

The

GERMAN MOUSE CLINIC

Report for DLG3

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1 Summary

In a **primary screen**, 46 DLG3 mice have been analyzed in the German Mouse Clinic (GMC) in the screens Behavior, Dymorphology, Bone and Cartilage, Neurology, Eye, Clinical Chemistry, Immunology, Allergy, Nociception, Lung Function, Metabolism, and comprehensive Pathology.

Analysis of the observed **behavioral parameters** indicated reduced grooming in female DLG3-deficient mice. There was no genotype effect in males. The DLG3 mice did not show any other pathological finding in any other screen. Therefore it would be interesting to conduct further experiments because confounding factors are absent.

In the screens **Dymorphology, Neurology, Eye, Clinical Chemistry, Immunology, Allergy, Nociception, Metabolism, Lung Function, and Pathology**, no genotype-specific differences could be found.

Please contact Valérie Gailus-Durner to discuss further steps and details.

2 General Part

2.1 The Role of the Gene

The W062C05 mutant line was created from the ES-cell line TBV-2 which was trapped with gene-trap vector pT1 β geo. Trapped gene turned to be novel a gene which is moderately similar to DLG3 (discs large 3) mouse gene and therefore named DLGh3 (discs large homologue 3). The function of DLGh3 has not been clarified yet.

The DLG3 protein; also known as presynaptic protein SAP 102, belongs to the MAGUK (membrane-associated guanylate kinase) family of cell junction proteins. Like the other members Scrib (Scribble) and Lgl (Lethal giant larvae), the DLGs are highly conserved between mammals and *Drosophila* and are assumed to be involved in cell polarity, cell proliferation and cancer (for a recent review please see Humbert *et al.*, 2003).

2.2 Known Phenotypes

No obvious phenotypes and breeding abnormalities were observed. Using Southern Blot analysis it has been shown that homozygous mice survive into adulthood. According to LacZ staining analysis, the trapped gene is widely expressed in embryonic tissue (E 11.5) and in the placenta.

All further findings we consider as new.

2.3 Mice

2.3.1 Number and kind of mice

As described by the sender, the mice provided to the GMC, were a second backcross generation to C57/BL6. Forty-six healthy mice (13 female -/-, 14 male -/-, 10 female +/+ and nine male +/+) arrived in the 31st week in 2003.

2.3.2 Housing conditions

In the GMC mice are housed in type II polycarbonate cages in individually ventilated caging (IVC) systems (VentiRack Bioscreen TM, Biozone, Margate, UK) on wood fibre (Altromin, Lage, Germany). The IVCs operate with positive pressure. Mice are transferred in weekly intervals to new cages with forceps in Laminar Flow Class II changing stations. Mice are fed with irradiated standard rodent high energy breeding diet (Altromin 1314) and given semidemineralized filtered (0.2 μ m) water *ad libitum*. Light is adjusted to a 12h/12h light/dark cycle; temperature and relative humidity are regulated to $22 \pm 1^\circ\text{C}$ and

55 ± 5%, respectively. In specified modules husbandry conditions are adjusted according to the experiment requirements (See corresponding sections). All people attending the facility completely change their garment (jackets and trousers autoclaved) and shoes and wear caps and masks before entering the GMC (Brielmeier *et al.*, 2002).

Outbred 8-week-old male SPF Swiss mice are used as sentinels and kept on a mixture of new bedding and aliquots of soiled bedding (50:50) from all cages of the IVC rack. In addition, the sentinels were also exposed to soiled air from all “upstream” cages of the IVC rack. Health monitoring is carried out by on-site examination of the sentinel mice by certified laboratories according to FELASA recommendations (www.felasa.org).

Mice are kept according to the German laws. Tests were carried out by authority of the Regierung von Oberbayern.

2.4 Workflow

2.4.1 Standardized workflow for the primary screen in the German Mouse Clinic

Mouse mutants entering the GMC are examined in a primary screen according to the following standard workflow (Fig. 1). Analyzed parameters are listed in Table 1.

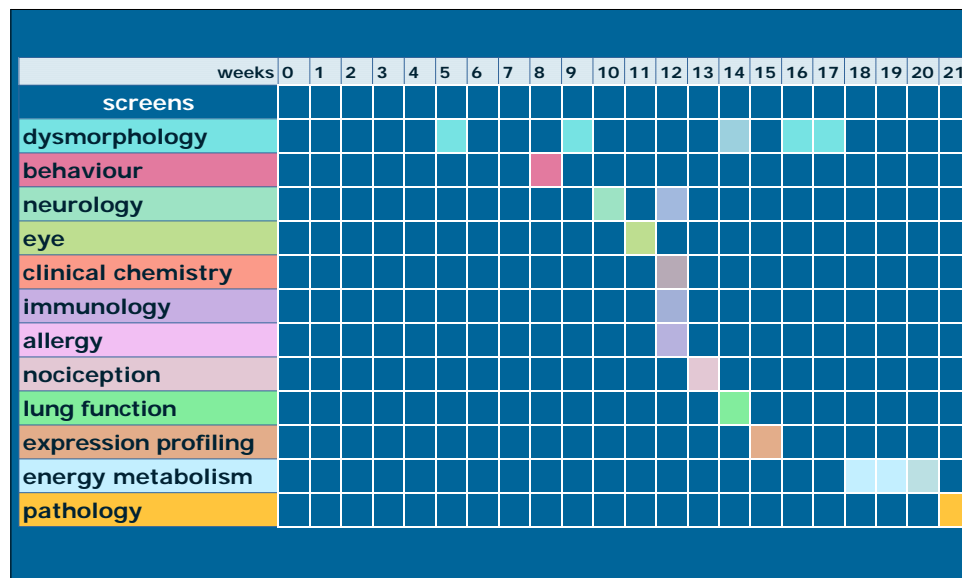


Figure 1: Workflow of the primary screen
 Explanation below, Analysis of blood-based parameters.

After the mice arrive at the GMC, they are acclimatized in the new environment for one week. The males then start in the Behavior Screen. There they stay for three weeks. Directly after the Behavior Tests, the anatomical inspection of the Dysmorphology Screen is performed. In the next week, the Neurology Screen is applied. One week later the mice go through the tests of the

Eye Screen. When the mice were 12 weeks old, blood is taken, and samples are distributed to the blood-based screens for Clinical Chemistry, Immunology, Allergy and the Lactate test. One week later, the animals are tested in the Nociceptive Screen. Two weeks after testing of the first blood sample, a second sample is taken to confirm outliers, and to supply the Dysmorphology Screen with material for determination of blood-based bone-related parameters. In parallel, 10 mutant animals (5 males / 5 females) and 10 controls (5 males / 5 females) leave the animal facility for the Lung Function Analysis, which for technical reasons is located elsewhere. These animals are, for hygienic reasons, not allowed to re-enter the German Mouse Clinic. The females go directly to Pathology. The males are used to freeze organs for future expression profiling on demand (remaining organs from those animals are analyzed by the Pathology). All other animals go through the bone and cartilage tests of the Dysmorphology Screen, and then stay three weeks in the Metabolic Screen. After completion of the primary screen, all animals end up in the Pathology.

The screening of female animals starts one week later and follows the same workflow (with the exception of Expression Profiling sampling). Deviations from our Standard operation protocol (SOP) are listed below; please take the specific number of analyzed animals from the sections of the applied screen.

2.4.2 Applied screens

The GMC standard workflow for the primary screen as described above was applied to analyze the DLG3 mice. As the demanded number of 60 animals (15 mice per sex per genotype) could not be delivered, the workflow was adapted to the available number of animals. Some parameters from the blood based screens could not be determined in all animals, as it was not possible to get the needed amount of blood from these animals. A few animals died during the primary screen and thus they could not be analyzed for all parameters

2.4.3 Quality Management

As a routine quality control, we take blood samples from for serological tests of the sanitary status of all mice after they went through the GMC primary screen. When indicated, the serum is tested for MHV (BioDoc, Hannover). We chose MHV as a "sentinel" pathogen, as it is one of the most common viruses in mouse facilities worldwide and it is transmitted easily. To be open for collaboration for as many partners as possible, we allow MHV positive animals to enter our facility.

Microgranulomas in the liver are observed commonly in mice on a C57BL/6 genetic background. In those cases the results of the MHV tests are used to exclude MHV as one possible reason for these infiltrates (See chapter 3.12 Pathology Screen).

2.5 Statistical Analysis of Data

If not otherwise stated, data of males and females was analyzed separately comparing mutant and control data using a Student's t-test. Sex differences within the mutant or the control group also were determined with a t-test. Tables summarizing the data will show mean \pm standard error of the mean. Significant differences are indicated stepwise from 0.05, 0.02, 0.01, 0.001 to 0.0001.

2.6 References

Brielmeier M., H. Fuchs, G. Przemeck, V. Gailus-Durner, M. Hrabé de Angelis, J. Schmidt (2002) The GSF – Phenotype Analysis Center (German Mouse Clinic, GMC): A sentinel-based health-monitoring concept in a multi-user unit for standardized characterization of mouse mutants. In: J. Guenet and C. Herweg (Eds.) Laboratory Animals Science - Basis and Strategy for Animal Experimentation Vol. 11, Proceedings of the 8th FELASA Symposium, Laboratory Animals Ltd., Aachen, pp. 19-22.

Humbert P, Russell S, Richardson H. (2003): Dlg, Scribble and Lgl in cell polarity, cell proliferation and cancer. *Bioessays* 25: 542-53

Abbreviations

DLG3	DLGh3 or Discs, large homologous 3
GMC	German Mouse Clinic
IVC	individually ventilated cage
+/+	homozygous wild type, i.e. control
-/-	homozygous mutant
wt	wild type
KO	knockout
FELASA	European Laboratory Animal Science Associations, 25 Shaftesbury Avenue, London W1D 7EG, UK, www.felasa.org

Table 1: Primary Screen at GMC

Screens	Goal	Methods
Dysmorphology, Bone and Cartilage	morphological analysis of body, skeleton, bone and cartilage	morphological observation, bone densitometry, X-ray, AVL analyzer, micro-computer tomography
Behavior	locomotor, exploratory, emotional and social behavior, object recognition memory	modified hole board
Neurology	assessment of muscle, spinocerebellar, sensory, and autonomic function	modified SHIRPA protocol
Eye	assessment of morphological and functional alterations of the eye	electroretinography, slit lamp biomicroscopy
Clinical Chemistry	determination of clinical-chemical and hematological parameters in blood	blood autoanalyzer, ABC-animal blood counter
Immunology	analysis of peripheral blood samples for immunological parameters	flow cytometry, ELISA
Allergy	analysis of total plasma IgE	ELISA
Nociception	detection of altered pain response	hot plate assay
Lung function	assessment of alterations in breathing patterns	whole body plethysmography (Buxco®)
Expression Profiling	RNA expression profiling	DNA-chip technology
Energy Metabolism	measurement of altered body weight regulation, body temperature and energy balance	bomb calorimetry
Pathology	microscopic and macroscopic examination	histology, immunochemistry

3 Specific part

3.1 Behavior Screen

3.1.1 Summary

The modified hole board test is used as primary screen in the behavioral phenotyping module of the GMC, because it allows the comprehensive analysis of a range of behavioral parameters known to be indicative of behavioral dimensions such as locomotor activity, exploratory behavior, arousal, emotionality, memory and social affinity in a single short test (See Ohi *et al.*, 2001).

Using this test, a grooming phenotype was detected in female DLG3-deficient mice. This phenotype occurred only in females, and it was the only behavioral alteration detected. It would be interesting to check the reproducibility of this phenotype with new mice and to eventually use pharmacological challenges to test for alterations in dopaminergic transmission, since D1 receptor mediated transmission is involved in grooming behavior.

Additionally, because of the known interaction of DLG3 with NMDA receptor 2B (NR2B) subunits, it would be very interesting to conduct secondary analysis concerning learning and memory parameters that can not be covered by the primary screen analysis. This is particularly interesting because of the lack of any other pathological finding in these mice, so that confounding factors are absent.

3.1.2 Mice

Mice were housed with food and water *ad libitum* under standard laboratory conditions. Animals were separated based on sex, but not genotype. They entered the laboratory at the age of six weeks, were given two weeks for acclimatization and were tested at the age of eight weeks. Three days before testing, an object (metal cube) was placed into the home cage and removed one day before testing.

In this screen, 26 female mice (10 control, 13 mutants) and 16 male mice (6 control, 10 mutants) were available for analysis, because too few males had been group housed.

3.1.3 Material and Methods

The modified hole board test was carried out according to the procedures described by Ohi *et al.*, 2001. The test apparatus consisted of a test arena (100 x 50 cm), in the middle of which a board (60 x 20 x 2 cm) with 23 holes (1.5 x 0.5 cm) staggered in three lines with all holes covered by movable lids was placed, thus representing the central area of the test arena as an open field. The area around the board was divided into 12 similarly sized quadrants by lines taped onto the floor of the box (See Ohi *et al.*, 2001). Both box and board were made of dark grey PVC. All lids were closed before the start of a trial. For each trial, an unfamiliar object (a blue plastic tube lid, similar in size to the metal cube) and the familiar object (metal cube) were placed into the

test arena with a distance of 2 cm between them. The illumination levels were set at approximately 150 lux in the corners and 200 lux in the middle of the test arena.

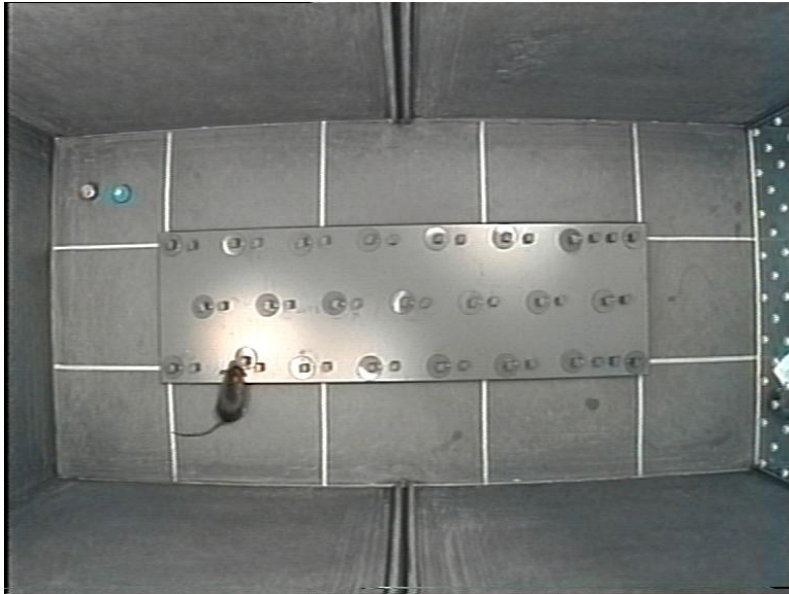


Figure 2: Test Arena for modified hole board test.

For testing, each animal was placed individually into the test arena and allowed to explore it freely for 5 min. The animals were always placed into the test arena in the same corner next to the partition, facing the board diagonally. The two objects were placed in the corner quadrant diametrical to the starting point. During the 5 min trial, the animal's behavior was recorded by a trained observer with a hand-held computer. Data were analyzed by using the Observer 4.1 Software (Noldus, Wageningen). Additionally, a camera was mounted 1.20 m above the center of the test arena, and the animal's track was videotaped and its locomotor path analyzed with a video-tracking system (Ethovision 2.3, Noldus, Wageningen). After each trial, the test arena was cleaned carefully with a disinfectant.

Data were statistically analyzed using SPSS software (SPSS Inc, Chicago, USA). The chosen level of significance was $p < 0.05$.

3.1.4 Parameters

Manually recorded behavior
Line crossings, rearings, board entries, hole explorations, hole visits, stretched attends (risk assessment), partition (group contact), grooming, defecation, unfamiliar and familiar object exploration
Video-track analysis
Total distance moved, mean velocity, maximum velocity

3.1.5 Results and Discussion

Analysis of the observed behavioral parameters indicated only reduced grooming in female DLG3 KO mice. There was no genotype effect in males.

Since these mice did not show any other pathological finding in any other screen, they might be very interesting for further analysis concerning a CNS phenotype. It would be interesting to conduct further experiments with new mice to see whether the grooming phenotype is reproducible, and if so, whether it might be related to the interaction of DLG3 with the cytoplasmic tail of the NR2B subunit which is involved in taste learning in the insular cortex. To this end sniffing box experiments particularly aimed at the analysis of grooming behavior, eventually combined with dopaminergic pharmacological challenges, because grooming behavior is known to be under prolactin and D1 receptor-mediated control. In addition, we would like to conduct social discrimination and fear-potentiated startle experiments with DLG3 mice in order to check for a learning and memory related phenotype that would not necessarily show up in the primary screen.

3.1.6 Reference

Ohl, F., Sillaber, I., Binder, E., Keck, M.E. & Holsboer, F. (2001) Differential analysis of behavior and diazepam-induced alterations in C57BL/6N and BALB/c mice using the modified hole board test. *J. Psychiatr. Res.* 35: 147-154.

Table 2: Results of Behavioral Observation in the Modified Hole Board Test

Data are presented as mean \pm standard error of mean.

Parameter	Control (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=6)	(n=10)	<i>p - value</i>	(n=10)	(n=13)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
Line crossing [Frequency]	142.33 \pm 16.87	124.8 \pm 13.55	N.A.	114.29 \pm 14.8	134.77 \pm 7.79	N.A.	n.s.	n.s.
Line crossing [Latency]	1.48 \pm 0.64	0.85 \pm 0.08	N.A.	1.26 \pm 0.12	0.9 \pm 0.12	N.A.	n.s.	n.s.
Immobility [Total duration %]	0 \pm 0	0 \pm 0	N.A.	0 \pm 0	0 \pm 0	N.A.	N.A.	n.s.
Rearings in box [Frequency]	24.5 \pm 3.73	24.8 \pm 3.45	N.A.	19 \pm 3.81	28.54 \pm 2.66	N.A.	n.s.	n.s.
Rearings in box [Latency]	28.72 \pm 11.48	23.26 \pm 3.66	N.A.	33.44 \pm 6.94	26.44 \pm 5.64	N.A.	n.s.	n.s.
Hole exploration [Frequency]	21.83 \pm 4.29	15.8 \pm 2.59	N.A.	26 \pm 6.72	19.62 \pm 2.4	N.A.	n.s.	n.s.
Hole exploration [Latency]	42.88 \pm 14.76	66.55 \pm 26.76	N.A.	72.64 \pm 39.7	34.95 \pm 5.13	N.A.	n.s.	n.s.
Hole visit [Frequency]	0 \pm 0	0 \pm 0	N.A.	0 \pm 0	0 \pm 0	N.A.	N.A.	N.A.
Hole visit [Latency]	300 \pm 0	300 \pm 0	N.A.	300 \pm 0	300 \pm 0	N.A.	N.A.	N.A.
Board entry [Frequency]	7 \pm 1.71	5 \pm 0.87	N.A.	8 \pm 1.85	5.92 \pm 0.87	N.A.	n.s.	n.s.
Board entry [Latency]	75.65 \pm 12.73	92.53 \pm 28.52	N.A.	86.26 \pm 40.42	81.98 \pm 19.62	N.A.	n.s.	n.s.

Board entry [Total duration %]	10.06 ± 2.6	6.54 ± 1.1	N.A.	12.39 ± 2.95	7 ± 0.94	N.A.	n.s.	n.s.
Rearing on board [Frequency]	0 ± 0	0.4 ± 0.16	N.A.	0.71 ± 0.71	0.23 ± 0.17	N.A.	n.s.	n.s.
Rearing on board [Latency]	300 ± 0	275.32 ± 12.5	N.A.	275.86 ± 24.14	292.77 ± 6.85	N.A.	n.s.	n.s.
Risk assessment [Frequency]	0 ± 0	0.4 ± 0.4	N.A.	0.43 ± 0.43	0 ± 0	N.A.	N.A.	N.A.
Risk assessment [Latency]	300 ± 0	282.05 ± 17.95	N.A.	274.89 ± 25.11	300 ± 0	N.A.	N.A.	N.A.
Group partition [Frequency]	9.5 ± 1.61	13.7 ± 0.92	N.A.	11 ± 1.6	13.46 ± 0.77	N.A.	n.s.	n.s.
Group partition [Latency]	22.65 ± 12.13	13.96 ± 4.42	N.A.	21.21 ± 8.53	11.23 ± 2.76	N.A.	n.s.	n.s.
Group partition [Total duration %]	16.6 ± 3.62	24.2 ± 2.75	N.A.	19.09 ± 5.29	25.51 ± 1.21	N.A.	n.s.	n.s.
Grooming [Frequency]	0.67 ± 0.21	2 ± 0.49	N.A.	1 ± 0.31	0.62 ± 0.24	N.A.	n.s.	p<0.05
Grooming [Latency]	268.25 ± 18.48	199.2 ± 30.18	N.A.	217.07 ± 23.71	262.85 ± 15.79	N.A.	n.s.	p=0.07
Grooming [Total duration %]	1.5 ± 0.55	4.06 ± 1.27	N.A.	3.07 ± 1.42	1.81 ± 0.96	N.A.	n.s.	n.s.
Defecation [Frequency]	0.17 ± 0.17	0.4 ± 0.22	N.A.	0.71 ± 0.36	0.15 ± 0.15	N.A.	n.s.	n.s.
Defecation [Latency]	251.93 ± 48.07	217.78 ± 41.93	N.A.	222.61 ± 49.49	278.66 ± 21.34	N.A.	n.s.	n.s.
Unfamiliar object ex- ploration [Frequency]	6 ± 0.89	5.3 ± 0.92	N.A.	4.57 ± 1.25	4.92 ± 0.5	N.A.	n.s.	n.s.

Familiar object exploration [Frequency]	6 ± 0.68	4.8 ± 0.95	N.A.	4.86 ± 1.1	5.54 ± 0.4	N.A.	n.s.	n.s.
Unfamiliar object exploration [Latency]	26.2 ± 6.06	56.61 ± 27.42	N.A.	99.19 ± 39.28	33.51 ± 7.63	N.A.	n.s.	n.s.
Familiar object exploration [Latency]	24.22 ± 10.79	78.53 ± 27.24	N.A.	76.46 ± 38.9	37.45 ± 9.06	N.A.	n.s.	n.s.
Unfamiliar object exploration [Total duration %]	1.87 ± 0.36	1.44 ± 0.24	N.A.	1.28 ± 0.38	1.37 ± 0.15	N.A.	n.s.	n.s.
Familiar object exploration [Total duration %]	1.2 ± 0.17	1.06 ± 0.21	N.A.	0.95 ± 0.19	1.17 ± 0.1	N.A.	n.s.	n.s.
Object Index	0.16 ± 0.07	0.13 ± 0.07	N.A.	0.08 ± 0.06	0.05 ± 0.07	N.A.	n.s.	n.s.

Table 3: Video-Tracking Results Regarding Locomotor Behavior

Data are presented as mean \pm standard error of mean.

Parameter	Control (A)			Mutant (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=6)	(n=10)	<i>p - value</i>	(n=10)	(n=13)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
Distance moved [cm]	3557.42 \pm 361.3	3148.68 \pm 296.66	N.A.	3116 \pm 220.59	3398.2 \pm 176.67	N.A.	n.s.	n.s.
Mean velocity [cm/sec.]	20.42 \pm 0.99	19.65 \pm 1.15	N.A.	18.79 \pm 0.81	19.88 \pm 0.63	N.A.	n.s.	n.s.
Maximum velocity [cm/sec.]	59.21 \pm 1.89	62.17 \pm 3.41	N.A.	60.76 \pm 3.59	60.45 \pm 2.71	N.A.	n.s.	n.s.
Turns [Frequency]	1810.67 \pm 137.17	1622.5 \pm 111.07	N.A.	1678.9 \pm 93.48	1780.85 \pm 66.46	N.A.	n.s.	n.s.
Mean Turn Angle [degrees]	24.31 \pm 1.75	22.91 \pm 1.03	N.A.	23.85 \pm 2.98	22.67 \pm 0.7	N.A.	n.s.	n.s.
Angular Velocity [degrees/sec.]	160.7 \pm 8.93	145.54 \pm 6.34	N.A.	159.75 \pm 21.91	148.24 \pm 3.66	N.A.	n.s.	n.s.
Absolute Meander [degrees/sec.]	17.03 \pm 1.4	16.22 \pm 0.86	N.A.	16.83 \pm 1.93	16.08 \pm 0.57	N.A.	n.s.	n.s.
Mean distance to wall [cm]	7.65 \pm 0.48	6.35 \pm 0.47	N.A.	6.53 \pm 0.62	6.73 \pm 0.24	N.A.	n.s.	n.s.
Mean distance to board [cm]	8.26 \pm 0.39	9.54 \pm 0.47	N.A.	9.54 \pm 0.49	9.11 \pm 0.19	N.A.	n.s.	n.s.

3.2 Dysmorphology, Bone and Cartilage

3.2.1 Summary

In the Dysmorphology, Bone and Cartilage Screen of the German Mouse Clinic mice are analyzed for morphological abnormalities in different organ systems with special focus on bone and cartilage development and homeostasis. We adapted the successful dysmorphology screening protocol from the Munich ENU-Mutagenesis Screen (Hrabé de Angelis *et al.* 2000) for use in the German Mouse Clinic. The nomenclature of the parameters was adapted according to the “Phenoslim” wording (<http://www.informatics.jax.org/userdocs/phenoslim.shtml>). Further tests for defects in bone development and homeostasis were taken over from human diagnosis, and were adapted for the use in mice analysis. Such tests include: X-ray analysis, bone densitometry and, in a limited number of animals, micro-computer tomography.

A total of 46 DLG3 mutants and controls have been analyzed in the Dysmorphology, Bone and Cartilage Module of the German Mouse Clinic. Anatomical observation, analysis of ionic calcium levels in blood, and qualitative analysis of X-ray images did not result differences between DLG3 mutants and littermate controls. By bone densitometry and quantification of X-ray data we found significant differences between female mutants and controls in the parameters sBMD and lumbar vertebrae height, but we were not able to conclude any association of DLG3 with these findings.

3.2.2 Mice

Twenty-three male (9 +/+, 14 -/-) and 23 female (10 +/+, 13 -/-) mice were analyzed by morphological inspection at the age of 9 weeks. Blood was taken at the age of 14 weeks for determination of ionic calcium from 22 knockout (KO) and 16 control animals, and 16-week-old KOs (17 animals) and controls (14 animals) entered the bone density and X-ray analysis.

3.2.3 Material and Methods

The Dysmorphology, Bone and Cartilage module of the German Mouse Clinic analyzed the mice in different phases:

1. At the age of 5 weeks, i.e. when the mice entered the facility, the general physical condition and health were checked;
2. At the age of 9 weeks, a morphological whole-body checkup was performed;
3. The ionized fraction of calcium in blood was analyzed in 14-week-old mice, and
4. At the age of 16 to 17 weeks, X-ray analysis and bone densitometry were performed.

Morphological test

The animals were screened using the protocol for morphological analysis from Fuchs *et al.* (2000) as adapted for the German Mouse Clinic.

Ionized calcium Analysis

Equipment: AVL 9180 Electrolyte Analyzer (distributed by Roche Diagnostics GmbH, Mannheim, Germany)

cleaning solution and conditioning solution (Roche),

ISEtrol Quality Control Solutions (Roche),

lithium-heparin polypropylen tubes,

glass capillary (0.8 mm diameter, 32 mm length, without heparin; special product of Laborteam K+K, Munich).

Quality control: Calibration of the system and quality control were performed at intervals recommended and with solutions provided by the manufacturer. The results from the quality control were recorded by the system. Before blood measurement, daily cleaning, conditioning and calibration of the analyzer were performed.

Procedure: Blood (100 µl) was collected from anesthetized mice in lithium heparin tubes and transferred directly to the analyzer. Values were transferred directly to the database.

X-ray Images

Equipment: Faxitron X-ray Model MX-20 (Specimen Radiography System, Illinois, USA),

NTB Digital X-ray Scanner EZ 40 (NTB GmbH, Diepholz, Germany),

Quality control: Calibration of the system is done in monthly intervals,

Settings: Voltage 25 kV, integration time 40 ms,

Procedure: The anesthetized mouse was fixed on an X-ray-permeable plate and placed in the machine. Using iX-Pect software supplied by the manufacturer of the X-ray scanner, the image was taken and analyzed. Analysis was done qualitatively by visual inspection of the images as well as quantitatively by using the ruler tool of iX-Pect software.

Bone density analysis

Equipment: pDEXA Sabre X-ray Bone Densitometer (Norland Medical Systems. Inc., Basingstoke, Hampshire, UK; distributed by Stratec Medizintechnik GmbH, Pforzheim, Germany),

Quality control: Calibration of the system was done in daily intervals using the QC and the QA phantoms delivered by the manufacturer. Results from the quality control were recorded by the system.

Settings: Scan speed 20 mm/s, Resolution 0.5 mm x 1.0 mm, HAW 0.020

Procedure: After anesthesia, the weight and length of the mouse were recorded, and the mouse was placed in the analyzer. After a scout scan, the area of interest was optimized and the measure scan started.

Data-analysis: For analysis of the data, regions have to be defined. The standard analysis comprises a whole body analysis as well as a whole body analysis excluding the skull.

Statistical analysis of data

Analysis of quantitative data sets was carried out using StatView software package (SAS corporation).

3.2.4 Parameters

Morphological inspection
<i>Growth/weight/body size:</i> abnormality <i>Eye:</i> dysmorphology, corneal or lens defect <i>Coat:</i> hair growth defects, hair texture defects, color anomalies, hair follicle, structure/orientation anomalies <i>Skin:</i> pigmentation anomalies, texture/condition, anomalies <i>Vibrissae:</i> dysmorphology <i>Extremities:</i> limb dysmorphology, digit dysmorphology, tail dysmorphology <i>Teeth:</i> tooth dysmorphology <i>Ears:</i> auditory defects/deafness, dysmorphology <i>Musculature:</i> muscle dysmorphology <i>Skeletal:</i> osteogenesis/developmental anomalies, axial defects, extremities defects, craniofacial defects <i>Neurological / behavioral:</i> seizures/epilepsy, motor capabilities / coordination / movement anomalies, feeding / drinking anomalies <i>Respiratory system:</i> dysmorphology <i>Reproductive system:</i> dysmorphology <i>Other aberrant phenotype</i>
X-ray analysis
Skull shape, mandibles, maxilla, teeth, orbit, number of vertebrae (cervical, thoracic, lumbar, pelvic, sacral), vertebrae shape, number of ribs, rib shape, scapulas, clavicle, pelvis, femur diameter, femur shape, tibia, fibula, humerus, ulna, radius, number of digits, completeness of digits, subcutaneous fat, joints
Dual energy X-ray absorption
Bone mineral density (BMD), partial bone mineral density (pBMD, whole body excluding skull), specific bone mineral density (sBMD), bone mineral content (BMC), lean mass, fat mass, bone content, lean content, fat content
AVL analyzer
Free ionic calcium
Computer tomography
3D-visualization of whole skeleton, 2D-examination of inner organs and soft tissue, high-resolution analysis of regions of interest

3.2.5 Results and Discussion

Forty-six animals were analyzed in the Dymorphology Screen by anatomical observation, AVL blood analysis, bone densitometry and X-ray analysis. In the anatomical observation, no genotype-specific differences between mutants and controls could be detected. However, there were some minor phenotypes observed, which are summarized in Table 4. The observed dark skin alterations are typical for some genetic backgrounds (e.g. C57BL/6, 129SvJ) and indicate that there is still some heterogeneity in the genetic background. The analysis of ionic calcium in the blood revealed no difference between mutants and controls.

X-ray analysis was done by qualitative analysis of the images and by quantification of defined distances on the image. In the qualitative analysis of the X-ray images we did not detect any malformations. In quantitative X-ray data, we found in a single parameter (lumbar vertebrae height) slightly significant differences in the females between mutants and controls. As the value of the controls was very low, we would not expect that a mutation in the DLG3 gene influences this parameter, and we would not consider to follow up this finding.

In the bone densitometry (Table 5) most values measured were in the range we expected from previously taken baseline data (data not shown). The BMD values of male mutants and sBMD of female controls were unexpectedly high. Only one parameter (sBMD) showed statistically significant differences between mutants and controls. The difference is only present in females. As already mentioned, in this parameter the data from the control females differs from the value we expect from our baseline experiments. From our data we would not conclude that DLG3 influences the specific bone mineral density.

Raw data will be available on demand.

3.2.6 References

Fuchs H, Schughart K, Wolf E, Balling R, Hrabé de Angelis M. (2000): Screening for dysmorphological abnormalities - a powerful tool to isolate new mouse mutants. *Mammalian Genome* 11(7): 528-30.

Hrabé de Angelis, M., H. Flaswinkel, H. Fuchs, B. Rathkolb, D. Soewarto, S. Marschall, S. Heffner, W. Pargent, K. Wuensch, M. Jung, A. Reis, T. Richter, F. Alessandrini, T. Jakob, E. Fuchs, H. Kolb, E. Kremmer, K. Schaeble, B. Rollinski, A. Roscher, C. Peters, T. Meitinger, T. Strom, T. Steckler, F. Holsboer, T. Klopstock, F. Gekeler, C. Schindewolf, T. Jung, K. Avraham, H. Behrendt, J. Ring, A. Zimmer, K. Schughart, K. Pfeffer, E. Wolf and R. Balling (2000): Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nature Genetics* 25: 444 – 447

Abbreviations

BMC	bone mineral content
BMD	bone mineral density
pBMD	partial bone mineral density (excluding skull)
sBMD	specific bone density

Table 4: Results from the Morphological Inspection

Phenotype	Male		Female	
	+/+	-/-	+/+	-/-
Digit bent		1		1
Digit missing	1		1	
Deaf/Hardness of hearing	2		2	1
Dark skin		2	2	2
Trembling (slightly)				1
Neck with bald patch			2	1
<i>Animals analyzed</i>	9	14	10	13

Table 5: Bone-Related Quantitative ParametersData are presented as mean \pm standard error of mean.

Parameter	Control (A)			Mutant (B)			A~B Male	A~B Female
	male (n=7)	female (n=7)	p-value	male (n=9)	female (n=8)	p-value	p-value	p-value
BMD [mg/cm²]	66 \pm 2	66 \pm 3	n.s.	72 \pm 3	60 \pm 2	< 0.01	n.s.	n.s.
BMC [mg]	894 \pm 61	604 \pm 42	< 0.01	740 \pm 78	681 \pm 67	n.s.	n.s.	n.s.
Lean mass [g]	18.76 \pm 1.08	16.19 \pm 1.11	n.s.	20.64 \pm 1.03	15.99 \pm 1.19	< 0.01	n.s.	n.s.
Fat mass [g]	10.66 \pm 1.42	4.71 \pm 0.62	< 0.01	8.50 \pm 1.84	5.33 \pm 1.64	n.s.	n.s.	n.s.
Body length [cm]	8.7 \pm 0.1	8.4 \pm 0.1	< 0.05	8.7 \pm 0.2	8.4 \pm 0.1	n.s.	n.s.	n.s.
Body Weight [g]	33.14 \pm 1.59	23.94 \pm 0.87	< 0.001	32.68 \pm 1.53	24.35 \pm 0.90	< 0.001	n.s.	n.s.
BMD/Weight [10⁻³ x cm⁻²]	1.99 \pm 0.06	2.78 \pm 0.09	< 0.001	2.23 \pm 0.14	2.49 \pm 0.04	n.s.	n.s.	< 0.02
sBMC [%]	2.7 \pm 0.2	2.5 \pm 0.2	n.s.	2.2 \pm 0.2	2.8 \pm 0.2	n.s.	n.s.	n.s.
Lean mass/Weight [%]	57.0 \pm 3.6	67.4 \pm 2.7	< 0.05	64.3 \pm 4.6	66.7 \pm 5.8	n.s.	n.s.	n.s.
Fat mass/Weight [%]	31.7 \pm 3.5	19.9 \pm 2.7	< 0.05	24.7 \pm 4.7	20.9 \pm 5.9	n.s.	n.s.	n.s.
pBMD [g/cm²]	55 \pm 2	53 \pm 3	n.s.	58 \pm 2	48 \pm 2	< 0.01	n.s.	n.s.
Femur width [mm]	1.3 \pm 0.1	1.1 \pm 0.1	n.s.	1.2 \pm 0.1	1.1 \pm 0.1	n.s.	n.s.	n.s.
Lumbar vertebrae height [mm]	3.1 \pm 0.2	2.8 \pm 0.2	n.s.	3.0 \pm 0.1	3.1 \pm 0.1	n.s.	n.s.	< 0.05
Lumbar vertebrae width [mm]	2.5 \pm 0.1	2.5 \pm 0.1	n.s.	2.5 \pm 0.1	2.3 \pm 0.1	n.s.	n.s.	n.s.
Femoral muscles width [mm]	12.4 \pm 0.2	11.7 \pm 0.6	n.s.	12.3 \pm 0.5	11.9 \pm 0.5	n.s.	n.s.	n.s.
Fat tissue [mm]	4.3 \pm 0.5	4.3 \pm 0.2	n.s.	4.5 \pm 0.5	3.9 \pm 0.4	n.s.	n.s.	n.s.

Table 6: Concentration of Ionic Calcium in BloodData are presented as mean \pm standard error of mean.

	Control (A)			Mutant (B)			A~B	A~B
	male (n=9)	female (n=7)	p - value	male (n=14)	female (n=8)	p - value	male p - value	female p - value
Ionized Calcium (Ca⁺⁺) [mmol/L]	1.22 \pm 0.03	1.21 \pm 0.02	n.s.	1.18 \pm 0.03	1.17 \pm 0.02	n.s.	n.s.	n.s.

3.3 Neurology Screen

3.3.1 Summary

In the primary neurological screen, 23 DLG3-deficient mice (14 males/13 females) and 19 control mice (9 males/10 females) were screened. Animals were analyzed according to our modified SHIRPA protocol where a battery of behavioral tests is carried out. This primary observation screen is a modification of the Irwin procedure (Irwin, 1968) and was proposed as a rapid, comprehensive and semi-quantitative screening method for qualitative analysis of abnormal phenotypes in a mouse strain (Rogers *et al.*, 1994). We carried out 37 of 40 designed test parameters (See web page: http://www.mgu.har.mrc.ac.uk/facilities/mutagenesis/mutabase/shirpa_summary.html) to detect phenotypic differences between DLG3 knockout and control mice. Each test parameter contributes to an overall assessment in muscle, lower motor neuron, spinocerebellar, sensory and autonomic function. The primary neurological screen is focused on investigating neurological reflexes to determine the neurological functioning of a mouse. We also examine lactate levels in the blood of mice to draw conclusions about energy metabolism.

The comparison of DLG3 knockout to control mice revealed no obvious neurological phenotype.

3.3.2 Mice

Fourteen 10-week-old male DLG3-knockout and nine 10-week-old male control mice entered the neurological screen at the beginning of the 33rd calendar week in 2003. Ten 10-week-old female DLG3-knockout and nine 10-week-old female control mice entered the neurological laboratory one week later. All animals were fed *ad libitum* for a period of one week during their stay in the neurological screen.

3.3.3 Material and Methods

At the age of 10 weeks assessment of each animal started with observation of undisturbed behavior (*Viewing Jar Behavior*) in a glass cylinder (11 cm in diameter). The mice were then transferred to an arena consisting of a clear Perspex box (420 x 260 x 180 mm) in which a Perspex sheet on the floor is marked with 15 squares. In this arena, locomotor activity and motor behavior was observed (*Behavior recorded in the Arena*). This was followed by a sequence of manipulations testing reflexes, grip strength, toe pinch and wire manoeuvre (*Behavior recorded on or above the arena*). For the wire manoeuvre test, a rigid horizontal wire (3 mm in diameter) is secured across the rear right corner of the arena. For grip strength testing, a grid (270 x 275 mm) is secured across the width of the arena. In the last part of the observation (*Behavior recorded during Supine Restraint*), the animals were restrained in a supine position to record autonomic responses such as salivation. Measurements were completed with the recording of limb tone, provoked biting, and body length. The last part of the primary screen also involves the analysis of righting reflex, negative geotaxis and contact righting reflex. A glass cylinder

(35 mm diameter, 135 mm length) is used for testing the contact-righting reflex. Throughout the entire procedure, abnormal behavior, irritability, fear, aggression and vocalization were recorded. Between testing of each mouse, faecal pellets and urination were removed from the viewing jar and arena. All experimental equipment is thoroughly cleaned with Pursept-A and dried prior to testing.

Values for body length, body weight and locomotor activity are presented as means \pm SEM. Kruskal-Wallis-test (S-PLUS, Insightful) was used to test for effects of genotype and gender factors on these parameters. The Chi-Squared test was applied for all other parameters.

3.3.4 Parameters

Muscle/lower motor neuron function
Body position, gait, Positional passivity, wire maneuver, tail elevation, limb tone, body tone, abdominal tone, grip strength, urination, defecation
Spinocerebellar function
Body position, gait, righting reflex, tail elevation, visual placing, limb tone, body tone, abdominal tone, grip strength
Sensory function
Transfer arousal, touch escape, gait, visual placing, toe pinch, pinna reflex, righting reflex
Autonomic function
Palpebral closure, urination, salivation, respiration rate, defecation
Neurological reflexes
Righting reflex (pons), contact righting reflex, visual placing, toe pinch/flexion reflex (cerebellar/spinal cord), negative geotaxis, corneal reflex (medulla), pinna reflex (hearing test)
Physiological parameters
Body weight, body length
General appearance
Body weight, body length, body position, transfer arousal, fear, touch escape, irritability, vocalization, positional passivity, aggression, spontaneous activity, locomotor activity, skin color

3.3.5 Results

All SHIRPA test parameters were without significant pathological findings. Blood lactate screening showed that both male and female DLG3-knockout mice displayed no significant changes in their blood lactate level as compared to control mice.

Raw data for each individual are available on demand in Excel sheets.

3.3.6 Discussion

No obvious phenotypes and breeding abnormalities were detected so far in DLG3 knockout mice. In our neurological screen, male and female DLG3-knockout mice did not show any significant SHIRPA parameter in comparison to the controls. The DLG3 trapped gene is widely expressed in embryonic tissue and in the placenta. Since homozygous DLG3-knockout mice survive into adulthood without any phenotype, the trapped gene might only influence embryonic tissues. Given that DLG3 is an interaction partner of NMDA receptor subunit NR2B, which is involved in taste learning in the insular cortex, defects in CNS might be possible. Perhaps there is also the possibility that background effects may have influenced results since the mice were only backcrossed to the second generation. In order to confirm a neurological phenotype further neurological/behavioral examinations have to be done in backcrossed mice. We propose screening of the mice with particular emphasis on memory tests (see 3.1.5, Behavior Screen).

3.3.7 References

Irwin S. (1968) Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia*. 13(3): 222-257.

Rogers D. C., E.M. Fisher, S.D. Brown, J. Peters, A.J. Hunter, J.E. Martin (1997) Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm Genome*. 8(10): 711-713.

Abbreviations

SHIRPA **S**mithKline Beecham Pharmazeuticals; **H**arwell, MRC Mouse Genome Centre and Mammalian Genetics Unit; **I**mperial Collegeschool of Medicineat St. Mary`s; **R**oyal London Hospital, **P**henotype **A**ssessment
http://www.mgu.har.mrc.ac.uk/facilities/mutagenesis/mutabase/shirpa_summary.html

s.a. Sub-maxillary area

Table 7: Recording of Body Length and Body WeightData are presented as mean \pm standard error of mean.

Parameter	Male			Female		
	Control (n=9)	Mutant (n=14)	<i>p-value</i>	Control (n=10)	Mutant (n=13)	<i>p-value</i>
Body Length [g]	8.75 ± 0.14	8.6 ± 0.13	<i>n.s.</i>	8.6 ± 0.16	8.5 ± 0.08	<i>n.s.</i>
Body Weight [g]	28.7 ± 0.89	29.5 ± 0.79	<i>n.s.</i>	22 ± 0.69	22.8 ± 0.4	<i>n.s.</i>

Table 8: Behavior Recorded in Viewing JarData shown represents the results of test parameters from major tests where a behavioral response was observed. Test parameters which did not elicit any response were excluded from this data. Statistical analysis: chi-squared test; significance $p < 0.05$

Parameter	Male			Female		
	Control (n=9)	Mutant (n=14)	<i>p-value</i>	Control (n=10)	Mutant (n=13)	<i>p-value</i>
Body Position						
Sitting or standing	9	14	<i>n.s.</i>	10	12	<i>n.s.</i>
Rearing on hind legs	0	0		0	1	
Spontaneous Behavior						
Moderate movement	9	14	<i>n.s.</i>	10	12	<i>n.s.</i>
Rapid movement	0	0		0	1	
Respiration rate						
Normal	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Tremor						
None	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>

Table 9: Recording of Locomotor Activity and Behavior in the Arena

Locomotor activity data are shown as mean (\pm SEM). Data from behavior recorded in the Arena represent the results of test parameters from major tests where a behavioral response was observed. Test parameters, which did not elicit any response, were excluded from this data. Statistical analysis: chi-squared test; significance $p < 0.05$

Parameter	Male			Female		
	Control (n=9)	Mutant (n=14)	<i>p-value</i>	Control (n=10)	Mutant (n=13)	<i>p-value</i>
Locomotor Activity	11.3 \pm 2.2	9.9 \pm 1.9	<i>n.s.</i>	7.3 \pm 2.19	9.5 \pm 2.28	<i>n.s.</i>
Transfer arousal						
Brief freeze, then active movement	0	2	<i>n.s.</i>	1	3	<i>n.s.</i>
No freeze, immediate movement	9	12		9	10	
Palpebral Closure						
Eyes wide open	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Piloerection						
None	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Gait						
Normal	9	14	<i>n.s.</i>	9	12	<i>n.s.</i>
Fluid but abnormal	0	0		1	1	
Pelvic Elevation						
Markedly flattened	1	1	<i>n.s.</i>	0	0	<i>n.s.</i>
Barely touches	2	2		0	0	
Normal	6	11		8	10	
Elevated	0	0		2	3	
Tail Elevation						
Dragging	1	1	<i>n.s.</i>	0	0	<i>n.s.</i>
Horizontally extended	7	13		9	13	
Elevated/Straub tail	1	0		1	0	
Touch Escape						
No response	0	5	<i>n.s.</i>	3	1	<i>n.s.</i>
Mild	5	5		2	5	
Moderate	3	3		4	6	
Vigorous	1	1		1	1	
Positional Passivity						
Struggles when held by tail	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>

Table 10: Behavior Recorded in or above the Arena
 Data shown represent the results of test parameters from major tests where a behavioral response was observed. Test parameters, which did not elicit any response, were excluded from this data. Statistical analysis: chi-squared test; significance $p < 0.05$

Parameter	Male			Female		
	Control (n=9)	Mutant (n=14)	<i>p-value</i>	Control (n=10)	Mutant (n=13)	<i>p-value</i>
Trunk Curl						
Absent	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Present	0	0		0	0	
Limb Grasping						
Absent	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Present	0	0		0	0	
Visual Placing						
Upon vibrassee contact	0	0	<i>n.s.</i>	0	1	<i>n.s.</i>
Before vibrassee contact	7	13		6	9	
Early vigorous	2	1		4	3	
Grip strength						
Moderate grip	9	14	<i>n.s.</i>	0	0	<i>n.s.</i>
Active grip	0	0		10	13	
Body Tone						
Slight resistance	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Extreme resistance	0	0		0	0	
Pinna reflex						
Active retraction	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Corneal Reflex						
Active single eye blink	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Toe Pinch						
None	0	1	<i>n.s.</i>	2	1	<i>n.s.</i>
Slight withdrawal	2	1		1	2	
Moderate withdrawal	0	3		5	1	
Brisk	7	9		2	9	
Wire maneuver						
Active grip			<i>n.s.</i>			<i>n.s.</i>
Difficulty to grasp	0	1		3	2	
Unable to grasp	6	8		6	8	
Unable to lift	3	4		1	2	
Falls immediately	0	1		0	1	

Table 11: Behavior during Supine Restraint

Data shown represent the results of test parameters from major tests where a behavioral response was observed. Test parameters, which did not elicit any response, were excluded from this data. Statistical analysis: chi-squared test; significance $p < 0.05$.

Parameter	Male			Female		
	Control (n=9)	Mutant (n=14)	<i>p-value</i>	Control (n=10)	Mutant (n=13)	<i>p-value</i>
Skin Color						
Pink	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Limb Tone						
No resistance	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Abdominal Tone						
Slight resistance	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Lacrimation						
None	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Salivation						
None	0	0		5	6	
Slight margin of s.a	7	11		3	3	
Wet zone entire of s.a.	2	3	<i>n.s.</i>	2	4	<i>n.s.</i>
Provoked biting						
Absent	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Righting reflex						
No impairment	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Contact righting reflex						
Present	9	14	<i>n.s.</i>	10	13	<i>n.s.</i>
Negative Geotaxis						
Turns and climbs the grid	9	13		9	13	
Moves, but fails to turn	0	0		1	0	
Falls off	0	1	<i>n.s.</i>	0	0	<i>n.s.</i>
Fear						
None	9	14		10	11	
Freezes during transfer	0	0	<i>n.s.</i>	1	2	<i>n.s.</i>
Irritability						
None						
Struggles during supine restraint	8 1	13 1	<i>n.s.</i>	10 0	11 2	<i>n.s.</i>
Aggression						
None	9	14		10	13	
Provoked biting or attack	0	0	<i>n.s.</i>	0	0	<i>n.s.</i>
Vocalization						
None	5	9		5	7	
Provoked during handling	4	5	<i>n.s.</i>	5	6	<i>n.s.</i>

Table 12: Lactate Levels

Data shown represent the results of the mean blood lactate concentrations, value (\pm SEM). Statistical analysis: Chi-squared test; significance $p < 0.05$.

	Male			Female		
	Control (n=9)	Mutant (n=14)	<i>p-value</i>	Control (n=10)	Mutant (n=13)	<i>p-value</i>
Lactate [mmo/l]	6.1 \pm 0.48	5.2 \pm 0.3	<i>n.s.</i>	3.5 \pm 0.33	3.7 \pm 0.34	<i>n.s.</i>

3.4 Eye Screen

3.4.1 Summary

In the Eye Screen, a high throughput electroretinography method (ERG) was employed to examine mice for retinal impairment (Dalke *et al.*, 2004). Furthermore, mice were examined for anterior segment abnormalities by slitlamp biomicroscopy (Favor, 1983).

In humans blindness is caused by several different ocular diseases. Among these, the cataracts are responsible for half of all cases (Johnson and Foster, 2003). The retinal disorders cover a broad variety of clinical symptoms and many different genes are involved in the corresponding pathological conditions in humans. The two most important groups are retinitis pigmentosa (RP) and age-related-macular-degeneration (ARMD; for recent reviews, see Rivolta *et al.*, 2002 and Stone *et al.*, 2001). Mouse models are appropriate tools to understand the genetic and biochemical mechanisms of ocular disorders. There is a rapid increasing number of mouse mutants available suffering from various types of eye diseases (for a recent review see Graw, 2003).

No significant differences between control and mutant DLG3 mice were detected.

3.4.2 Mice

Nineteen DLG3 +/+ (nine male, 10 female) and 27 DLG3 -/- mice (14 male, 13 female) entered the Eye Screen at the age of 11 weeks. Mice were first examined by slit lamp biomicroscopy and on the following day, an ERG was performed. Mice were kept under standard laboratory conditions with food and water *ad libitum*.

3.4.3 Materials and Methods

Electroretinography (ERG) was used to examine the retinal function as described (Dalke *et al.*, 2004). Mice were dark-adapted for at least 12 hours and anaesthetized with 137 mg Ketamine and 6.6 mg Xylazine per kg body weight. After pupil dilation (1 drop Atropine 1%), individual mice were fixed on a sled with Velcro straps. Gold wires (as active electrodes) were placed on the cornea; care was taken not to obstruct the pupillary opening. The ground electrode was a subcutaneous needle in the tail; a reference electrode was placed subcutaneously between the eyes. The mice were introduced into an ESPION ColorBurst Handheld Ganzfeld LED stimulator (Diagnosys LLC, Littleton, MA, USA) on a rail to guide the sled (High-Throughput Mouse-ERG, STZ for Biomedical Optics and Function Testing, Tübingen, Germany). To minimize temperature influences on the ERG, body temperature was kept at 37°C using a warming plate. 10 ms light pulses were delivered at a frequency of 0.48 Hz in two steps at 500 and 12,500 cd/m². Bandpass filter was set ranging from 0.15 to 1000 Hz. Responses were recorded simultaneously from both eyes with an ESPION Console (Diagnosys LLC, Littleton, MA, USA) and stored for offline analysis after averaging 10-40 individual measurements at each step.

Slit Lamp Biomicroscopy: Mice were examined biomicroscopically for eye abnormalities as previously described (Favor, 1983). Briefly, pupils were dilated with a 1% atropine solution applied to the eyes at least 10 min prior to examination. Both eyes of the mice were examined by slit lamp biomicroscopy (Zeiss SLM30) at 48x magnification with a narrow beam slit lamp illumination at 25-30° angle from the direction of observation. Observed phenotypic variants of the eyes were carefully documented.

Statistical Analysis: ERG data were statistically analyzed using MS-Excel. Differences between mouse groups were evaluated with the Student's t-test. Statistical significance was set at $p < 0.05$. Data are presented as mean values \pm standard error of the mean (SEM).

3.4.4 Parameters

Electroretinography (ERG)
a/b-wave, left/right eye at 500/12.50 cd/m ²
Slit lamp biomicroscopy
(qualitative) abnormalities of lens and cornea like opacity and development disorders
Histology
(qualitative) retinal lamination and morphology of cell layers and lens
Morphology
(qualitative) like size and degree of closure

3.4.5 Results

ERG responses were recorded from the groups of DLG3 (control – mutant) mice with light pulses at two different light intensities. These two luminance levels were chosen because at 500 cd/m² a well discernable b-wave amplitude (nearly no a-wave) mainly stemming from the rod system is induced, while light pulses at 12,500 cd/m² induce a maximally developed b-wave response and an a-wave, coming presumably from rods and cones. At first, a comparison of the left and right eyes for each group was performed on the amplitudes of a- and b-wave for both luminance intensities (data not shown). Since slight differences were observed between the left and right eye, ERG amplitudes of both eyes were evaluated separately. The mean value and standard error was calculated for each group of mice, male and female, control and mutant (Table 13).

A total of 46 animals (nine male +/+, 14 male -/-, 10 female +/+, and 13 female -/-) were examined by **slit lamp biomicroscopy**. A number of animals (three -/- males, five +/+ females, 10 -/- females) expressed nuclear-zonular opacities. However, there was no association (Fisher-s exact test, $p = 0.2198$) between genotype and phenotype. We conclude that there are no effects due to mutation of the DLG3 gene.

3.4.6 Discussion

ERG screening is a quick, robust and reproducible *in-vivo* method to detect functional retinal impairment in mice. For the analysis of ERG data, the average amplitudes from left and right eyes was used, as no major differences between the eyes were detected in the ERG response. The comparison of a- and b-wave amplitudes of males and females, mutant and wild type revealed no consistent differences between the groups (Table 13). Most of the p-values (T-test) calculated are not significant (Table 13). The statistical significant values comparing control and mutant males seem to be of no relevance, because no pathologic ERGs were observed and the minor ERG amplitudes were found in the group of wt mice.

Results from slit lamp biomicroscopy indicated that there was no effect on eye phenotype due to loss of function of the DLG3 gene.

3.4.7 References

- Dalke C., J. Löster, H. Fuchs, V. Gailus-Durner, D. Soewarto, J. Favor, A. Neuhäuser-Klaus, W. Pretsch, F. Gekeler, K. Shinoda, E. Zrenner, T. Meitinger, M. Hrabé de Angelis and J. Graw (2004): Electroretinography as a screening method for mutations causing retinal dysfunction in mice. *IOVS* 45: 601-609.
- Favor, J. (1983): A comparison of the dominant cataract and recessive specific-locus mutation rates induced by treatment of male mice with ethylnitrosourea. *Mutation Research* 110: 367-382.
- Graw J. (2003): The genetic and molecular basis of congenital eye defects. *Nat. Rev. Genet.* 4: 876-888.
- Johnson G.J. and A. Foster (2003): Prevalence, incidence and distribution of visual impairment. In: G.J. Johnson, D.C. Minassian, R.A. Weale, S.K. West (eds.): *The epidemiology of the eye disease*. Arnold, London, UK, 2003, 3-28.
- Rivolta C., D. Sharon, M. Hrabé de Angelis and T.P. Dryja (2002): Retinitis pigmentosa and allied diseases: numerous diseases, genes, and inheritance patterns. *Hum. Mol. Genet.* 11: 1219-1227.
- Stone E.M., V.C. Sheffield and G.S. Hageman (2001): Molecular genetics of age-related macular degeneration. *Hum. Mol. Genet.* 10: 2285-2292.

Abbreviations

cd/m ²	candela per square meter
ERG	electroretinography
Hz	hertz
n.s.	not significant

Table 13: Comparison of ERG-Responses at Illumination Levels of 500 and 12,500 cd/m².

Mean ± standard error is calculated for a- and b-wave amplitudes.

Parameter	Control (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=9)	(n=10)	<i>p - value</i>	(n=14)	(n=13)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
a-wave 500 cd/m ²	-5 ± 1.5	-9 ± 2.0	n.s.	-13 ± 1.9	-14 ± 2.1	n.s.	<0,01	n.s.
b-wave 500 cd/m ²	170 ± 9.8	193 ± 10.8	n.s.	171 ± 8.1	184 ± 5.8	n.s.	n.s.	n.s.
a-wave 12500 cd/m ²	-31 ± 4.0	-30 ± 3.9	n.s.	-44 ± 2.5	-39 ± 2.6	n.s.	<0,02	n.s.
b-wave 12500 cd/m ²	241 ± 17.1	232 ± 15.8	n.s.	249 ± 14.4	312 ± 85.3	n.s.	n.s.	n.s.

3.5 Clinical-Chemical Screen

3.5.1 Summary

The aim of the Clinical-Chemical Screen is the detection of hematological changes, defects of various organ systems, and changes in metabolic pathways and electrolyte homeostasis by means of suitable laboratory diagnostic tools. Since most inherited metabolic disorders are known to lead directly or indirectly, via altered organ function, to changes in the parameters investigated, this screening process provides a comprehensive investigation of clinical phenotypes with counterparts in humans and animal species (Rathkolb *et al.*, 2000). The methods used are routine procedures, allowing the appropriate screen of large numbers of mice for a broad spectrum of clinical-chemical and hematological parameters (Champy *et al.*, 2004; Hough *et al.*, 2002).

In the primary clinical chemical screen, nineteen (nine males/10 females) control mice and twenty-seven (14 males /13 females) DLG3 mice were analyzed. Twenty different clinical-chemical parameters were measured including various enzyme activities, as well as plasma concentrations of specific substrates and electrolytes. Additionally, we measured eight basic hematological parameters.

We could not detect any effect of the DLG3 knockout on the clinical-chemical and haematological parameters measured in the primary screen.

3.5.2 Mice

Nine 12-week-old control and fourteen 12-week-old mutant males entered the clinical-chemical screen at the beginning of the 36th calendar week in 2003. Ten 12-week-old control and thirteen 12-week-old mutant females entered the screen one week later.

3.5.3 Materials and Methods

Blood Withdrawal and Storage

The Clinical-chemical Screen of the German Mouse Clinic routinely analyzed 12-week-old mice. A blood sample was taken from an ether-anesthetized mouse by puncturing the retro-orbital sinus with a non-heparinized capillary (0.8 mm in diameter; Laborteam K&K; Munich, Germany; Art.No. 1.28.13.1.2). The time for sample taking was recorded in a work list. Blood was collected in a heparinized tube (Li-heparin, KABE; Nümbrecht, Germany; Art.No. 078028). An additional smaller sample was collected (using the same capillary) in EDTA-coated tubes (KABE, Art.No 078035). The tube was immediately inverted five times to achieve a homogeneous distribution of the anticoagulant.

After removal of 40 µl blood for the Neurology Screen, the Li-heparin-coated tubes were stored in a rack at room temperature for two hours. Afterwards, cells and plasma were separated by a centrifugation step (10 min, 4656 x g; Biofuge, Heraeus; Hanau, Germany). Plasma was distributed between the Immunology Screen (30 µl), the Allergy Screen (30 µl), the Clinical Chemical Screen (130 µl) and the Steroid Screen (residual), while the cell pellet was given to the Immunology Screen for FACS-analysis. The plasma sam-

ple for the clinical chemical analysis was transferred into an Eppendorf tube and diluted 1:2 with aqua dest. The solution was mixed for a few seconds (Vortex genie, Scientific Industries, New York, America) to prevent clotting and then centrifuged again for 10 min at 4656 x g. Additionally the Clinical Chemical Screen received the EDTA-blood sample for hematological investigations.

Clinical Chemistry

The high-throughput of the screen was insured by the use of an Olympus AU 400 autoanalyzer and adapted reagents from Olympus (Hamburg, Germany) and Roche (Mannheim, Germany). In the primary screen, 20 different parameters were measured including various enzyme activities, as well as plasma concentrations of specific substrates and electrolytes.

Hematology

A volume of 50 µl EDTA-blood was used to measure basic hematological parameters with a blood analyzer, which has been carefully validated for the analysis of mouse blood (ABC-Blutbild-Analyzer, Scil Animal Care Company GmbH, Viernheim). Red blood cells, white blood cells, and platelets are measured by electrical impedance, and hemoglobin by spectrophotometry. Mean corpuscular volume (MCV) is calculated directly from the cell volume measurements, the hematocrit (HCT) from $MCV \times \text{red blood cell count}$. Mean corpuscular hemoglobin (MCH) and mean concentration of corpuscular hemoglobin (MCHC) are calculated from hemoglobin/red blood cells count (MCH) and hemoglobin/hematocrit (MCHC).

Analysis of Data

Data were statistically analyzed using Excel and Sigma Stat 2.0 with the level of significance set at $p < 0.05$.

3.5.4 Parameters

Proteins and plasma enzyme activities
Alkaline phosphatase (EC 3.1.3.1), α -Amylase (EC 3.2.1.1), Creatine kinase (EC 2.7.3.2), Aspartate-aminotransferase (AST/GOT; EC 2.6.1.1), Alanine-aminotransferase (ALT/GPT; EC 2.6.1.2), Ferritin, Transferrin, Lipase (EC 3.1.1.3), Total protein
Plasma concentrations of specific substrates
Glucose, Cholesterol, Triglycerides, Uric acid, Urea, Creatinine
Plasma concentrations of electrolytes
Potassium, Sodium, Chloride, Calcium, Inorganic phosphate
Basic hematology
White blood cell count (WBC), Red blood cell count (RBC) Hematocrit (HCT), Hemoglobin (HGB), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), and Platelet count (PLT)

3.5.5 Results

Clinical Chemistry

The values obtained for the clinical chemical parameters were within the normal ranges usually found in mice of this genetic background at the age of three months (unpublished results; Quimby, 1999 and literature cited therein). There were no differences between mutants and control animals (Table 14). In the control animals, we saw sex differences in calcium, urea, cholesterol, triglyceride, amylase, and transferrin concentrations. In the DLG3 mice, we saw sex differences in potassium, calcium, chlorid, total protein, urea, cholesterol, triglyceride, alkaline phosphatase, amylase and in blood glucose concentration.

Hematology

In the primary screen for hematological parameters, we detected sex differences only in the wild-type in the platelet count (Table 15). There were no differences between knockouts and controls.

Raw data for each individual are available on demand in Excel sheets.

3.5.6 Discussion

All parameters of both mutants and knockouts were within the physiological ranges. In the clinical chemical and hematological parameters no differences between knockouts and control animals were detected.

3.5.7 References

- Champy, M.-F., M. Selloum, L. Piard, V. Zeitler, C. Caradec, P. Chambon and J. Auwerx (2004): Mouse functional genomics requires standardization of mouse handling and housing conditions. *Mammalian Genome* 15: 768-783
- Hough T.A., P. Nolan, V. Tsipouri, A.. Toye, I. Gray, M. Goldsworthy, L. Moir, R. Cox, S. Clements, P. Glenister, J. Wood, R. Selley, M. Strivens, L. Vizor, S. McCormack, J. Peters, E. Fisher, N. Spurr, S. Rastan, J. Martin, S. Brown and A. Hunter (2002): Novel phenotypes identified by plasma biochemical screening in the mouse. *Mammalian Genome* 13: 595-602
- Quimby, F. (1999) The Mouse. In: *The clinical chemistry of laboratory animals*, ed. by W. F. Loeb and F. W. Quimby. Taylor and Francis, New York, pp. 3-31
- Rathkolb B., T. Decker, H. Fuchs, D. Soewarto, C. Fella, S. Heffner, W. Pargent, R. Wanke, R. Balling, M. Hrabe de Angelis, H. J. Kolb and E. Wolf (2000): The clinical-chemical screen in the Munich ENU Mouse Mutagenesis Project: screening for clinically relevant phenotypes. *Mammalian Genome* 11: 543-546
- Suckow, M.A., P. Dannemann and C. Brayton (2001) *The laboratory mouse* CRC Press, Boca Raton, London, New York, Washington

Table 14: Clinical-Chemical Parameters.Data are presented as mean \pm standard error of mean.

Parameter	Mutant (A)			Control (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=14)	(n=13)	p- value	(n=9)	(n=10)	p-value	p-value	p-value
Sodium [mmol/l]	153 \pm 0.68	152 \pm 0.42	n.s.	153 \pm 0.68	152 \pm 0.73	n.s.	n.s.	n.s.
Potassium [mmol/l]	4.1 \pm 0.08	3.6 \pm 0.07	<0.001	4.0 \pm 0.10	4.4 \pm 0.85	n.s.	n.s.	n.s.
Calcium [mmol/l]	2.3 \pm 0.03	2.1 \pm 0.03	<0.001	2.3 \pm 0.03	1.8 \pm 0.21	<0.05	n.s.	n.s.
Chloride [mmol/l]	108.9 \pm 0.52	111.2 \pm 0.33	<0.001	109.2 \pm 0.66	110.2 \pm 0.55	n.s.	n.s.	n.s.
Inorganic Phosphate [mmol/l]	1.5 \pm 0.06	1.4 \pm 0.10	n.s.	1.5 \pm 0.14	1.4 \pm 0.09	n.s.	n.s.	n.s.
Total Protein [g/dl]	5.0 \pm 0.04	5.1 \pm 0.03	<0.01	4.9 \pm 0.08	5.1 \pm 0.07	n.s.	n.s.	n.s.
Creatinine [mg/dl]	0.337 \pm 0.01	0.346 \pm 0.01	n.s.	0.356 \pm 0.01	0.338 \pm 0.00	n.s.	n.s.	n.s.
Urea [mg/dl]	78.1 \pm 2.4	56.5 \pm 2.33	<0.001	84.1 \pm 4.65	55.8 \pm 2.9	<0.001	n.s.	n.s.
Uric acid [mg/dl]	1.3 \pm 0.20	1.4 \pm 0.17	n.s.	1.4 \pm 0.23	1.4 \pm 0.30	n.s.	n.s.	n.s.
Cholesterol [mg/dl]	141.3 \pm 8.6	89.1 \pm 5.65	<0.001	132.9 \pm 7.43	97.4 \pm 6.3	<0.01	n.s.	n.s.
Triglyceride [mg/dl]	235.8 \pm 13.90	138.2 \pm 11.62	<0.001	218.6 \pm 11.26	138.6 \pm 7.6	<0.001	n.s.	n.s.
Creatine Kinase [U/l]	51 \pm 11.00	38 \pm 10.16	n.s.	52 \pm 12.56	26 \pm 5.00	n.s.	n.s.	n.s.
Alanine-Amino-transferase [ALAT,GPT] [U/l]	19 \pm 2.00	16 \pm 1.41	n.s.	17 \pm 0.94	15 \pm 1.00	n.s.	n.s.	n.s.
Aspartate-Aminotransferase (AST,GOT) U/l	22 \pm 1.00	24 \pm 1.26	n.s.	22 \pm 1.80	24 \pm 1.00	n.s.	n.s.	n.s.
Alkaline Phosphatase [U/l]	110 \pm 5.00	151 \pm 10.87	<0.01	109 \pm 4.31	136 \pm 17	n.s.	n.s.	n.s.
α -Amylase [U/l]	3166 \pm 162	2217 \pm 122.54	<0.001	3092 \pm 131	2505 \pm 170	<0.02	n.s.	n.s.
Glucose [mg/dl]	195.6 \pm 5.80	169 \pm 5.91	<0.001	189.8 \pm 6.78	172.1 \pm 6.4	n.s.	n.s.	n.s.
Ferritin [ng/ml]	19.3 \pm 1.00	41.5 \pm 16.02	n.s.	20.3 \pm 0.50	18.4 \pm 1.7	n.s.	n.s.	n.s.
Transferrin [mg/dl]	148.7 \pm 2.40	155.2 \pm 3.10	n.s.	142.6 \pm 2.03	152.2 \pm 2.8	<0.02	n.s.	n.s.

Table 15: Hematological Parameters.Data are presented as mean \pm standard error of mean.

Parameter	Mutant (A)			Control (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=14)	(n=13)	<i>p</i> - value	(n=9)	(n=10)	<i>p</i> - value	<i>p</i> - value	<i>p</i> - value
White blood cell count [10 ³ /μl]	6.78 ±0.29	7.29 ±0.45	n.s.	6.47 ±0.38	7.73 ±0.80	n.s.	n.s.	n.s.
Red blood cell count [10 ³ /μl]	10.51 ±0.24	10.18 ±0.20	n.s.	10.10 ±0.09	10.13 ±0.07	n.s.	n.s.	n.s.
Hemoglobin [g/dl]	16.12 ±0.17	15.86 ±0.26	n.s.	15.85 ±0.10	15.87 ±0.25	n.s.	n.s.	n.s.
Hematocrit [%]	50 ±0.97	48 ±0.92	n.s.	48 ±0.42	48 ±0.56	n.s.	n.s.	n.s.
Mean corpuscular volume [fl]	47.50 ±0.49	47.08 ±0.46	n.s.	47.89 ±0.56	47.40 ±0.52	n.s.	n.s.	n.s.
Mean corpuscular hemoglobin [pg]	15.40 ±0.25	15.60 ±0.14	n.s.	15.69 ±0.16	15.67 ±0.24	n.s.	n.s.	n.s.
Mean corpuscular hemoglobin concentration [g/dl]	32.44 ±0.44	33.10 ±0.18	n.s.	32.81 ±0.31	33.10 ±0.32	n.s.	n.s.	n.s.
Platelet count [10 ³ /μl]	731 ±47.84	623 ±29.55	n.s.	735 ±35.94	617 ±26.42	<0.02	n.s.	n.s.

3.6 Immunology Screen

3.6.1 Summary

Mouse models have been a primary source of information for understanding the intricate mechanisms of the immune system (Bluethmann and Ohashi, 1994; Mak *et al.*, 2001; Fischer 2002; Rogner and Avner, 2003). The Immunology Screen at the GMC was set up to conduct a broad immunological phenotyping of mouse mutant lines with the intention of identifying distinct gene functions, which play key roles in the immune defenses of the organism through a complex network of cellular and soluble components (Janeway *et al.*, 2004).

According to the data summary presented to the GMC, no immunological phenotype was known in DLG3-deficient mice. Their analysis in the Immunology Screen revealed only slight differences between knockouts and their littermate controls.

3.6.2 Mice

We analyzed 27 KO animals (13 females and 14 males) and the 19 age- and sex-matched littermate controls (10 females and 9 males).

3.6.3 Material and Methods

Peripheral blood leukocytes (PBLs) were isolated from 500 μ l blood by erythrocyte lysis with NH_4Cl (0.17M) - Tris buffer (pH 7.45) directly in 96-well microtiter plates. After subsequent washing with FACS staining buffer (PBS, 0.5% BSA, 0.02% sodium azide, pH 7.45), PBLs were incubated for 20 min with 1 μ M ethidium monazide bromide (EMA, Molecular Probes, The Netherlands) and Fc block (clone 2.4G2, PharMingen, San Diego, USA). EMA bound to the DNA of dead cells was photocrosslinked by brief light exposure. Cells were then stained with fluorescence-conjugated monoclonal antibodies (PharMingen).

The following main cell populations were analyzed: B cells (CD19⁺ clone 1D3), B1 B cells (CD19⁺CD5⁺, clone 53-7.3), B2 B cells (CD19⁺CD5⁻), T cells (CD3⁺, clone 145-2C11), CD4⁺ T cells (clone RM4-5), CD8⁺ T cells (CD8 α , clone 53-6.7; CD8 β , clone H35-17.2), γ/δ T cells (clone GL3), granulocytes (Gr-1⁺, clone RB6-8C5), and NK cells (CD49b⁺, clone DX5). We also analyzed additional subpopulations based on the following surface antigens: IgD (clone 11-26c.2a), B220 (clone RA3-6B2), CD11b (clone M1/70), CD103 (clone 2E7), CD25 (clone PC61), CD62L (clone MEL-14), CD45RA (clone 14.8), Ly-6C (clone AL-21), and CD44 (clone IM7). Data were acquired on a FACS Calibur (Becton Dickinson, San Diego, USA) and were analyzed using FlowJo software (TreeStar Inc, USA). All samples were acquired until a total number of 25,000 cells was reached.

The plasma levels of IgM, IgG₁, IgG_{2a}, IgG_{2b}, IgG₃, and IgA were determined by standard sandwich ELISAs using goat anti-mouse immunoglobulin antibodies and alkaline phosphatase (AP) conjugates (SouthernBiotech, Birmingham, USA). The presence of rheumatoid factor and anti-DNA antibodies

was evaluated by indirect ELISA with rabbit IgG (Sigma-Aldrich, Steinheim, Germany) and calf thymus DNA (Sigma-Aldrich), respectively, as antigens and AP-conjugated goat anti-mouse secondary antibody (Sigma-Aldrich). Serum samples from MRL/MpJ-Tnfrsf6^{lpr} mice (Jackson Laboratory, Bar Harbor, USA) were used as positive controls in the autoantibody assays.

3.6.4 Parameters

Flow cytometry
B cells (CD19 ⁺), B1 B cells (CD19 ⁺ CD5 ⁺), B2 B cells (CD19 ⁺ CD5 ⁻), T cells (CD3 ⁺), CD4 ⁺ T cells, CD8 ⁺ T cells, γ/δ T cells, granulocytes (Gr-1 ⁺), and NK cells (CD49b ⁺). Furthermore, all potential subpopulations which can be identified by co-staining for other surface markers (IgD, B220, CD11b, MHC II, I-A ^K , CD25, CD8 β , CD62L, CD45RA, Ly-6C, CD44) using 6 parameter/5 color flow cytometry were analyzed.
ELISA
IgM, IgG ₁ , IgG _{2a} , IgG _{2b} , IgG ₃ , IgA; anti-DNA antibodies, rheumatoid factor

3.6.5 Results

The analysis of standard immunological parameters measured in the primary screen (Table 16) revealed no significant differences between DLG3 ^{-/-} mice and their littermate controls. The only difference detectable in DLG3 ^{-/-} mice was a slight decrease in the IgG₁ level in KO females. Besides this finding, no immunological abnormalities could be detected in peripheral blood with respect to other soluble factors or cellular components measured in the screen.

3.6.6 Discussion

Under standard screening conditions, DLG3 ^{-/-} mice showed no distinct immunological phenotype when compared with their littermate controls. With the exception of the lower IgG₁ plasma level in KO females, no indications of immunological defects could be found. Although one cannot exclude that this dissimilarity is caused by the knockout, it can also be due to the low number of backcrosses or to natural variation of IgG₁ concentration between the analyzed groups of mice. Taking into account the results of the primary screen, a more detailed immunological analysis of DLG3 ^{-/-} mice does not seem very promising.

Raw data will be available on demand.

3.6.7 References

Bluethmann, H., and P. S. Ohashi (Eds.) (1994): Transgenesis and targeted mutagenesis in immunology. Academic Press, San Diego.

Fischer, A. (2002): Natural mutants of the immune system: a lot to learn! *Eur J Immunol* 32: 1519-1523.

Janeway C, Travers P, Walport M, Shlomchik M and M.J. Shlomchik (2004) *Immunobiology: The Immune System in Health and Disease*. 6th edition, Garland Publishing, London.

Mak, T. W., J. M. Penninger and P. S. Ohashi (2001): Knockout mice: a paradigm shift in modern immunology. *Nat Rev Immunol* 1: 11-19.

Rogner, U. C., and P. Avner (2003): Congenic mice: cutting tools for complex immune disorders. *Nat Rev Immunol* 3: 243-252.

Table 16: Basic Parameters Analyzed in the Immunology Screen.Data are presented as mean \pm standard error of mean.

Parameter	Mutants (A)			Control (B)			A - B	
	Male	Female	<i>p</i> - value	Male	Female	<i>p</i> - value	Male	Female
	(n=14)	(n=13)		(n=9)	(n=10)		<i>p</i> - value	<i>p</i> - value
CD19 ⁺ [%]	37.6 \pm 3.7	24.7 \pm 6	<0.01	33.8 \pm 3.5	23.5 \pm 4.4	n.s.	n.s.	n.s.
CD19 ⁺ CD5 ⁻ [%]	94.9 \pm 3.2	92.8 \pm 1.4	n.s.	95.2 \pm 2.2	91.1 \pm 4	n.s.	n.s.	n.s.
CD19 ⁺ CD5 ⁺ [%]	5.1 \pm 3.2	7.2 \pm 1.4	n.s.	4.7 \pm 2.2	8.9 \pm 4	n.s.	n.s.	n.s.
CD3 ⁺ [%]	29.5 \pm 7.2	33.6 \pm 4.7	n.s.	27.7 \pm 11.2	35.8 \pm 7	n.s.	n.s.	n.s.
γ/δ TCR ⁺ [%]	6.1 \pm 2.7	0.7 \pm 0.3	<0.01	7.2 \pm 3.8	1 \pm 0.4	<0.01	n.s.	n.s.
Gr-1 ⁺ [%]	22.4 \pm 6.2	24.5 \pm 6.6	n.s.	23.5 \pm 7.2	25.8 \pm 5.5	n.s.	n.s.	n.s.
CD49b ⁺ [%]	20.8 \pm 9	33.1 \pm 6.1	<0.01	19.7 \pm 7.6	31.7 \pm 4.5	<0.001	n.s.	n.s.
CD4 ⁺ [%]	14.8 \pm 4.8	21.2 \pm 6.5	<0.02	18.6 \pm 5.4	25.9 \pm 5.6	<0.01	n.s.	n.s.
CD8 β ⁺ [%]	9.5 \pm 2.1	10.7 \pm 2.1	n.s.	11.6 \pm 1.6	12.4 \pm 1.8	n.s.	n.s.	n.s.
IgG ₁ [μ g/ml]	83.5 \pm 34.3	206.5 \pm 18.4	<0.01	99.2 \pm 65.1	289.1 \pm 21.4	<0.05	n.s.	<0.05
IgG _{2a} [μ g/ml]	263.3 \pm 93.3	459.6 \pm 61.1	n.s.	171.8 \pm 60.8	383.2	NA	n.s.	NA
IgG _{2b} [μ g/ml]	103.8 \pm 34	219 \pm 46.8	n.s.	121.5 \pm 33.3	303.4 \pm 55.2	<0.02	n.s.	n.s.
IgG ₃ [μ g/ml]	560.8 \pm 115	1723 \pm 165	<0.001	450.1 \pm 201	391.8	NA	n.s.	NA
IgM [μ g/ml]	209.4 \pm 54	151.9 \pm 20.6	n.s.	321.7 \pm 154	170.9 \pm 39.1	n.s.	n.s.	n.s.
IgA [μ g/ml]	58.9 \pm 13.4	55.2 \pm 4.9	n.s.	55.7 \pm 21.1	66.1 \pm 15.7	n.s.	n.s.	n.s.
Anti-DNA Ab [%]	0	0	n.s.	0	0	n.s.	n.s.	n.s.
Rheumatoid factor [%]	0	0	n.s.	0	0	n.s.	n.s.	n.s.

Raw data will be available on demand.

3.7 Allergy Screen

3.7.1 Summary

The goal of the Allergy screen within the German Mouse Clinic (GMC) is to search for IgE mutants in order to establish mouse models for allergic diseases and to find new strategies for antiallergic therapy. The increased production of IgE in response to common environmental antigens is the hallmark of atopic diseases in man (Hamelmann *et al.* 1999). Mouse mutants with phenotypic alterations in IgE production represent a valuable tool to study and characterize the molecular mechanisms of IgE-mediated allergic hypersensitivity (Zhang *et al.* 1997).

In the primary Allergy screen, 19 control and 27 DLG3 knockout mice were screened. Their analysis did not reveal any profound difference between knockout and control mice.

3.7.2 Mice

An age- and sex-matched group of 19 control (10 females, 9 males) and 27 knockout (13 females, 14 males) mice aged 12 weeks was analyzed in Allergy screen.

3.7.3 Material and Methods

Twelve-week-old male and female mice were screened for alterations in plasma total IgE concentrations. Blood samples were taken from animals by puncturing the retroorbital plexus under ether anesthesia. Plasma IgE concentrations were measured by isotype-specific sandwich ELISA technique with a lower detection limit of 1 ng/ml. briefly, microtiter plates were coated with the IgG fraction of sheep anti-mouse IgE in sodium bicarbonate buffer (pH 9.6). After incubation, plates were washed with Tris buffer (pH 7.4) and blocked with 3% (w/v) bovine serum albumin at room temperature. Diluted plasma samples and standard were added to the plates. After overnight incubation biotinylated rat anti-mouse IgE was added and plates were incubated at room temperature for 2 h. Then plates were incubated in the presence of peroxidase-labeled streptavidin. After washing, tetramethylbenzidine (TMB) substrate solution was added and after an appropriate incubation time the stop solution (sulphuric acid, 2M) was added. The plates were read in a standard microplate reader at a wavelength of 450 nm. Total murine IgE data are reported in ng/ml, based on a standard curve of purified murine IgE (Alessandrini *et al.*, 2001).

3.7.4 Results

The analysis of total IgE levels in plasma of DLG3-KO mice and their sex- and age-matched littermate controls revealed higher mean IgE concentrations of male animals (mutants and controls). This sex-difference was statistically significant (Table 17). Raw data will be available on demand.

Table 17: Total plasma IgE of DLG3 miceData are presented as mean \pm standard error of mean.

	Control (A)			Mutant (B)			A~B	A~B
	Female	Male		Female	Male		Female	Male
	(n=10)	(n=9)	<i>p</i> - value	(n=13)	(n=14)	<i>p</i> - value	<i>p</i> - value	<i>p</i> - value
Total IgE [ng/ml]	98 \pm 16.4	293 \pm 65	<0.01	68 \pm 13.1	316 \pm 126.2	0.07	n.s.	n.s.

3.7.5 Discussion

No statistically significant difference between *DLG3*-knockout and control mice was found. In both *DLG3*-knockout and control animals, the mean concentration of total IgE in males was higher than in females.

The mean concentration of total plasma IgE of both *DLG3*-knockout and control females was close to the normal range for total IgE in C57BL/6 mice established in our laboratory (females 87.6 \pm 20.12 ng/ml vs. males 30.3 \pm 4.41 ng/ml, unpublished results). The mean concentration of total IgE in *DLG3*-knockout and control males was higher than our baseline data, but *DLG3* animals have been backcrossed just two generations to C57BL/6, therefore they do not have a pure background.

Taken together, under standard screening conditions for primary Allergy screen, *DLG3*-knockout mice did not show changes in total plasma IgE levels that would reveal a major allergy phenotype.

3.7.6 References

- Alessandrini, F., Jakob, T., Wolf, A., Wolf, E., Balling, R., Hrabé de Angelis, M., Ring, J., Behrendt, H. (2001): ENU mouse mutagenesis: Generation of mouse mutants with aberrant plasma IgE levels. *Int Arch Allergy Immunol.* 124: 25-28
- Hamelmann, E., K. Takeda, A. Oshiba and E.W. Gelfand (1999): Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness – a murine model. *Allergy* 54: 297-305
- Zhang, Y., W.J.E. Lamm, R.K. Albert, E.Y. Chi, W.R.Henderson and D.B. Lewis (1997) Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. *Am J Respir Crit Care Med.* 155: 661-669

3.8 Nociceptive Screen

3.8.1 Summary

Pain is the perception of an aversive or unpleasant sensation that originates from a specific region of the body. The highly subjective nature of pain is one of the factors that make it difficult to define and to treat clinically. Pain is more than a conspicuous sensory experience that warns of danger.

Nociceptors are activated by tissue injury but also by mechanical, thermal, or chemical stimuli. Harmful stimuli applied to the skin or to subcutaneous tissue, activate nociceptors, the peripheral endings of primary sensory neurons whose cell bodies are located in the dorsal root or in the trigeminal ganglia.

A noxious stimulus activates the nociceptor by depolarizing the membrane of the sensory ending. When peripheral tissues are damaged, the sensation of pain in response to subsequent stimuli is enhanced. This phenomenon termed hyperalgesia, may involve a lowering of threshold of the nociceptors or an increase in the magnitude of pain evoked by suprathreshold stimuli. Hyperalgesia can occur both at the site of tissue damage (primary hyperalgesia) and in the surrounding undamaged areas (secondary hyperalgesia; Wall and Melzak, 1984). By means of different inbred mouse strains it could be demonstrated that rodents display large and heritable differences in both nociceptive and analgesic sensitivity (Mogil, 1999; Mogil *et al.*, 1999)

In the first screen we tested the responsiveness of the intact somatosensory system to thermal pain of the DLG3 mouse mutant line by means of the hot plate test. We found no significant difference in pain reactivity between the knockout animals and their littermate controls as well as between the sexes.

3.8.2 Mice

Twenty-seven DLG3 knockout mice (14 male, 13 female), and 19 control animals (nine male, 10 female) were tested in our first screen.

3.8.3 Material and Methods

Hot plate test

The mice were placed on a metal surface maintained at $52 \pm 0.2^\circ\text{C}$ (Hot plate system was made by TSE GMBH, Germany; Eddy and Leimbach, 1953). Locomotion of the mouse on the hot plate was constrained by 20 cm high plexiglas wall to a circular area with a diameter of 28 cm. Mice remained on the plate until they performed one of three behaviors regarded as indicative of nociception: hind paw lick (h.p. licking), hind paw shake/flutter (h.p. shaking) or jumping.

We evaluated only hind paw but not the front paw responses, because fore paw licking and lifting are components of normal grooming behavior. Each mouse was tested only once since repeated testing leads to profound changes in response latencies. The latency was recorded to the nearest 0.1 s. To avoid tissue injury 60 s cut-off time was used. The data values are given in seconds.

Statistical analysis

Statistical analysis was performed using a statistical package Statgraphics® (Statistical Graphics Corporation, Rockville, MD). The differences between the groups were compared with ANOVA, LSD test was used as *post hoc*. Statistical significance was assumed at $p < 0.05$.

3.8.4 Parameters

Hind paw licking
Reaction with licking of hind paw to the thermal pain
Hind paw shaking
Reaction with shaking of hind paw to the thermal pain
Jumping
Jumping reaction to the thermal pain

3.8.5 Results and Discussion

The results are shown in Table 18. The first nociceptive response observed in mice is hind paw shaking, the response latencies did not differ significantly between the sexes and genotypes. Both genotypes showed hind paw licking, another typical nociceptive response. This behavior was observed in all groups with comparable latencies except for male controls, which displayed a slightly delayed behavior. The third examined response was the jumping of the animals.

We observed a sex-related difference in hind paw licking only, the latency was longest in male control mice. Both sexes reacted the same way to thermal pain considering the other two signs of pain.

To sum up, we could not find any association between genotype or gender and pain response. Therefore we do not plan to perform further pain related studies in this mouse mutant line.

Raw data will be available on demand.

Table 18: Nociceptive Screen								
Data are presented as mean \pm standard error of mean.								
Parameter	Mutant (A)			Control (B)			A~B	A~B
	Fe-male	Male		Female	Male		Fe-male	Male
	(n=13)	(n=14)	<i>p</i> -value	(n=10)	(n=9)	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value
H.p. licking	14.4 \pm 2.1	15.5 \pm 2.0	n.s.	15.6 \pm 2.4	23.8 \pm 2.5	<0.03	n.s.	<0.01
H.p. shaking	12.3 \pm 1.6	13.2 \pm 1.5	n.s.	15.0 \pm 1.8	16.7 \pm 1.9	n.s.	n.s.	n.s.
Jumping	58.4 \pm 1.4	59.8 \pm 1.4	n.s.	58.5 \pm 1.6	57.4 \pm 1.7	n.s.	n.s.	n.s.

3.8.6 References

Eddy, N.B. Leimbach, D. (1953): Synthetic analgesics II. Diethienylbutenyl – and dithienylbutylamines. J. Pharmacol. Exp. Ther. 107: 385-393

Mogil J.S. (1999): The genetic mediation of individual differences in sensitivity to pain and its inhibition. Proc. Nat. Acad. Sci. 96: 7744-7751

Mogil J.S., S.G. Wilson, K. Bon, S.E. Lee, K. Chung, P. Raber, J.O. Pieper, H.S. Hain, J.K. Belknap, L. Hubert, G.I. Elmerl, J.M. Chung and M. Devor (1999): Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. Pain. 80:67-82.

Wall P.D. and R. Melzack (Eds.) Textbook of Pain, Churchill Livingstone, London, 1984

Abbreviations

h.p. hind paw

3.9 Lung Function Screen

3.9.1 Summary

Neural and mechanical processes that control breathing frequency have been investigated in man for a long time (Mead, 1960; Otis *et al.*, 1959), but only with the availability of mouse inbred strains the contribution of genetic determinants to differential baseline breathing patterns could be elucidated (Tankersley *et al.*, 1997; Tankersley, 1999). By use of genetically engineered mice, candidate genes for human developmental disorders of breathing have been identified (Katz, 2003).

Spontaneous breathing patterns during rest and activity were studied in 15-week-old male and female DLG3-deficient and wild-type mice. Concerning the absolute parameters of the spontaneous breathing pattern, statistically relevant sex differences were not pronounced in control mice, neither in mutants with one exception: male mice showed higher mean breathing rates than females in both wild type and mutants, which could be related to a higher activity level in males. Sex differences for the specific parameters sTV and sMV, which are calculated by use of body weight were seen in the mutants with significantly higher values in females.

Comparing mutant to control mice, significant differences occurred among the males. Wild type male mice showed a higher breathing rate than mutant male mice during activity phase and higher values for sTV and sMV during both rest and activity phase. The differences in the spontaneous breathing pattern seem to be secondary effects due to the significantly higher body weight in the male mutants.

3.9.2 Mice

Mutant and control mice of both sexes were studied at the age of 15 weeks (Table 19). Body weights of female mice were comparable between wild types and mutants (23.0 ± 1.0 vs. 23.6 ± 0.9). Among males, the controls exhibited significantly lower body weights than the mutants (26.5 ± 1.5 vs. 32.2 ± 1.2).

3.9.3 Material and Methods

Whole Body Plethysmography

A commercially available system from Buxco[®] Electronics (Sharon, Connecticut) was used to assess breathing patterns in unrestrained animals according to the principle described by Drorbaugh and Fenn (1955). It measures the pressure changes which arise from inspiratory and expiratory temperature and humidity fluctuations during breathing (Figs. 3 and 4).

Calibration of the system allows to transform these pressure swings into flow and volume signals so that automated data analysis provides tidal volumes (TV), respiratory rates (f), minute ventilation (MV), inspiratory and expiratory times (Ti, Te), as well as peak inspiratory and peak expiratory flow rates (PIF, PEF). These data were stored online as mean values at 10 s intervals.



Figure 3: System used at GMC to assess breathing patterns.

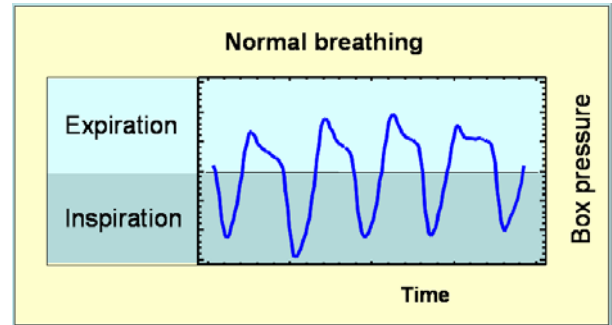


Figure 4: Recorded data used to calculate the breathing parameters.

Measurements were always performed between 8 a.m. and 11 a.m. to account for potential diurnal variations in breathing. The system was set up in a quiet room where temperature and humidity were kept constant throughout the measurements. Before each measurement, the system was calibrated and the actual barometric pressure, temperature, and humidity were supplied to warrant adequate calculations of flow rates and volumes. After placing the animals into the chamber, data recording was immediately started and was continued for 40 min. Mice underwent typical phases during the measuring period. Primarily, the animals were stressed so that the respiratory rate was highest at the beginning. Usually after 5 min. the animals became calmer, they slightly reduced their respiratory rate, and began to explore the chamber and start cleaning themselves – *phase of activity*. Later activity was more and more interrupted by phases of rest or even short periods of snoozing – *resting phase*. Some of the animals even went to *phases of sleep*, which resulted in a further marked decrease in respiratory rate. The frequency histogram of the respiratory rates was determined for each individual, and breathing was analyzed for the above mentioned parameters during the phases of activity and rest. In addition to the directly recorded parameters, mean inspiratory and expiratory flow rates (MEF, MIF) were calculated offline from the ratio of tidal volume and the respective time interval. The relative duration of inspiration (T_i/TT) was determined from the ratio of inspiratory time to total time required for the breathing cycle. Specific tidal volumes and minute ventilations (sTV, sMV) were calculated by relating the absolute values to the body weight of the

animal. Furthermore, the mean of all breathing frequencies (mean_f) measured during the 40-minute-period was calculated as a rough and ready parameter to assess whether the duration of rest and activity was similar in all mouse strains.

Statistical Analysis of Data

Statistical analyses were performed using a commercially available statistics package (Statgraphics®, Statistical Graphics Corporation, Rockville, MD). Differences between strains were evaluated by Students t-test. Statistical significance was assumed at $p < 0.05$. Data are presented as mean values \pm standard error of the mean (SEM).

3.9.4 Parameters

Directly recorded data
Tidal volumes (TV), respiratory rates (f), minute ventilation (MV), inspiratory and expiratory times (Ti, Te), as well as peak inspiratory and peak expiratory flow rates (PIF, PEF).
Calculated data
mean inspiratory flow rates (MEF), expiratory flow rates (MIF), relative duration of inspiration (Ti/TT), specific tidal volumes (sTV), minute ventilations (sMV), mean of all breathing frequencies (mean_f)

3.9.5 Results and Discussion

Tables 20 and 21 summarize the results obtained for spontaneous breathing under resting and active conditions. Raw data are available on demand.

Sex related differences: In both control and mutant mice, males exhibited significantly higher mean breathing rates than females. The mean breathing rate calculated over the whole period of measurement during rest and activity can be regarded carefully as a rough parameter for the activity level. Thus, the results seem to show that the males are more active than the females, but this has to be investigated much more closer by use of behavior tests.

Further sex differences in the spontaneous breathing pattern are only found in mutants. The female mutant mice showed significantly higher values for the specific tidal volume (sTV) and the specific minute ventilation (sMV) than the male mutants. Since these specific parameters take the body weight into account and the absolute values primarily are not affected, the observed sex differences are likely due to the significantly higher body weight in mutant males compared to mutant females.

Differences between wild-type and DLG3 mice: Among females, all parameters were comparable between wild-type and mutant mice. The spontaneous breathing pattern differed significantly only between the male animals of wild type and mutant. The male controls showed significantly higher breathing rates than the mutants during the activity phase and higher values for sTV and sMV in both rest and activity phase. These differences mainly reflect the significantly higher body weights of the mutant males and may not be due to

primary alterations in lung function. Further investigations of the male mutants would be required to reveal possible metabolic disorders that lead to an elevated body mass.

3.9.6 References

Drorbaugh J.E. and W.O. Fenn (1955): A barometric method for measuring ventilation in newborn infants. *Pediatrics* 16: 81-87

Katz D.M. (2003): Neuronal growth factors and development of respiratory control. *Respir. Physiol. Neurobiol.* 135: 155-165

Mead, J. (1960): Control of respiratory frequency. *J. Appl. Physiol.* 15: 325-336

Otis, A.B., W.O. Fenn and H. Rahn (1950): Mechanics of breathing in man. *J. Appl. Physiol.* 2: 592-607

Tankersley, C.G. (1999): Genetic control of ventilation: What are we learning from murine models? *Current Opinion in Pulmonary Medicine* 5: 344-348

Tankersley, C.G., Fitzgerald R.S., Levitt R.C., Mitzner W.A., Ewart S.L. and S.R. Kleeberger (1997): Genetic control of differential baseline breathing pattern. *J. Appl. Physiol.* 82: 874-81

Abbreviations

bw	body weight (g)
mean_f	mean of all respiratory rates (1/min)
f	respiratory rate (1/min)
TV	tidal volume (ml)
sTV	specific tidal volume ($\mu\text{l/g}$)
MV	minute ventilation (ml/min)
sMV	specific ventilation (ml/min/g)
Ti	inspiratory time (ms)
Te	expiratory time (ms)
Ti/TT	relative duration of inspiration
PIF	peak inspiratory flow rate (ml/s)
PEF	peak expiratory flow rate (ml/s)
MIF	mean inspiratory flow rate (ml/s)
MEF	mean expiratory flow rate (ml/s).

Table 19: Characterization of Studied Mice

Data are presented as mean \pm standard error of mean.

Parameter	Control (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=2)	(n=3)	<i>p - value</i>	(n=5)	(n=5)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
bw	26.5 \pm 1.5	23.0 \pm 1.0	n.s.	32.2 \pm 1.2	23.6 \pm 0.9	<0.001	<0.05	n.s.
mean_f	489.5 \pm 45.5	344.3 \pm 10.5	<0.05	421.1 \pm 19.5	355.6 \pm 7.7	<0.02	n.s.	n.s.

Table 20: Spontaneous Breathing Pattern during Rest

Data are presented as mean \pm standard error of mean.

Parameter	Control (A)			Mutant (B)			A~B	A~B
	Male (n=2)	Female (n=3)	<i>p</i> - value	Male (n=5)	Female (n=5)	<i>p</i> - value	Male <i>p</i> - value	Female <i>p</i> - value
Rest								
f	402.6 \pm 27.1	346.6 \pm 4.4	0.0754	357.0 \pm 11.5	347.2 \pm 3.7	n.s.	0.1152	n.s.
TV	0.26 \pm 0.007	0.26 \pm 0.01	n.s.	0.26 \pm 0.01	0.24 \pm 0.007	n.s.	n.s.	n.s.
sTV	9.8 \pm 0.3	11.4 \pm 0.06	n.s.	8.2 \pm 0.2	10.3 \pm 0.5	<0.01	< 0.001	n.s.
MV	104.3 \pm 11.3	87.9 \pm 4.7	n.s.	91.6 \pm 7.3	82.0 \pm 3.3	n.s.	n.s.	n.s.
sMV	3.9 \pm 0.2	3.8 \pm 0.04	n.s.	2.8 \pm 0.1	3.5 \pm 0.2	< 0.05	<0.01	n.s.
Ti	51.8 \pm 1.7	53.7 \pm 1.1	n.s.	56.5 \pm 1.8	56.3 \pm 1.4	n.s.	n.s.	n.s.
Te	97.9 \pm 8.4	119.5 \pm 2.8	0.0573	112.3 \pm 4.0	116.6 \pm 2.8	n.s.	n.s.	n.s.
Ti/TT	0.35 \pm 0.01	0.31 \pm 0.008	n.s.	0.34 \pm 0.007	0.33 \pm 0.01	n.s.	n.s.	n.s.
PIF	8.4 \pm 0.4	8.3 \pm 0.5	n.s.	7.9 \pm 0.6	7.3 \pm 0.3	n.s.	n.s.	n.s.
PEF	5.6 \pm 0.6	5.6 \pm 0.9	n.s.	5.5 \pm 0.5	4.7 \pm 0.4	n.s.	n.s.	n.s.
MIF	5.0 \pm 0.3	4.9 \pm 0.3	n.s.	4.7 \pm 0.3	4.3 \pm 0.1	n.s.	n.s.	n.s.
MEF	2.7 \pm 0.3	2.2 \pm 0.1	n.s.	2.4 \pm 0.2	2.1 \pm 0.1	n.s.	n.s.	n.s.

Table 21: Spontaneous Breathing Pattern during ActivityData are presented as mean \pm standard error of mean.

Parameter	Control (A)			Mutant (B)			A~B	A~B
	Male (n=2)	Female (n=3)	<i>p</i> - value	Male (n=5)	Female (n=5)	<i>p</i> - value	Male <i>p</i> - value	Female <i>p</i> - value
Activity								
f	524.6 \pm 15.8	481.5 \pm 6.6	0.0594	483.6 \pm 6.0	474.4 \pm 6.0	n.s.	< 0.05	n.s.
TV	0.28 \pm 0.02	0.26 \pm 0.02	n.s.	0.26 \pm 0.02	0.25 \pm 0.009	n.s.	n.s.	n.s.
sTV	10.4 \pm 0.07	11.3 \pm 0.3	n.s.	8.0 \pm 0.3	10.5 \pm 0.6	< 0.01	< 0.01	n.s.
MV	144.3 \pm 13.2	123.5 \pm 8.2	n.s.	123.9 \pm 9.3	114.8 \pm 3.6	n.s.	n.s.	n.s.
sMV	5.4 \pm 0.2	5.4 \pm 0.1	n.s.	3.8 \pm 0.2	4.9 \pm 0.3	< 0.02	< 0.01	n.s.
Ti	42.9 \pm 0.8	43.3 \pm 0.2	n.s.	44.1 \pm 0.7	43.7 \pm 0.5	n.s.	n.s.	n.s.
Te	71.6 \pm 4.2	81.3 \pm 1.8	0.0895	80.0 \pm 1.8	82.9 \pm 1.3	n.s.	0.0729	n.s.
Ti/TT	0.38 \pm 0.02	0.35 \pm 0.005	n.s.	0.36 \pm 0.007	0.35 \pm 0.004	n.s.	n.s.	n.s.
PIF	10.4 \pm 0.1	10.2 \pm 0.8	n.s.	9.6 \pm 0.6	9.6 \pm 0.4	n.s.	n.s.	n.s.
PEF	8.1 \pm 0.7	7.7 \pm 1.1	n.s.	7.1 \pm 0.6	6.5 \pm 0.5	n.s.	n.s.	n.s.
MIF	6.4 \pm 0.3	6.0 \pm 0.4	n.s.	5.9 \pm 0.4	5.6 \pm 0.2	n.s.	n.s.	n.s.
MEF	3.9 \pm 0.5	3.2 \pm 0.2	n.s.	3.3 \pm 0.3	3.0 \pm 0.08	n.s.	n.s.	n.s.

3.10 Expression Profiling

The molecular phenotyping screen archives organs of mutant mice for subsequent DNA-chip expression profiling analysis. Seven male mice of the DLG3 mutant mouse line were provided to the molecular phenotyping screen.

Organs were collected at the age of 105-110 days. To minimize the influence of circadian rhythm on gene expression, mice were killed between 9 a.m. and 12 a.m. by carbon dioxide asphyxiation. The following 17 organs were collected and archived in liquid nitrogen following our established standard operating protocols: bulbourethral gland, spleen, kidney, seminal vesicles, testis, white fat, liver, heart, lung, thymus, skin/cartilage (outer ear), bone (femur), skeletal muscle, salivary gland, brain, brown fat, and eye.

Table 22: DLG3-deficient and wild-type mice stored for expression profiling					
Mouse ID	Strain	Sex	Date of Birth	Genotype	Date of Collection
30011153	DLG3	m	08.06.2003	-/-	25.09.2003
30011158	DLG3	m	07.06.2003	-/-	25.09.2003
30011160	DLG3	m	11.06.2003	-/-	25.09.2003
30011161	DLG3	m	08.06.2003	-/-	25.09.2003
30011162	DLG3	m	08.06.2003	-/-	25.09.2003
30011165	DLG3	m	08.06.2003	+/+	25.09.2003
30011166	DLG3	m	08.06.2003	+/+	25.09.2003

In a first discussion no organ was selected for analysis. When further examination is considered necessary, expression profiling analysis can be performed using our DNA-chip containing 21,000 probes. Please contact Johannes Beckers, (beckers@gsf.de) to discuss this option.

3.11 Metabolic Screen

3.11.1 Summary

The metabolic screening provides a comparative analysis of bioenergetic parameters in mice. Mechanisms which lead to disturbances in body weight regulation and energy metabolism are determined. Hence, the basal energetic demands are monitored during *ad libitum* feeding and under food restricted conditions. In humans unbalanced energy uptake and energy expenditure cause the development of obesity (Spiegelman and Flier, 2001) or anorexia nervosa with severe weight loss (Hebebrand *et al.*, 2003). Some rodent and other species tend to increase activity upon food restriction leading to weight loss when given access to an activity wheel (Exner *et al.*, 2000). Several studies described that fasting in mice results in transient depression of metabolic rate, heart rate, body temperature and locomotor activity (Duffy *et al.*, 1990; Williams *et al.*, 2002). Therefore the primary Metabolic Screening focused on the determination of food and energy uptake under *ad libitum* conditions and metabolic adaptations during food restriction and serves as the origin for further investigations in the Secondary and Tertiary screening which go into details of energy expenditure and energy storage.

In the primary metabolic screen 12 (six male/six female) control mice and 12 (six male/six female) DLG3-knockout mice were analysed. They were first fed under *ad libitum* conditions for two weeks, followed by one week of food restriction to 60%. The primary metabolic screen focus on investigation of metabolic demands of mice determining daily body weight, energy uptake, metabolizable energy and body temperature and adaptive capacity of metabolic processes. No statistical differences between knockout and control mice have been found in food and energy assimilation.

3.11.2 Mice

Six adult control males and six adult KO males entered the Metabolic screen at the beginning of calendar week 42nd in 2003. The females (six control and six knockout) entered the metabolic laboratory one week later.

The mice were single caged on grid panels (0.5 cm grid hole diameter). They were fed *ad libitum* for a period of 14 days, followed by a period of food restriction to 60% of *ad libitum* for seven days to analyze adaptive responses of metabolism.

3.11.3 Material and Methods

Recorded Data

During the different feeding regimes body weight, food consumption (F_{con}), rectal temperature (T_{re}), daily feces production (Fec), energy uptake (E_{up}), energy content of the feces (E_{fec}), metabolizable energy (E_{met}) and the food assimilation coefficient (F_{ass}) were recorded.

Analysis of Feces

The separation of mice in single cages allowed collection of feces in three day intervals. Samples of lab chow and feces (~1 g) were dried at 60°C for two days, homogenized in a coffee grinder and squeezed to a pill for determination of energy content in a bomb calorimeter (IKA Calorimeter C7000) based on dry measurement principle. Energy uptake is determined as the product of food consumed and the caloric value of the food. To obtain metabolizable energy (E_{met}) the energy content of feces and urine (2% of E_{up} ; Drozd 1975) were subtracted from energy uptake.

Statistical Analysis

All values are presented as means \pm SEM. Two-way-ANOVA (SigmaStat, Jandel Scientific) was used to test for effects of the factors genotype and gender. The Tukey test was applied for post hoc multiple comparison. The Mann-Whitney-Test for paired samples was used to analyze the effect of nutritional status on parameters of energy metabolism.

3.11.4 Parameters

Recorded Data during the different feeding regimes
body weight, food consumption (F_{con}), rectal temperature (T_{re}), daily feces production (Fec), energy uptake (E_{up}), energy content of the feces (E_{fec}), metabolizable energy (E_{met}), food assimilation coefficient (F_{ass})

3.11.5 Results

No genotype-specific differences between wild-type and knockout mice could be found, neither in males nor in females. Sex-specific differences were detected in almost all parameters. Both gender decreased body temperature during food restriction (Table 23).

Raw data for each individual are available on demand in Excel sheets.

3.11.6 Discussion

No information about metabolic parameters were available prior to metabolic screening of DLG3 mice. No statistical differences between wild-type and knockout mice have been found in food and energy assimilation. This indicates that the mutation of the DLG3 gene does not influence energy metabolism or related parameters, e.g. body temperature.

3.11.7 References

Drozd M. (1975) A Food habits and food assimilation in mammals. In: Methods for Ecological Bioenergetics, edited by W. Grodzinski, R.Z. Klekowski and A Duncan. Oxford, UK: Blackwell, p: 23-47

- Duffy, P.H., R. J. Feuers and R. W. Hart (1990): Effect of chronic caloric restriction on the circadian regulation of physiological and behavioral variables in old male B6C3F1 mice. *Chronobiol Int* 7: 291-303
- Exner, C., J. Hebebrand, H. Remschmidt, C. Wewetzer, A. Ziegler, S. Herpertz, U. Schweiger, W. F. Blum, G. Preibisch, G. Heldmaier and M. Klingenspor (2000): Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. *Mol Psychiatry* 5: 476-481.
- Hebebrand J., C. Exner, K. Hebebrand, C. Holtcamp, R.C. Casper, H. Remschmidt, B. Herpertz-Dahlmann, M. Klingenspor (2003): Hyperactivity in patients with anorexia nervosa and in semistarved rats: Evidence for a pivotal role of hypoleptinemia. *Physiology and Behavior* 79: 25-37
- Spiegelman B.M. and J.S. Flier (2001): Obesity and the regulation of energy balance. *Cell* 104: 531-543
- Williams T. D., J.B. Chambers, R.P. Henderson, M.E. Rashotte and J.M. Overton (2002): Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. *Am J Physiol Regul Integr Comp Physiol* 282: R1459-67

Abbreviations

F_{con}	Food consumption
T_{re}	rectal temperature
Fec	daily feces production
E_{up}	energy uptake
E_{fec}	energy content of the feces
E_{met}	metabolizable energy
F_{ass}	food assimilation coefficient

Table 23: Metabolic Parameters Recorded in the Primary Screen

Data are presented as mean \pm standard error of mean.

Parameter	Control (A)					Mutant (B)					A~B	
	<i>ad libitum</i>			food reduction, 7 days to 60%		<i>ad libitum</i>			food reduction, 7 days to 60%			
	Male	Female		Male	Female	Male	Female		Male	Female	Male	Female
	(n=6)	(n=6)	<i>p</i> - <i>value</i>	(n=6)	(n=6)	(n=6)	(n=6)	<i>p</i> - <i>value</i>	(n=6)	(n=6)	<i>p</i> - <i>value</i>	<i>p</i> - <i>value</i>
Body weight [g]	32.7 \pm 2.0	23.4 \pm 0.95	< 0.01	27.4 \pm 2.13	18.9 \pm 0.82	31.0 \pm 1.86	23.3 \pm 0.88	< 0.01	25.8 \pm 1.56	18.9 \pm 0.63	n.s.	n.s.
Rectal body temperature [°C]	36.37 \pm 0.09	36.8 \pm 0.12	< 0.02	35.2 \pm 0.15	34.6 \pm 0.34	36.6 \pm 0.09	36.95 \pm 0.17	n.s.	35.08 \pm 0.1	34.91 \pm 0.28	n.s.	n.s.
Food consumption [g day ⁻¹]	3.12 \pm 0.14	2.6 \pm 0.14	< 0.05	60% of <i>ad libitum</i>		3.3 \pm 0.18	2.76 \pm 0.12	< 0.05	60% of <i>ad libitum</i>		n.s.	n.s.
Energy uptake [kJ day ⁻¹]	57.7 \pm 2.66	49.14 \pm 2.32	< 0.05	34.6 \pm 1.6	29.48 \pm 1.39	61.15 \pm 3.29	51.08 \pm 2.22	< 0.05	36.69 \pm 1.97	30.65 \pm 1.33	n.s.	n.s.
Energy uptake BW ⁻¹ [kJ g ⁻¹ day ⁻¹]	1.77 \pm 0.06	2.06 \pm 0.12	< 0.02	1.27 \pm 0.05	1.53 \pm 0.09	2.02 \pm 0.19	2.19 \pm 0.07	n.s.	1.45 \pm 0.13	1.62 \pm 0.06	n.s.	n.s.
Feces production [g day ⁻¹]	0.58 \pm 0.04	0.45 \pm 0.02	< 0.05	0.34 \pm 0.03	0.27 \pm 0.02	0.57 \pm 0.02	0.48 \pm 0.03	< 0.05	0.35 \pm 0.02	0.26 \pm 0	n.s.	n.s.
Energy content feces [kJ g ⁻¹]	16.16 \pm 0.04	15.78 \pm 0.08	< 0.01	15.83 \pm 0.11	15.77 \pm 0.12	16.23 \pm 0.06	15.62 \pm 0.05	< 0.001	15.91 \pm 0.07	15.84 \pm 0.07	n.s.	n.s.
Metabolized energy [kJ day ⁻¹]	48.59 \pm 2.06	41.33 \pm 2.22	< 0.05	29.37 \pm 1.23	24.71 \pm 1.35	52.04 \pm 2.98	43.67 \pm 1.8	< 0.05	31.23 \pm 1.81	26.57 \pm 1.35	n.s.	n.s.
Metabolized energy [kJ g ⁻¹ day ⁻¹]	1.49 \pm 0.05	1.77 \pm 0.05	< 0.01	1.08 \pm 0.04	1.3 \pm 0.05	1.72 \pm 0.16	1.87 \pm 0.06	n.s.	1.24 \pm 0.12	1.4 \pm 0.06	n.s.	n.s.
Food assimilation coefficient [%]	84.2 \pm 0.5	85.6 \pm 0.4	n.s.	84.8 \pm 0.4	85.3 \pm 0.5	85.0 \pm 0.3	85.5 \pm 0.4	n.s.	85.0 \pm 0.8	86.5 \pm 0.6	n.s.	n.s.

3.12 Pathology Screen

3.12.1 Summary

The Pathology screen performed a complete morphological analysis with standard stains. We analyzed forty five mice. Mutant and control mice showed the same morphologic characteristics. Specific phenotypes of DLG3 mice were not observed.

3.12.2 Mice

A total of 45 mice, 27 mutant (14 males, 13 females) and 28 control mice (8 males, 10 females) were analyzed. Due to the workflow in the GMC, mice of different ages were received from different screens, and thus, from different ages (Table 24)

Table 24: DLG3 mice and their control littermates analyzed.						
	Control		Mutant		Number of Animals	Age (weeks)
	Male	Female	Male	Female		
Lung Screen	0	3	0	5	8	16
Dysmorphology Screen	0	0	2	1	3	21 - 22
Metabolic Screen	6	7	7	7	27	21 - 22
Other Screen	2	0	5	0	7	16
Total Number of Animals	8	10	14	13	45	

3.12.3 Materials and Methods

Mice received in the laboratory of pathology were sacrificed with CO₂. The animals were analyzed macroscopically and weighed. The thymus and left lobe of the liver were measured. Blood samples were taken, centrifuged and the serum was saved at -20°C. Tails were preserved at -70°C for further genetic analysis. Following a complete dissection, an X-ray of the complete bone structure was taken, when indicated (Hewlett Packard, Cabinet X-Ray System Faxitron Series). All organs were fixed in 4% buffered formalin and embedded in paraffin for histological examination. Two μm thick sections from skin, heart, lung, brain, cerebellum, thymus, spleen, cervical lymph nodes, thyroid, parathyroid, adrenal gland, stomach, intestine, liver, pancreas, kidney, reproductive organs, and urinary bladder were cut and stained with hematoxylin and eosin (H&E). Immunohistochemistry was performed with an immunostainer (Ventana Medical Systems, Inc., Tucson AZ). The slides were deparaffinized and rehydrated. The antigen retrieval was done in a microwave pressure

cooker with 0.01 mol/l citrate buffer (pH 6) containing 0.1% Tween 20 at 600 W for 30 minutes. After cooling down the slides in Tris-buffered saline the sections were incubated in 3% goat serum for 20 minutes. The following primary antibodies were used when indicated: CD3 (DAKO, Hamburg, Germany), B220, and Mac-3 (Pharmingen, Germany). Samples for electron microscopy were fixed in glutaraldehyde and 0.5 µm thick semi fine sections were taken.

3.12.4 Genotype-specific results

Overview

Table 25: DLG3 mice. Genotype-specific morphological alterations.					
Organ	Skin	Musculoskeletal System	Eyes	Brain	Cerebellum
Alteration	no	no	no	no	no
Organ	Heart	Trachea	Lung	Teeth	Salivary glands
Alteration	no	no	no	no	no
Organ	Esophagus	Stomach	Small Intestine	Large Intestine	Liver
Alteration	no	no	no	no	no
Organ	Pancreas	Cervical Lymph Nodes	Thymus	Spleen	Thyroid
Alteration	no	no	no	no	no
Organ	Parathyroid	Adrenal Gland	Kidneys	Urinary Bladder	Testes
Alteration	no	no	no	no	no
Organ	Epididymis	Funiculus spermaticus	Ovaries	Uterus	Vagina
Alteration	no	no	no	no	no

In the analyzed organs, DLG3 mice do not differ morphologically from their control littermates.

3.12.5 Secondary Results

In both genotypes, DLG3 mice and their littermate controls, two types of secondary lesions were identified.

Thymus

In 8/12 male-mice (4 wild-type, and 4 DLG3 mice) received from the metabolic screen, cortical thymus atrophy was observed.

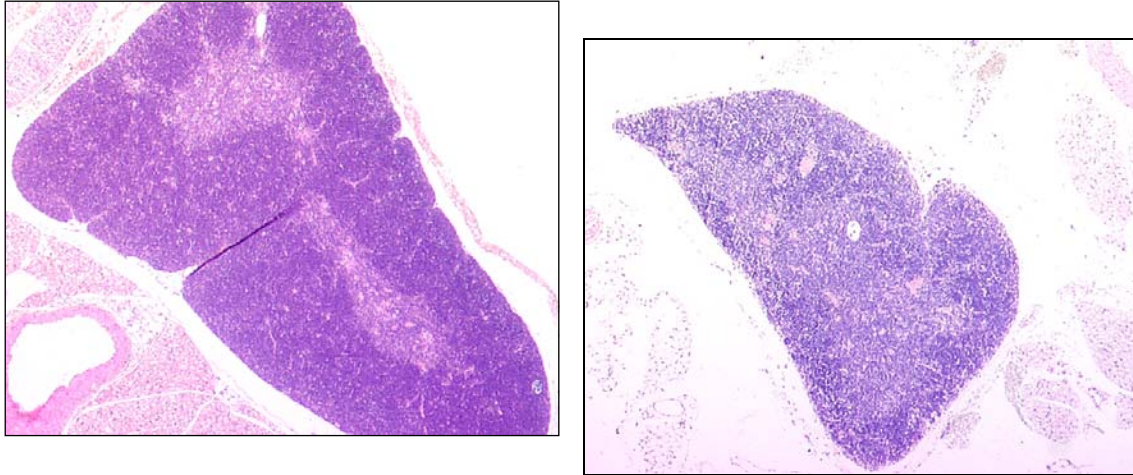


Figure 5: Cortical thymus atrophy

The left panel shows a normal thymus of a wild-type mouse from the metabolic screen. Note the thick, dense, dark blue cortex given by the presence of normal number of lymphocytes (H&E, 25x). In contrast, the right panel demonstrates the depletion of the cortex of a DLG3 mouse from the metabolic screen, resulting in a more open, light blue appearance of the cortex due to the decreased number of lymphocytes (H&E, 25x).

Spleen and Liver

In 8/21 male-mice (3 wild-type, and 5 DLG3 $-/-$ mice) and 120 /23 female-mice (6 wild-type, and 4 DLG3 $-/-$ mice) received from the metabolic screen strong extramedullary erythropoiesis in the spleen and liver was identified.

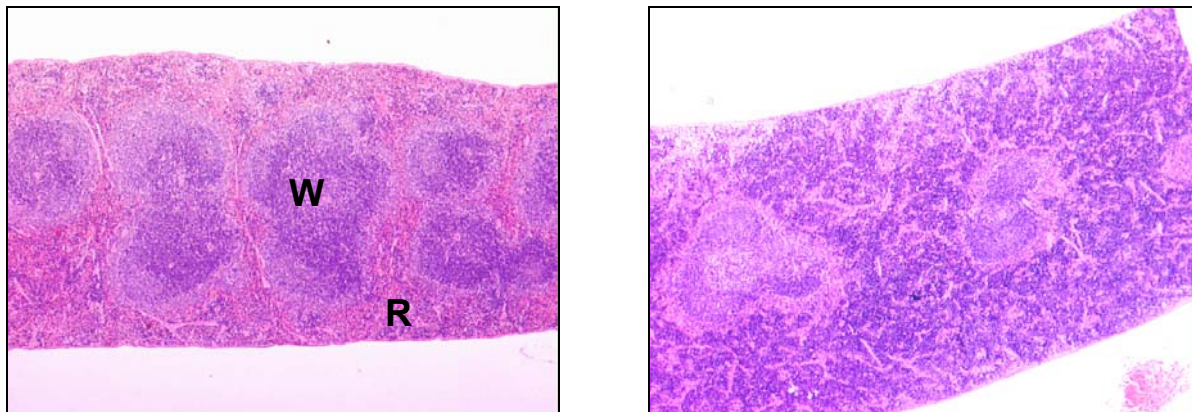


Figure 6: Extramedullary erythropoiesis in the spleen

On the left side a picture of an unaltered spleen. The relation between the white (W) and the red pulp (R) is normal. The right panel shows a spleen with very strong erythropoiesis.

3.12.6 Discussion

The complete morphological analysis in the primary screen did not reveal any genotype-specific phenotypes in DLG3 mice. Thymus atrophy is a frequent finding in mice with C57BL/6J genetic background passing through the metabolic screen. We do believe that the thymus atrophy is secondary to food restriction during the metabolic screening procedure and /or the stress induced during this analysis (unpublished observation).

Extramedullary erythropoiesis was observed as a reaction to blood withdraws a couple of days before the animal was sacrificed.

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