

**SMP: Mammalian Models****Project: German Mouse Clinic - Dysmorphology Screen**

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**Introduction**

The skeleton is the third largest organ of the human body. Bone, consisting largely of an organic type I collagen matrix, is a mineralized tissue that serves many functions such as: affording mechanical scaffold and stability to joints, tendons and ligaments for the basis of mobility, protecting inner organs, supporting haematopoiesis, and mediating blood calcium levels. Formation, deposition and mineralization of bone are governed by osteoblasts that differentiate from mesenchymal precursor cells. Throughout life, skeletal tissue is continuously remodeled by the balanced (coupled) processes of resorption and consecutive bone formation. Disorders of the skeleton can be divided into four categories that include: 1.) Developmental patterning defects, 2.) Metabolic and growth defects, 3.) Modeling and remodeling defects, and 4.) Aging and immune system defects.

In the German Mouse Clinic (GMC) we characterize mouse mutants in a comprehensive, standardized phenotype screen in order to establish validated models for human diseases. In doing so, we aim to increase the number of applicable mouse models in understanding the molecular basis of inherited diseases and to further characterize each model by comprehensive phenotyping. The Dysmorphology Screen of the GMC characterizes mouse models for bone related human diseases like osteoarthritis and osteoporosis. Mouse mutants are analyzed using medically relevant dysmorphology, bone and cartilage parameters. In the primary screen, up to 26 mutant lines (1,500 animals) can be phenotyped per year. In addition, there is the capacity for another 1,500 phenotypic analyses in the secondary and tertiary screens for more detailed evaluation of model systems for bone related human diseases.

**Results**

We have established a specialized lab for bone, cartilage and morphological analysis. For the primary screen, we cover a broad spectrum of parameters of bone development, metabolism and homeostasis. We have implemented an experimental set-up entailing DXA (dual energy X-ray absorption), X-ray imaging, micro computer tomography, and blood analyzers, which enables us to perform high throughput non-invasive first-line phenotyping for bone and cartilage abnormalities. In addition, we developed a protocol (54 parameters) for a quick anatomical observation of animals, which is able to detect and evaluate malformations and malfunction of different organ systems. In secondary tests, we evaluate mutants with altered parameters in the primary screen reflecting bone related disease like scoliosis, limb defects, osteoporosis, osteogenesis imperfecta or osteoarthritis by more detailed phenotypic methodologies.

We established a micro computer tomograph for the analysis of life mice in close collaboration with the manufacturer (VAMP, Möhrenndorf, Germany) and the Institute of Medical Physics (University Erlangen, Germany). In Collaboration with the GSF Institute of Medical Informatics we are attempting to improve the power of the machine and to automate some of the analysis functions. Two diploma students are currently writing program codes for this issue.

We are the leading partner of the bone and cartilage work-package of EUMORPHIA ([www.eumorphia.org](http://www.eumorphia.org)), which is a European project for the collection and standardization of mouse phenotyping protocols. Together with our partners

from the University of Geneva, Switzerland, and Animage (Lyon, France), we established standardized first-line phenotyping protocols for the analysis of mice for bone and cartilage development and homeostasis (e.g. bone densitometry, X-ray analysis, AVL analyzer). Additional protocols are still in progress. Within the same project, we also contribute with our know-how especially in micro computer tomography to the imaging work-package. In another EU funded project, ANABONOS, we contribute the establishment of mouse models with altered bone mineral density.

**Primary Screen**

Since the beginning of the GMC the Core Facility provided 37 mutant mouse lines and six inbreeding or hybrid lines for the primary screen. We collected baseline data from substrains of common inbred mouse strains (C3HeB/FeJ, C57BL/6J, BALB/cAnPt, 129X1/SvJ) and two hybrid strains (129xB6, B6x129). Additional strains are in preparation.

Most of the 37 mutant lines provided by the Core facility for the primary screen have already finished the phenotypic analysis in the Bone and Cartilage module. In 11 lines we did not detect any alterations in any of our analyzed parameters. In 15 lines we found altered parameters, but without recommendations for further analysis in secondary tests. Eight mutant lines showed interesting changes in a series of parameters. These lines are considered as potential model organisms, and additional mice have been requested for secondary analysis.

**Secondary Screen**

In the secondary screen, detailed analysis of mutant lines with known defects in bone metabolism was performed. Currently, we have two mutant lines under investigation that might serve as animal models for osteoarthritis. Two further mutant lines have decreased levels of bone mineral density and thus are candidates as model systems for osteoporosis or osteopenia.

Another mutant line is also being characterized, and might serve as a model for osteogenesis imperfecta. A series of further mutant lines, which are currently being analyzed show congenital malformations of the skeleton like limb defects. Also, the characterization of a mutant line with hypertrophic hardening of the knee joint (bursae) or knee osteoarthritis has just recently started. Two mutant lines with osteopenia are in preparation. Additional promising mutant lines will follow soon.

**A new model for osteogenesis imperfecta**

We analyzed an autosomal dominant mutant line derived from the Munich ENU Skeletal Phenotyping screen. This line was mapped and cloned using a standard out-backcross positional cloning technique followed by a candidate gene approach. Heterozygous animals show a bone-bending phenotype. They display long-term viability, and the skin, coat, nails and teeth develop normally on a macroscopic level. There are no evidence of dermal fibrosis, similar to scleroderma, eye defects, and any skin ulcerations, erosions or nodules, or any partial or patchy alopecia. There are indications of abnormal joint and limb contractures. It is in these regions where both trabecular and cortical bone are thinner, thereby affecting bone integrity. There are no reported sex differences in this line. There are no articular cartilage defects or inflammatory infiltration into the synovium in mild animals. The epiphysis growth plate appears intact,

while the trabecular structure of the femur and tibia display excessive resorption zones in the metaphysis. Also, the calvarium shows incidences of high bone turnover. Micro structural damage in bone has been identified using scanning electron microscopy (SEM). SEM revealed abnormal collagen crosslinking. The mutants display heightened osteoclast activity as measured by TRAP (tartarate-resistant acid phosphatase) and resorption spaces. Osteoblast activity is also increased as independently measured by total ALP serum levels, RT-PCR, and ALP staining. The full histomorphometric work-up of the mutant line is still pending. Both the trabecular number and spacing are reduced as measure by  $\mu$ CT analysis. Volumetric BMD is also significantly reduced. The polar moment of inertia – a measurement of strength – is also significantly reduced.

### New models for inflammatory arthritis

Two dominant mutant lines, which are candidate model organisms for arthritis are under investigation. Histological analysis exhibited inflammatory infiltration into joints, bone marrow and surrounding soft tissues. Both mutant mice also show significantly reduced bone mineral density. One mutant has been mapped to mouse chromosome 4. So far the region has been narrowed to approximately 100 kb. Using bacterial artificial chromosome (BAC) clones, which include the critical region, transgenic rescue experiments are currently under way. The two mutant lines show parallelism with human diseases like rheumatoid arthritis and/or psoriatic arthritis.

To elucidate how the immune system is involved in this systemic inflammation, the Immunology Screen analyzed various immunological parameters by means of ELISA and flow cytometry. Although increased granulocyte populations and elevated levels of IgE were detected in peripheral blood of homozygous mice, autoimmune markers such as rheumatoid factor and anti-DNA antibodies were moderately up-regulated. In lymph nodes and spleen, increased populations of activated T lymphocytes (CD4+CD25+) were detected, suggesting a highly activated immune system. Next, we used disease transfer systems to address what these cell types contribute to the phenotype. Bone marrow cells (BMCs) were injected intravenously to irradiated wild-type animals. In contrast to control experiments using BMCs from wild-type mice, mutant BMCs could transfer the arthritis phenotypes. However, serum failed to transfer the phenotypes. These results indicate that bone marrow derived cells, not serum factors, play an important role in the inflammatory process.

### Limb mutants

These mutants have been isolated in a genome-wide ENU mutagenesis project on C3HeB/FeJ and display a polydactyly at the hind limbs. The limb phenotypes are inherited in a dominant manner and show a penetrance of about 60% in all offspring. In a few cases, 5-10% of these the first digits are additionally shortened. Homozygous offspring are not viable and die around birth; they display severe forms of polydactyly, scoliosis and exencephaly or anencephaly. All four lines map on chromosome 13. Further sequence analysis of candidate genes revealed that all four lines carry a point mutation in the zincfinger region of *Gli3*. *Gli3* is a transcription factor that is known to be involved the Shh pathway and in early limb development. *Gli3* is expressed in the anterior part of limb buds and functions as a negative regulator of *Shh*. Although a number of *Gli3* alleles are already available, they mostly represent either larger deletions of several exons (of the zinc finger domain) or

transgenic insertions, whereas the point mutations described resemble more the situation in human pathogenesis. Therefore, these mutants can contribute to new aspects of *Gli3* function. Further molecular and phenotypic analysis (RT-PCR, Northern blot, Western blot, in situ hybridization, skeletal preps and histology) will be performed. Several human diseases are already connected to mutations in *Gli3*, such as Greig Syndrome (where patients display facial dysmorphism, syndactyly at hand/ feet and preaxial polydactyly) or Pallister Hall Syndrome (where patients have multiple malformations, often central polydactyly).



**Fig 1:** Whole body micro computer tomography of mice.  
Picture: B. Müller

### Outlook

New standardized tests will be developed, implemented and validated in the primary and secondary analysis of mouse models in the bone and cartilage module of the GMC. New techniques for the histological and molecular analysis of bone and cartilage tissue have to be established. We will expand the Bone, Cartilage and Dymorphology module from the GMC, and special focus will be on genome-environment interaction. We plan on the implementation of challenge tests in the fields of nutrition and physical activity. Changes of diet or physical activity are thought to have a major impact on the development and homeostasis of bone and cartilage. Animals will be challenged with low fat, normal and high fat diets to study the impact on the homeostasis of bones. In addition, mineral content of the diet will be changed. Test batteries will be set up for increased physical activity by changes of cage size and enrichment of the environment. We will perform metabolic time-lapse studies in order to monitor metabolic changes after physical challenge of mice.

*Lit.: 1. Gailus-Durner V. et al. Introducing the German Mouse Clinic: open access platform for standardized phenotyping. Nat Methods. 2005 Jun;2(6):403-4.*