addition to MausDB that stores data on the level of individual mice, another database-driven web application called CoordDB has been developed in-house to support the project management team by centrally storing information from incoming phenotyping requests and scheduling of phenotyping slots for MMLs in order to maximize phenotyping capacity utilization.

Request procedure

To apply for phenotyping of a MML in the GMC a scientist should visit our website (www.mouseclinic.de) and submit a completed request form. With the submission of the form the collaboration partner provides the GMC management team with essential information needed for processing the request and scheduling the phenotyping slots. Further steps will

include a test for sanitary status of the mice, a collaboration agreement, and preparation as well as scheduling of the cohort.

Quality management in a large scale mouse phenotyping project

The quality management at the GMC consists of multiple elements: Project management supported by the central database systems MausDB and CoordDB, standardized analyses via Standard Operating Procedures (SOP) that have been validated within the EUMORPHIA program, as well as quality control and continuous training of the staff members. As a member of the National Genome Research Network (NGFN), we were also supported by the workgroup quality management of the NGFN.

The GMC management team (core facility) coordinates the scientific issues, logistics and administration of the GMC. The coordination software tool CoordDB supports the GMC management team in handling the incoming requests for phenotyping and for management of the complex workflow of the primary and secondary screening. The central mouse cohort and result management system MausDB ensures full traceability of samples, documentation of all data and administration of the successful execution of phenotyping tasks.

The GMC developed a set of SOPs which cover all steps from the mouse importing and handling to phenotyping and data analysis. These SOPs are strictly followed during the whole screening process in the GMC and all procedures are documented.

The GMC is one of the major partners of the EUMODIC consortium that emerged from the EUMORPHIA program (Brown et al., 2005, www.eumorphia.org), a consortium for the selection, establishment and standardization of phenotyping protocols for mice as models for human diseases and for mouse husbandry. Cross-validation of protocols and screening procedures was performed by the different EUMORPHIA institutions.

In addition to routine experiment-specific quality control procedures, control animals of selected strains (e.g. C57BL/6J and C3HeB/FeJ) are analyzed through the standard protocol for all phenotypes at regular intervals.

A tissue archive has been established for the storage of tail and blood plasma samples taken from all mice that have been analyzed in the GMC. These samples can be used on demand for re-genotyping or re-evaluation of the sanitary status.

Regular specific training courses are held at the GMC by inviting specialists who give lectures or offer practical training (e.g. animal handling, sanitary management or security).

Contribution to the European Mouse Disease Clinic (EUMODIC) and other European projects

Within the European initiative EUMORPHIA (www.eumorphia.org, Brown et al. 2005), we have standardized and validated our protocols and screening procedures. The GMC is one of the four large-scale phenotyping centers of the EUMODIC consortium (www.eumodic.org), which will undertake a primary phenotype assessment of up to 650 mutant mouse lines made available by the EUCOMM project. To enable cross-center comparisons of phenotyping results we have adapted the primary workflow to EMPReSSslim with two phenotyping pipelines. Many screens of the GMC also contribute to secondary screening activities within the EUMODIC project.

Results

In total, 81 MMLs that were created with different techniques like knock outs, gene targeting, ENU mutagenesis, or by spontaneous mutations were obtained from institutes all over the

world and were successfully phenotyped in the primary screen. In nearly all of the MMLs, we detected altered parameters in the mutants compared to their wild-type controls. In addition, we collected baseline data from the most commonly used inbred mouse strains.

In 95% of the 81 mutant lines that were analyzed in the primary screen we found new or additional phenotypic alterations (figure 2) . Also, subtle changes were detected essentially in every line assessed. Secondary screens were recommended for almost every mutant line.

Table 2 provides an overview of the analyzed disease models.

Table 2: Disease models analyzed

Neurological diseases / complex syndromes
Schizophrenia, depression, bipolar affective disorders, alcoholism, mental retardation, cognition deficits, inherited ataxia, tremor, progressive deafness, altered pain perception, learning and memory, developmental verbal dyspraxia, dying back oligodendrogliopathy, juvenile neuronal ceroid lipofuscinosis, Down syndrome, Mowat-Wilson syndrome, Leigh syndrome, Alzheimer‘s disease, Huntington‘s disease
Bone / cartilage / eye
Syndactyly, polydactyly, Amelogenesis imperfecta, Osteogenesis imperfecta, osteoporosis, scoliosis, acroosteolysis, inflammatory arthritis, cataract, microphthalmia, retinal degeneration, altered cornea
Other disease areas (e.g. metabolic, cardiac)
Obesity, metabolic syndrome, glycolipid storage disease, iron storage disease, Selenium deficiency syndrome, peroxisome biogenesis disorder, male infertility, gynecomastia, spontaneous progressive liver fibrosis, dystrophic cardiac calcification, polycystic ovarian syndrome, Diamond-Blackfan anemia, Bartter syndrome, Morbus Addison

Genome-environmental interactions

To explore the complex relationship between environmental changes and genetic factors, we set up standardized challenge platforms for mouse phenotyping. By mimicking specific environmental exposures or life styles that have a strong impact on human health, we want to determine their effects on disease etiology and progression, uncovering the physiological and molecular mechanisms of genome-environment interactions. We have chosen five areas – diet, air pollution, stress, exercise and immunity – representing the major interfaces of the organism with the environment (gut, lung/skin, brain/sensory organs, muscle/bone and immune system). The challenged animals will subsequently be analyzed by appropriate standard screens of the GMC. This platform will allow us to identify genetic predispositions as susceptibility factors for environmental influences.

Meta-analysis of data and data mining

Comprehensive screening activities within the German Mouse Clinic provide a tremendous amount of data, which are collected in a standardized format in the GMC database system MausDB. It represents a promising resource for meta-analysis approaches and data mining exercises. As a first outcome, data of the molecular phenotyping screen helped to discover syn-expression groups of genes in different organs (Horsch et al. 2008). Other data mining exercises for the complete data-set are in progress.

Conclusion

The German Mouse Clinic is a large scale phenotyping center for the systemic analysis of mouse mutant lines. The GMC is a consortium of scientists with expertise in various fields of mouse research . The GMC operates in close cooperation with clinicians, and it offers a

collaboration-based phenotyping of MMLs as an open access platform to the scientific community. The systemic analysis of MMLs in the GMC has proven to be a powerful tool to find new model systems for human diseases. The German Mouse Clinic can be contacted at www.mouseclinic.de.

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Figure Legends

Figure 1: Workflow for the primary screen

Figure 2: Overview on the results of the first 81 mutant lines

Fugure 3: Challenge platforms

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