

The **GERMAN MOUSE CLINIC**

Report for DnaseX

Helmut Fuchs, Valérie Gailus-Durner, Christoph Lengger, Ilka Schneider, Svetoslav Kalaydjiev, Martina Klempt, Ralf Elvert, Sabine M. Hölter, Julia Calzada-Wack, Claudia Dalke, Elisabeth Grundner-Culemann, Anahita Javaheri, Tobias Franz, Sandra Kunder, Nicole Ehrhardt, Tomek Mijalski, Claudia Reinhard, Vera Pedersen, Ildiko Racz, Cornelia Prehn, Dagmar Krüger, Birgit Rathkolb, Markus Brielmeier, Jerzy Adamski, Johannes Beckers, Holger Schulz, Jack Favor, Martin Klingenspor, Thomas Meitinger, Andreas Zimmer, Heidrun Behrendt, Jochen Graw, Thomas Klopstock, Markus Ollert, Leticia Quintanilla-Fend, Gerhard Heldmaier, Joachim Heyder, Heinz Höfler, Eckhard Wolf, Wolfgang Wurst, Dirk H. Busch, Martin Hrabé de Angelis

1. General Part

1.1. Summary

Fifty-nine DnaseX mice have been analyzed in the German Mouse Clinic (GMC) in behaviour tests, dysmorphology, bone and cartilage, neurology, eye tests, clinical chemistry, immunology, allergy, nociception, lung function, metabolism, and a comprehensive pathology. We could show that DnaseX knockout mice reduce feces production during food restriction. In male DnaseX ^{-/0} mice the lactate concentration was significantly higher than in wild type controls. SHIRPA tests indicated higher locomotor activity in DnaseX ^{-/-} females. In the modified hole board test female mutants showed significantly more rearings than their wild type controls, and the time spent in group contact was reduced.

First results from the secondary screens were already available. The Neurology screen detected a significant difference in grip strength between mutant females and controls. Thus, the female DnaseX ^{-/-} mice show a defect in motor coordination.

1.2. Mice

1.2.1. Number and kind of mice

The 93 mice arrived in calendar week 4 of 2003 in a good condition. Females were kept in groups of five animals per cage. Males had to be separated into single cages housing, as they started to fight and injure each other after they arrived in the animal facility. As previously agreed, some mice arrived without genotype information, and were tail clipped by us and genotyped by the sender.

59 animals were used for the tests. In the few cases that we lost an animal, it was replaced (if possible) by one of the remaining mice. As described by the sender, the mice analyzed were a (F1N2)F2 generation. Back crossing was done to C57BL/6. Due to the special situation that DnaseX-gene is located on the X-chromosome, the mice we analyzed did not result from heterozygous x heterozygous matings as demanded by the German Mouse Clinic for a litter-mate control.

1.2.2. Housing conditions

Mice in the GMC are housed in type II polycarbonate cages in individually ventilated caging (IVC) systems (VentiRack Bioscreen TM, Biozone, Margate, UK) on wood fiber (Altromin). 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 diet (Altromin 1314) and given semidemineralized filtered (0,2µm) water ad libitum. Light is adjusted to a 12h/12h light/dark cycle; temperature and relative humidity are regulated to 22+/- 1°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.

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

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).

1.3. Workflow

1.3.1. Workflow description

After the mice arrived at the German Mouse Clinic (GMC), they were acclimatized in the new environment for 1 week. The males then started in the behaviour screen. There, they stood for three weeks. Directly after the Behaviour test, the anatomical inspection of the Dysmorphology screen was performed. In the next week, the Neurology screen was applied. One week later the mice went through the tests of the Eye screen. When the mice were 12 weeks old, blood was taken, and the blood-based screens for Clinical chemistry, Immunology, Allergy and the Lactate test were performed. As DnaseX mice were quite small, we had problems to get the needed amount of blood from each animal. Thus, not from each individual animal all parameters could be determined. One week later the animals were tested in the Nociceptive screen. Two weeks after testing of the first blood sample, a second sample was taken to confirm outliers, and to supply the Dysmorpholgy screen with blood for the

determination of blood based bone- related parameters. In parallel, five mutant females and eight corresponding controls left the animal facility for the lung function analysis, which for technical reasons is located elsewhere. These animals were, for hygienic reasons, not allowed to reenter the German Mouse Clinic and went directly to the pathology. Five 15-week old mutant males and four controls were used to freeze organs for future expression profiling on demand. The remaining organs were analyzed by the pathology. All other animals went through the bone and cartilage tests of the Dysmorphology screen, and then stayed three weeks in the Metabolic screen. After completion of the primary screen, all animals ended up in the Pathology.

For females the suite order of the screens was the same (with the exception of the Lung function tests and expression profiling sampling), but they started one week later.

1.3.2. Applied screens

The GMC standard workflow as described above was applied to analyze the DnaseX mice.

1.4. Existing Knowledge

There was only poor previous knowledge about the phenotype of DnaseX KO mice. They are viable and fertile, and equal numbers of males and females are born. As no abnormalities were previously reported, all further findings we consider as new.

2. Specific part

2.1. Behaviour Screen

2.1.1. Summary

There were no effects of genotype on specific locomotor, anxiety-related and memory-related measures. However, among the male mutants were individuals which were very aggressive in the home cage before the screen, so that 7 male mice had to be separated into single cages because they were either badly bitten or biting the others. These animals could not be screened. Interestingly, all of these mice were mutants. Thus, concerning male mice, 15 wild-type (wt) animals, but only 7 mutants could be analysed.

The only small effects of genotype observed were that female mutants reared more and spent tendentially less time in group contact, which may indicate a difference in general exploratory strategy and reduced social affinity compared to wildtype. Male mutants demonstrated a shorter latency to rearings and to familiar object exploration. Because of the small effect sizes and the observation of increased home cage aggression among the males shortly prior to the test, which may have influenced these results, they have to be regarded as preliminary.

2.1.2. Mice

Three to five mice per cage 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 29 female mice (15 wt, 14 mutants) and 22 male mice (15 wt, 7 mutants) were available for analysis.

2.1.3. Material and Methods

The modified hole board test was carried out according to the procedures described by Ohl *et al.*, 2001. The test apparatus consisted of a box (150 x 50 x 50 cm) which was divided into a test arena (100 x 50 cm) and a group compartment (50 x 50 cm) by a transparent PVC partition (50 x 50 x 0.5 cm) with

111 holes (1 cm diameter) staggered in 12 lines to allow group contact. A board (60 x 20 x 2 cm) with 23 holes (1.5 x 0.5 cm) staggered in 3 lines with all holes covered by movable lids was placed in the middle of the test arena, 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 Ohl *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.

At the beginning of the experiment, all animals of a cage were allowed to habituate to the test environment together in the group compartment for 20 min. Then each animal was placed individually into the test arena and allowed to explore it freely for 5 min, during which the cage mates stayed present in the group compartment. 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 behaviour 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 animal was returned to the group compartment, and 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 $p < 0.05$.

2.1.4. Results

Analysis of the observed behavioral parameters showed that DnaseX-deficient mice did not differ from wildtype concerning locomotor activity levels, directed exploration, anxiety-related behavior, arousal or object recognition memory. The only (small) effects observed were an increase in rearings made in the box in females, as well as a shorter latency to rearings and to familiar object exploration in males.

Analysis of the animals' locomotor path revealed that there were no genotype effects on exploratory drive, speed of movement, thigmotaxis or turning behavior.

2.1.5. Discussion

Except for the unvalidated, occasional observation of increased home cage aggression among the male mutants, there were only very subtle genotype effects. These effects most likely reflect subtle alterations in exploratory strat-

egy, which matches the observation in the Neurology Screen of an increase in their parameter “locomotor activity” for 30 seconds after transfer in female mutants. However, a general influence of genotype on activity levels or anxiety-related behavior is not indicated by our results.

Because of the small number of male mutants that could be analyzed, the results in males can be only considered preliminary unless confirmed by additional experiments.

2.1.6. 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 2.1.1. Results of behavioral observation in the modified hole board test

Parameter	wildtype			mutant			wt-mut	wt-mut
	<u>male</u> (n=15)	<u>female</u> (n=15)	<i>p - value</i>	<u>male</u> (n=7)	<u>female</u> (n=14)	<i>p - value</i>	male <i>p - value</i>	female <i>p - value</i>
Line crossing (Frequency)	121.73 ± 9.82	131.67 ± 8.49	N.A.	126.00 ± 10.73	136.57 ± 5.47	N.A.	n.s.	n.s.
Line crossing (Latency)	1.19 ± 0.23	0.98 ± 0.16	N.A.	1.0 ± 0.19	0.71 ± 0.1	N.A.	n.s.	n.s.
Rearings in box (Frequency)	33.0 ± 3.62	34.93 ± 1.62	N.A.	33.57 ± 3.15	40.86 ± 1.74	N.A.	n.s.	p<0.05
Rearings in box (Latency)	23.16 ± 4.34	14.11 ± 2.43	N.A.	16.54 ± 3.18	8.91 ± 1.81	N.A.	n.s.	n.s.
Hole exploration (Frequency)	14.00 ± 2.41	14.60 ± 1.98	N.A.	15.00 ± 2.85	15.71 ± 1.90	N.A.	n.s.	n.s.
Hole exploration (Latency)	42.03 ± 6.28	70.87 ± 22.61	N.A.	35.91 ± 6.63	60.48 ± 17.59	N.A.	n.s.	n.s.
Hole visit (Frequency)	0.0 ± 0.0	0.0 ± 0.0	N.A.	0.0 ± 0.0	0.0 ± 0.0	N.A.	N.A.	N.A.
Hole visit (Latency)	300.0 ± 0.0	300.0 ± 0.0	N.A.	300.0 ± 0.0	300.0 ± 0.0	N.A.	N.A.	N.A.
Board entry (Frequency)	7.67 ± 0.97	6.00 ± 1.26	N.A.	6.57 ± 1.13	5.36 ± 1.15	N.A.	n.s.	n.s.

Board entry (Latency)	79.35 ± 16.90	103.73 ± 26.90	N.A.	58.49 ± 13.85	124.84 ± 25.15	N.A.	n.s.	n.s.
Board entry (Total duration %)	7.22 ± 1.22	4.88 ± 0.87	N.A.	8.58 ± 1.29	5.42 ± 1.37	N.A.	n.s.	n.s.
Rearing on board (Frequency)	0.13 ± 0.13	0.20 ± 0.11	N.A.	0.71 ± 0.42	0.29 ± 0.16	N.A.	n.s.	n.s.
Rearing on board (Latency)	289.60 ± 10.4	279.75 ± 11.07	N.A.	234.26 ± 32.21	276.37 ± 14.51	N.A.	p<0.05	n.s.
Risk assessment (Frequency)	0.0 ± 0.0	0.0 ± 0.0	N.A.	0.0 ± 0.0	0.0 ± 0.0	N.A.	N.A.	N.A.
Risk assessment (Latency)	300.0 ± 0.0	300.0 ± 0.0	N.A.	300.0 ± 0.0	300.0 ± 0.0	N.A.	N.A.	N.A.
Group partition (Frequency)	11.27 ± 1.39	12.73 ± 0.88	N.A.	11.29 ± 1.34	12.29 ± 0.83	N.A.	n.s.	n.s.
Group partition (Latency)	20.52 ± 3.75	8.96 ± 1.62	N.A.	17.56 ± 4.78	10.57 ± 1.85	N.A.	n.s.	n.s.
Group partition (Total duration %)	22.14 ± 2.09	30.15 ± 2.73	N.A.	22.51 ± 4.32	22.39 ± 1.88	N.A.	n.s.	n.s.
Grooming (Frequency)	0.73 ± 0.18	0.6 ± 0.21	N.A.	0.71 ± 0.42	0.5 ± 0.31	N.A.	n.s.	n.s.
Grooming (Latency)	212.66 ± 22.23	243.60 ± 22.51	N.A.	248.41 ± 31.30	278.37 ± 14.06	N.A.	n.s.	n.s.
Grooming (Total duration %)	2.34 ± 0.79	1.08 ± 0.41	N.A.	1.74 ± 1.22	0.91 ± 0.63	N.A.	n.s.	n.s.

Defecation (Frequency)	0.0 ± 0.0	0.0 ± 0.0	N.A.	0.0 ± 0.0	0.07 ± 0.07	N.A.	n.s.	n.s.
Defecation (Latency)	300.0 ± 0.0	300.0 ± 0.0	N.A.	300.0 ± 0.0	281.21 ± 18.79	N.A.	n.s.	n.s.
Unfamiliar object exploration (Frequency)	4.73 ± 0.52	4.07 ± 0.52	N.A.	4.00 ± 0.87	5.21 ± 0.54	N.A.	n.s.	n.s.
Familiar object exploration (Frequency)	3.47 ± 0.49	3.73 ± 0.54	N.A.	3.57 ± 0.48	4.86 ± 0.69	N.A.	n.s.	n.s.
Unfamiliar object exploration (Latency)	48.13 ± 13.07	40.79 ± 9.40	N.A.	34.09 ± 9.35	18.59 ± 3.12	N.A.	n.s.	n.s.
Familiar object exploration (Latency)	80.35 ± 19.59	75.13 ± 21.81	N.A.	19.59 ± 6.04	74.94 ± 20.19	N.A.	p<0.05	n.s.
Unfamiliar object exploration (Total duration %)	1.82 ± 0.83	0.85 ± 0.14	N.A.	0.84 ± 0.22	1.13 ± 0.2	N.A.	n.s.	n.s.
Familiar object exploration (Total duration %)	0.59 ± 0.08	0.62 ± 0.09	N.A.	0.51 ± 0.06	0.84 ± 0.13	N.A.	n.s.	n.s.
Object Index	0.35 ± 0.08	0.17 ± 0.09	N.A.	0.15 ± 0.13	0.16 ± 0.10	N.A.	n.s.	n.s.

Table 2.1.2. Video-tracking results regarding locomotor behavior

Parameter	wildtype			mutant			wt-mut	wt-mut
							male	female
	<u>male</u> (n=14)	<u>female</u> (n=15)	<i>p - value</i>	<u>male</u> (n=7)	<u>female</u> (n=9)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
Distance moved (cm)	3134.68 ± 248.81	3294.98 ± 189.47	N.A.	3207.07 ± 220.71	3269.79 ± 165.07	N.A.	n.s.	n.s.
Mean velocity (cm/sec.)	19.93 ± 1.10	22.62 ± 0.89	N.A.	19.63 ± 1.12	21.90 ± 0.87	N.A.	n.s.	n.s.
Maximum velocity (cm/sec.)	60.48 ± 2.23	63.22 ± 1.37	N.A.	58.13 ± 2.97	60.90 ± 1.46	N.A.	n.s.	n.s.
Turns (Frequency)	1606.00 ± 79.61	1514.64 ± 56.87	N.A.	1612.72 ± 88.27	1563.0 ± 46.46	N.A.	n.s.	n.s.
Mean Turn Angle (degrees)	22.71 ± 0.74	20.97 ± 0.86	N.A.	21.99 ± 1.18	22.57 ± 0.96	N.A.	n.s.	n.s.
Angular Velocity (degrees/sec.)	154.05 ± 4.39	150.70 ± 5.27	N.A.	148.73 ± 7.86	155.69 ± 5.75	N.A.	n.s.	n.s.
Absolute Meander (degrees/sec.)	16.05 ± 0.61	14.31 ± 0.73	N.A.	15.49 ± 0.85	15.76 ± 0.81	N.A.	n.s.	n.s.
Distance to zone border (wall, cm)	6.56 ± 0.3	6.06 ± 0.31	N.A.	6.42 ± 0.35	5.75 ± 0.44	N.A.	n.s.	n.s.
Distance to zone border (Board, cm)	9.12 ± 0.18	9.51 ± 0.27	N.A.	8.67 ± 0.41	9.61 ± 0.36	N.A.	n.s.	n.s.

2.2. Dismorphology, bone and cartilage

2.2.1. Summary

A total of 59 animals of DnaseX–KO mice have been analysed in the dysmorphology, bone and cartilage module of the German Mouse Clinic. In the morphological investigation via visual inspection and X-ray analysis, only mild phenotypes were found. These phenotypes were found in both mutant and control animals. No correlation of any phenotype with a certain genotype was detected. In the quantitative, bone-related parameters, no significant differences between KO and controls were detected.

2.2.2. Mice

The 30 male (15 $-/0$, 15 $+/0$) and 29 female (15 $+/+$, 14 $-/-$) DnaseX mice were analyzed by morphological inspection at the age of nine weeks. Blood was taken at the age of 14 weeks for determination of ionic calcium from 24 KO and 23 control animals, and 16-week-old KOs (18 animals) and controls (19 animals) entered the bone density tests and x-ray analysis.

2.2.3. Material and Methods

The dysmorphology, bone and cartilage module of the German Mouse Clinic analyzes the mice in three different phases:

At the age of nine weeks, the animals are screened morphologically using the protocol of Fuchs et al. 2000.

The ionized fraction of calcium in blood is analyzed in 14-week-old mice, and X-ray analysis and bone densitometry are performed with 16- to 17-week-old mice.

Ionized calcium analysis:

Equipment: AVL 9180 Electrolyte Analyzer; distributed by Roche Diagnostics GmbH, D-68298 Mannheim, Germany, Cleaning Solution and Conditioning Solution (Roche). ISEtrol Quality Control Solutions (Roche), Lithium-Heparin polypropylen tubes, glass capillary (0,8mm diameter, 32mm length, without heparin; special product of Laborteam K+K, Munich).

Quality control: Calibration of the system and quality control is done in intervals recommended by the manufacturer using the solutions delivered by the manufacturer.

The results from the quality control are recorded by the system. Before blood measurement a daily cleaning, conditioning and calibration of the analyser is performed.

Procedure: After anesthesia, 100 µl blood is collected in a lithium heparin tube and transferred directly to the analyzer. According to the electrodes used, the parameters are measured and values are transferred directly to the database.

X-ray images:

Equipment: Faxitron X-ray Model MX-20 Specimen Radiography System, Illinois. NTB Digital X-ray Scanner EZ 40, NTB GmbH, Diepholz, Germany.

Settings: Voltage 25 kV, integration time 40 ms.

Procedure: The anesthetized mouse is fixed on an X-ray-permeable plate and placed on the machine. Using iX-Pect software, which is supplied by the X-ray scanner manufacturer, the image is taken and analyzed.

Quality Control: Calibration of the system is done in monthly intervals.

Bone density analysis:

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

Parameters: Body weight, body length, fat mass, bone mass, bone mass density, lean mass.

Quality control: Calibration of the system is done in daily intervals using the QC and the QA phantoms delivered by the manufacturer. The results from the quality control are recorded by the system.

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

Procedure: After anesthetization the weight and length of the mouse are recorded, and the mouse is placed in the analyser. After a scout scan, the area of interest is optimized and the measure scan starts.

Data Analysis: For the analysis of the data, regions have to be defined. The standard analysis comprises a whole body analysis and a whole body analysis excluding the head area.

2.2.4. Results and discussion

In the morphological inspection of the mice, only mild phenotypes could be detected, and these did not correlate with a certain genotype. Two animals in which a mild kinky tail was found later went through X-ray analysis, and a mild deformation of the vertebrae was confirmed (table 2.1).

Quantitative parameters did not show any significant differences between KOs and controls. The sex differences we observed are common in many mouse strains, and thus are not unexpected (table 2.2).

2.2.5. Reference

Helmut Fuchs, Klaus Schughart, Eckhard Wolf, Rudi Balling, Martin Hrabe de Angelis, 2000, Screening for dysmorphological abnormalities—a powerful tool to isolate new mouse mutants *Mammalian Genome* 11, 528–530.

Raw data will be available on demand.

Table 2.2.1 Results from the morphological inspection

Phenotype	Males		Females	
	+/+	-/-	+/+	-/-
Brighter tooth color		2		2
Reduced hearing		1		1
Deafness			2	2
Mild tailkink		2*		2
Thin digits			1	
Mild cramping when hung by tail			1	
Mild cramping when gripped by the neck				1
Mildly unsteady gait			1	2
Small body size			3	1
<i>Animals Analyzed</i>	15	15	15	14

* confirmed by X-ray image

Table 2.2.2. Results from bone-related quantitative parameters

Parameter	Wild type (+/+ and +/-)			Mutant (-/- and +/-)			wt-mut	wt-mut
	male (n=15)	female (n=15)	p - value	male (n=15)	female (n=14)	p - value	p - value	p - value
Body weight (9 weeks)	24.1 ±0.7	18.5 ±0.6	0.0001	26.1 ±0.8	19.9 ±0.5	0.0001	n.s.	n.s.
	(n=14)	(n=9)		(n=15)	(n=9)			
Ionic Ca	1.21 ±0.031	1.170 ±0.022	n.s.	1.169 ±0.018	1.169 ±0.024	n.s.	n.s.	n.s.
	(n=10)	(n=9)		(n=9)	(n=9)			
Body length	8.5 ±0	8.3 ±0.1	0.0001	8.5 ±0	8.4 ±0.1	0.001	n.s.	n.s.
Body weight (16 weeks)	29.9 ±0.9	22.8 ±0.9	0.05	31.3 ±1.0	24.5 ±1.1	n.s.	n.s.	n.s.
Bone density	0.062 ±0.002	0.059 ±0.001	n.s.	0.059 ±0.001	0.060 ±0.001	n.s.	n.s.	n.s.
Bone mass	0.60 ±0.05	0.53 ±0.03	n.s.	0.64 ±0.04	0.55 ±0.04	n.s.	n.s.	n.s.
Lean mass	22.9 ±0.8	17.4 ±0.9	0.001	23.2 ±0.5	19.0 ±0.6	0.0001	n.s.	n.s.
Fat mass	4.70 ±0.93	2.59 ±0.33	0.05	4.75 ±0.94	2.95 ±0.62	n.s.	n.s.	n.s.
Bone density without head area	0.048 ±0.001	0.045 ±0.001	0.05	0.047 ±0.001	0.046 ±0.001	n.s.	n.s.	n.s.

2.4 Neurology Screen

2.4.1 Summary

In the primary neurological screen 29 DnaseX-knock-out mice (15 males/14 females) and 30 control mice (15 males/15 females) were screened. These animals were analysed according to our SHIRPA protocol where a battery of behavioural tests are carried out. This primary observation screen is a modification of the Irwin procedure (Irwin, 1968) and is proposed as a rapid, comprehensive and semi-quantitative screening method for qualitative analysis of abnormal phenotypes in a mouse (Rogers et al., 1997). We carried out 34 of 38 designed test parameters (see web page: www.mgu.har.mrc.ac.uk/mutabase/shirpa) to detect phenotypic differences between DnaseX-KO and control mice. Each test parameter contributes to an overall assessment in muscle and lower motor neuron function, spinocerebellar function including sensory and autonomic function. The primary neurological screen is thereby focused on the investigation of neurological reflexes of mice determining the neurological functioning of a mouse. Additionally we examined lactate in blood of mice to draw conclusions about energy metabolism. The comparison of DnaseX-knock-out and control mice (male and female) showed that DnaseX^{-/-} females have a significantly increased locomotor activity in comparison to the DnaseX^{+/+} females. Furthermore DnaseX^{-/0} males had significantly higher blood lactate values than DnaseX^{+/0} male mice.

2.4.2 Mice

Fifteen 10-week-old male DnaseX^{-/0} and fifteen 10-week-old male DnaseX^{+/0} mice entered the neurological screen at the beginning of the 10th calendar week. Fourteen 10-week-old female DnaseX^{-/-} and fifteen 10-week-old female DnaseX^{+/+} mice entered the neurological laboratory one week later. The male mice were single-caged because of their aggressiveness. All animals were fed *ad libitum* for a period of one week during their stay in the neurological screen.

2.4.3 Material and Methods

Assessment of each animal at age 10 weeks began with observation of undisturbed behaviour (*Viewing Jar Behaviour*) in a glass cylinder (11cm 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 behaviour were observed (*Behaviour recorded in the Arena*). This was followed by a se-

quence of manipulations testing reflexes, grip strength, toe pinch and wire manoeuvre (*Behaviour 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 (*Behaviour recorded during Supine Restraint*), the animals were restrained in a supine position to record autonomic responses such as salivation and heart rate. 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 of the contact righting reflex. Throughout the entire procedure, abnormal behaviour, irritability, fear, aggression and vocalization were recorded. Between testing of each mouse, fecal 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 the strain and gender factors in these parameters. The chi-squared test was applied for all other parameters.

2.4.4 Results

Female DnaseX^{-/-} mice had a significantly higher locomotor activity than DnaseX^{+/+} female mice ($p < 0.007$). The locomotor activity behaviour of male DnaseX^{-/0} and DnaseX^{+/0} did not show any significant changes. All other parameters were without significant pathological findings. Blood lactate examination revealed that the blood lactate value (mmol/l) of male DnaseX^{-/0} was significantly increased in comparison to male DnaseX^{+/0} animals. However, there was no statistical difference between female DnaseX^{-/-} and the female DnaseX^{+/+} mice.

2.4.5 Discussion

We found a significantly increased locomotor activity in female DnaseX^{-/-} mice ($p < 0.007$, see Table). So far, no information about any locomotor activity parameters in DnaseX-knock-out mice were available prior to this neurological screening. Locomotor activity comprises a multidimensional construct that can be simplified into independent dimensions. Increased locomotor activity could be due to changes in the striatal dopaminergic system or to diminished anxiety. We propose secondary and tertiary screening of the mice with particular emphasis on the basal ganglia system (skilled reaching tests, e. g. our **staircase test**) and on anxiety behaviour (behavioural screen).

Moreover, we found a significant increase of lactate in male DnaseX^{-/0} mice in comparison to the male DnaseX^{+/0} mice ($p < 0.05$, see Table), pointing towards a defect of energy metabolism. We recommend **grip strength** meas-

urements to test the muscle strength in the forelimbs of mice and **RotaRod** screening to observe balance and motor coordination.

Raw data will be available on demand.

2.4.6 References

Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia*. 1968 Sep 20;13(3):222-57.

Rogers DC, FisherEM, Brown SD, Peters J, Hunter AJ, Martin JE. Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm Genome*. 1997 Oct;8(10):711-3.

Table 2.4.1: Recording of body length (in cm) and body weight (in g)

Data are shown as mean (\pm SEM).Statistical analysis: Kruskal-Wallis test, significance $p \leq 0,05$

	male			female		
	Dnase-x +/0 (n=15)	Dnase-x -/0 (n=15)	<i>P</i> value	Dnase-x +/+ (n=15)	Dnase-x -/- (n=14)	<i>P</i> value
Body Length	8,25 \pm 0,06	8,5 \pm 0,06	<i>n.s.</i>	7,8 \pm 0,1	8,11 \pm 0,09	<i>n.s.</i>
Body Weight	24,84 \pm 0,7	26,89 \pm 0,9	<i>n.s.</i>	19,58 \pm 0,6	21,16 \pm 0,4	<i>n.s.</i>

Table 2.4.2: Behaviour recorded in Viewing Jar

Data shown represent the results of a test parameter from a major test where a behaviour response was observed. Test parameters which did not elicit any response were excluded from this data. Statistical analysis: chi-squared test; significance $p \leq 0,05$

	male			female		
	Dnase-x +/0 (n=15)	Dnase-x -/0 (n=15)	<i>P</i> value	Dnase-x +/+ (n=15)	Dnase-x -/- (n=14)	<i>P</i> value
Body Position						
Sitting or standing	15	15	<i>n.s.</i>	15	13	<i>n.s.</i>
Rearing on hind legs	0	0		0	1	
Spontaneous Behaviour						
Moderate movement	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Respiration rate						
Grasping, irregular	0	1	<i>n.s.</i>	0	0	<i>n.s.</i>
Normal	15	14		15	14	
Tremor						
None	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>

Table 2.4.3: Recording of locomotor activity and Behaviour recorded in the Arena

Locomotor activity data are shown as mean (\pm SEM). Data from Behaviour recorded in the arena represent the results of a test parameter from a major test where a behaviour response was observed. Test parameters which do not elicit any response were excluded from this data. Statistical analysis: Kruskal-Wallis test, chi-squared test, significance $p \leq 0,05$

	male			female		
	Dnase-x +/0 (n=15)	Dnase-x -/0 (n=15)	<i>P</i> value	Dnase-x +/+ (n=15)	Dnase-x -/- (n=14)	<i>P</i> value
Locomotor Activity	14,7 \pm 1,9	13,4 \pm 1,7	<i>n.s.</i>	21,53 \pm 1,3	27,42 \pm 1,3	0,007
Transfer arousal						
Brief freeze, then active movement	0	0	<i>n.s.</i>	0	1	<i>n.s.</i>
No freeze, immediate movement	15	15		15	13	
Palpebral Closure						
Eyes wide open	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Piloerection						
None	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Gait						
Normal	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Pelvic Elevation						
Markely flattened	1	10	<i>n.s.</i>	0	0	<i>n.s.</i>
Normal	13	12		11	11	
Elevated	1	3		4	3	
Tail Elevation						
Horizontally extended	15	14	<i>n.s.</i>	14	14	<i>n.s.</i>
Elevated/Straub tail	0	1		0	0	
Touch Escape						
No response	0	1	<i>n.s.</i>	0	0	<i>n.s.</i>
Mild	0	1		0	0	
Moderate	3	4		2	2	
Vigorous	12	9		13	12	
Positional Passivity						
Struggles when hold by tail	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>

Table 2.4.4: Behaviour recorded in or above the Arena

Data shown represents the results of a test parameter from a major test where a behaviour response was observed. Test parameters which do not elicit any response were excluded from this data. Statistical analysis: chi-squared test; significance $p \leq 0,05$

	male			female		
	Dnase-x +/0 (n=15)	Dnase-x -/0 (n=15)	<i>P</i> value	Dnase-x +/+ (n=15)	Dnase-x -/- (n=14)	<i>P</i> value
Trunk Curl						
Present	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Limb Grasping						
Absent	14	15		15	14	
Present	1	0	<i>n.s.</i>	0	0	<i>n.s.</i>
Visual Placing						
Upon vibrassee contact	10	6		3	5	
Before vibrassee contact	5	9		10	4	
Early extension	0	0	<i>n.s.</i>	2	5	<i>n.s.</i>
Grip strength						
Moderate grip	1	0		0	1	
Active grip	14	15	<i>n.s.</i>	15	13	<i>n.s.</i>
Body Tone						
Flaccid	1	0		2	0	
Slight resistance	14	15	<i>n.s.</i>	13	14	<i>n.s.</i>
Pinna reflex						
Active retraction	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Corneal Reflex						
Active single eye blink	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Toe Pinch						
Slight withdrawal	0	0		0	2	
Moderate withdrawal	6	3		6	6	
Brisk, withdrawal	8	12	<i>n.s.</i>	9	6	<i>n.s.</i>
Wire manoeuvre						
Active grip	3	0		6	3	
Difficulty to grasp	11	14		9	11	
Unable to grasp	1	0		0	0	
Falls immediately	0	1	<i>n.s.</i>	0	0	<i>n.s.</i>

Table 2.4.5: Behaviour during Supine Restraint

Data shown represent the results of a test parameter from a major test where a behaviour response was observed. Test parameters which do not elicit any response were excluded from this data. Statistical analysis: chi-squared test, significance $p \leq 0,05$

	male			female		
	Dnase-x +/0 (n=15)	Dnase-x -/0 (n=15)	<i>P</i> value	Dnase-x +/+ (n=15)	Dnase-x -/- (n=14)	<i>P</i> value
Skin Colour						
Pink	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Heart Rate						
Slow, bradycardia	1	0		0	0	
Normal	14	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Limb Tone						
Slight resistance	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Abdominal Tone						
Flaccid, no return of cavity to normal	1	0		0	0	
Slight resistance	14	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Lacrimation						
None	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Salivation						
None	0	0		2	1	
Slight margin of s.a.	9	5		13	12	
Wet zone entire of s.a	6	10	<i>n.s.</i>	0	1	<i>n.s.</i>
Provoked biting						
Absent	10	8		15	13	
Present	5	7	<i>n.s.</i>	0	1	<i>n.s.</i>
Righting reflex						
No impairment	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Contact righting reflex						
Present	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Negative Geotaxis						
Turns and climbs the grid	14	15		15	14	
Moves, but fails to turn	1	0	<i>n.s.</i>	0	0	<i>n.s.</i>
Fear						
None	15	15	<i>n.s.</i>	15	14	<i>n.s.</i>
Irritability						
None	0	0		0	1	
Struggles during supine restraint	15	15	<i>n.s.</i>	15	13	<i>n.s.</i>
Aggression						
None	11	8		15	14	
Provoked biting	4	7	<i>n.s.</i>	0	0	<i>n.s.</i>
Vocalization						
None	1	0		0	1	
Provoked during handling	14	15	<i>n.s.</i>	15	13	<i>n.s.</i>

Table 2.4.6: Lactate

Data shown represent the results the mean blood Lactate value (\pm SEM)

	male			female		
	Dnase-x +/0 (n=15)	Dnase-x -/0 (n=15)	<i>P</i> value	Dnase-x +/+ (n=15)	Dnase-x -/- (n=14)	<i>P</i> value
Lactate (mmol/l)	4,1 \pm 0,49	5,5 \pm 0,5	0,05	4,5 \pm 0,44	3,8 \pm 0,39	n.s.

2.4 Eye Screen

2.4.1 Summary

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

No significant differences between wildtype and mutant DnaseX mice were detected.

2.4.2 Mice

30 male and 29 female DnaseX mice entered the Eye Screen at the age of 11 weeks. Mice were first examined by slitlamp biomicroscopy and on the following day an ERG was performed. Mice were kept under standard laboratory conditions with food and water *ad libitum*.

2.4.3 Materials and Methods

Electroretinography (ERG).

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 corneae; 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 12500 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.

2.4.4 Results

ERG responses were recorded from the groups of DnaseX (wildtype – mutant) mice with two different light intensities. These two luminance levels were chosen because with 500 cd/m^2 a well discernable b-wave amplitude (nearly no a-wave) mainly stemming from the rod system is induced, while 12500 cd/m^2 induces 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 no major differences were observed between the left and right eye, ERG amplitudes of both eyes were averaged for further evaluation. The mean value and standard error was calculated for each group of mice, male and female, wildtype and mutant (Table 2.4.1).

Examination by slit lamp biomicroscopy indicated that most animals expressed minor lens opacities ranging from nuclear flecks to cloudy nuclear opacity (Table 2.4.2). Three males (2 -/0 and 1 +/-) expressed unilateral corneal opacity, and one female (-/-) expressed unilateral pre-retinal blood vessels.

Table 2.4.1: Comparison of ERG-responses at illumination levels of 500 and 12500 cd/m².

Mean ± standard error is calculated for a- and b-wave amplitudes; the amplitudes of left and right eyes were averaged.

<i>DnaseX</i>	mutant (-/0; -/-)			wildtype (+/0; +/+)			mut-wt	mut-wt
	male (n=10)	female (n=9)	p - value (m-f)	male (n=10)	female (n=10)	p - value (m-f)	male <i>p - value</i>	female <i>p - value</i>
a-wave 500 cd/m ²	-12 ± 1.8	-11 ± 1.5	n.s.	-12 ± 2.0	-9 ± 1.7	n.s.	n.s.	n.s.
b-wave 500 cd/m ²	180 ± 15.0	174 ± 13.0	n.s.	194 ± 9.5	167 ± 10.6	n.s.	n.s.	n.s.
a-wave 12500 cd/m ²	-43 ± 3.0	-32 ± 3.0	<0.02	-34 ± 1.8	-39 ± 3.3	n.s.	<0.05	n.s.
b-wave 12500 cd/m ²	215 ± 10.5	212 ± 12.6	n.s.	218 ± 11.6	187 ± 9.1	<0.05	n.s.	n.s.

Table 2.4.2: Results of slit lamp biomicroscopy

Genotype	No eye abnormality	Minor lens opacities
+/0, +/+	2	28
-/0, -/-	10	19

2.4.5 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 wildtype revealed no consistent differences between the groups (Table 2.4.1). Most of the p-values (T-test) calculated are not significant (Table 2.4.1). The significant differences in the comparisons of Male mutants vs Female mutants and Male wildtype vs Male mutants are both due to a single outlier within the Male mutant group. When this Male was excluded the differences between groups were no longer significant.

Results from slit lamp biomicroscopy indicated no correlation between genotype and the minor eye phenotypes. Based on our extensive mutagenesis screen for induced eye mutations, the minor eye phenotypes observed fall into the category of normal phenotypic variability. The high frequency of such phenotypes may reflect a genetic background effect.

References

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.

Abbreviations

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

2.5. Clinical-Chemical Screen

2.5.1 Summary

The aim of the Clinical-Chemical Screen was to detect hematological changes, defects of various organ systems, and changes in metabolic pathways and electrolyte homeostasis using appropriate routine laboratory diagnostic procedures that allow the screen of large numbers of mice for a broad spectrum of clinical-chemical and hematological parameters. In the primary Clinical-Chemical Screen, 28 (14 males/14 females) control mice and 29 (15 males/14 females) DnaseX-KO mice were analyzed. A total of 19 different clinical-chemical parameters were measured including various enzyme activities, as well as plasma concentrations of specific substrates and electrolytes. Additionally, we measured 8 basic hematological parameters. The comparison of DnaseX-knockout and control mice showed significant differences in sodium and chloride concentration. In contrast to control females, alanine-aminotransferase activity was significantly decreased in mutants. In mutants sex differences are more pronounced for urea, total protein, uric acid and alanin-aminotransferase as compared to controls. Therefore muscle, kidney or liver function might be influenced by the X-linked mutation. DnaseX mutant mice have also a tendency to a lower creatinin kinase activity but the results were not significant.

2.5.2 Mice

Fourteen 12-week old wildtype and fifteen 12-week old mutant males entered the Clinical-Chemical Screen at the beginning of the 12th calendar week. Fourteen wildtype female mice and fourteen mutant female mice entered the Clinical-Chemical Screen one week later.

2.5.3 Materials and Methods

All animals were fasted overnight before blood was drawn. Blood samples were collected into heparinized (500µl) and EDTA coated (50µl) tubes by retro-orbital puncture under ether anesthesia using heparinised glass capillaries. The samples for plasma analyses (heparinised blood) was mixed carefully, and an amount of 40µl was removed with an end-to-end capillary for the lactate analysis (Neurology Screen).

The samples were then left for two hours at room temperature before being centrifuged (7 minutes, 4656 x g). Subsequently the plasma was removed and divided into individual samples for the different screens: The Allergy and the Immunology screen received 30µl each, and 130µl plasma was reserved for the clinical-chemical analysis.

For clinical chemistry the plasma was diluted 1:2 with distilled water. The solution was mixed for a few seconds (Vortex genie) to prevent clotting, and centrifuged again for 10 minutes at 4656 x g. 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, 19 different parameters were measured, including various enzyme activities, as well as plasma concentrations of specific substrates and electrolytes.

A total of 50µl EDTA-treated blood was used directly after sample collection to measure basic hematological parameters with a blood analyzer, which was 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, while hemoglobin is quantified by spectrophotometry. Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) are calculated from hemoglobin/red blood cells count (MCH), hemoglobin/hematocrit (MCHC), and red blood cells count/hematocrit (MCV).

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

2.5.4 Results

One of the female wildtype animals was not analyzed for clinical chemical and hematological parameters, since blood collection did not yield a sample volume allowing to provide adequate amounts to all blood based screens. Additionally the clinical chemical data of one wildtype male had to be excluded from the statistical analysis, since this animal showed highly pathologic findings for most of the clinical chemical parameters (inorganic Phosphate, total protein, creatinine, urea, cholesterol, alanine-aminotransferase, aspartate-aminotransferase, alkaline phosphatase and glucose).

Clinical Chemistry

Most values obtained for the clinical chemical parameters were within the ranges found in healthy C57BL/6 mice at the age of three months, as supported by previously published data. Significant differences between mutants and controls and between males and females were detected for several parameters (Table 2.5.1). Specifically, differences between mutants and controls were found in blood glucose level in males, while significant differences in sodium and chloride concentration and alanine-aminotransferase activity were detected in mutant versus control females. Sex-dependent differences in the DnaseX-knockout mice were found for the following parameters: sodium, inorganic phosphate, total protein, creatinine, urea, uric acid, cholesterol,

alanine-aminotransferase, alkaline phosphatase, amylase, and ferritin. In the control animals sex differences in inorganic phosphate concentration, alkaline phosphatase activity as well as creatinine, cholesterol, glucose, ferritin and transferrin levels were seen.

Hematology

The primary screen for hematological parameters revealed several significant sex-dependent differences (Table 2.5.2): Sex differences in the parameters hematocrit and MCHC were detected in the DnaseX-mutants and control mice. Additionally a significant gender difference of MCH and platelet count were noted only in control animals. There were no significant differences between mutants and controls.

2.5.5 Discussion

Almost all values measured for all parameters of both, control and DnaseX knockout animals, were within the typical baseline ranges of C57BL/6 mice. An increase in urea concentrations and a decrease of plasma creatinine concentration was found in both the mutant and control male mice. Creatinine and urea are the principal nitrogenous wastes eliminated from the body by the kidneys; an increase in their plasma concentrations may be caused by pre-renal (increased protein catabolism, impaired renal blood flow), renal (reduced glomerular filtration rate), or postrenal causes (obstruction to urinary outflow). Significantly higher urea concentrations in the blood of male mice compared to females are normal in C57BL/6 mice. The elevated level of urea concentrations in the male mice could be due to an increased muscle metabolism resulting in a preferred combustion of proteins. Changes in food utilization can be determined by measuring the respiratory quotient, defined as the quotient of CO₂-production and oxygen consumption. This method is offered by the secondary metabolic screen. Sex differences for urea, total protein, uric acid and cholesterol were more pronounced in DnaseX mice than in the wildtype animals, hinting that protein metabolism, kidney function or liver function might be influenced by the X-linked gene. However, these findings reflect relations usually found in wildtype C57BL/6 mice.

Raw data will be available on demand.

Table 2.5.1: Results of clinical-chemical parameters, comparison of DnaseX knockout mice versus controls. Clinical-chemical data are shown as mean (\pm SEM); (n.s.=non significant)

	DnaseX -/- and -/0 (A)			Wildtype +/+ and +/0 (B)			A~B male	A~B female
	<u>male</u> (n=15)	<u>female</u> (n=14)	<i>p - value</i>	<u>male</u> (n=14)	<u>female</u> (n=14)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
Sodium mmol/l	161 \pm 0.70	158 \pm 0.51	<0.01	160 \pm 0.81	160 \pm 0.53	n.s.	n.s.	<0.05
Potassium mmol/l	3.8 \pm 0.08	3.6 \pm 0.10	n.s.	4.7 \pm 0.46	3.8 \pm 0.10	n.s.	n.s.	n.s.
Calcium mg/dl	2.2 \pm 0.02	2.2 \pm 0.02	n.s.	1.9 \pm 0.14	2.2 \pm 0.02	n.s.	n.s.	n.s.
Chloride mmol/l	111.6 \pm 0.77	112.2 \pm 0.42	n.s.	112.7 \pm 0.57	113.4 \pm 0.38	n.s.	n.s.	<0.05
Inorganic Phosphate mg/dl	1.8 \pm 0.06	1.6 \pm 0.06	<0.05	1.8 \pm 0.09	1.5 \pm 0.06	<0.02	n.s.	n.s.
Total Protein mg/dl	5.5 \pm 0.08	5.2 \pm 0.07	<0.05	5.3 \pm 0.10	5.2 \pm 0.08	n.s.	n.s.	n.s.
Creatinine mg/dl	0.335 \pm 0.01	0.363 \pm 0.01	<0.05	0.327 \pm 0.01	0.367 \pm 0.01	<0,01	n.s.	n.s.
Urea mg/dl	92.1 \pm 5.90	68.3 \pm 3.25	<0.01	84.2 \pm 2.18	77.5 \pm 3.40	n.s.	n.s.	n.s.
Uric acid mg/dl	0.9 \pm 0.10	2.2 \pm 0.25	<0.001	1.2 \pm 0.27	1.8 \pm 0.30	n.s.	n.s.	n.s.
Cholesterol mg/dl	106.3 \pm 3.70	86.1 \pm 2.93	<0.001	101.9 \pm 2.98	88.0 \pm 3.30	<0,01.	n.s.	n.s.
Triglyceride mg/dl	85.4 \pm 5.70	92.8 \pm 6.03	n.s.	74.4 \pm 10.91	84.6 \pm 7.90	n.s.	n.s.	n.s.
Creatinine Kinase mg/dl	57 \pm 17.00	73 \pm 30.36	n.s.	230 \pm 79.34	128 \pm 47.00	n.s.	n.s.	n.s.

	DnaseX -/- and -/0 (A)			Wildtype +/+ and +/0 (B)			A~B male	A~B female
	<u>male</u> (n=15)	<u>female</u> (n=14)	<i>p - value</i>	<u>male</u> (n=14)	<u>female</u> (n=14)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
Alanine- Aminotransferase (ALAT) U/l	18± 1.00	14± 0.59	<0.05	26± 4.27	18± 1.00	n.s.	n.s.	<0.01
Aspartate- Aminotransferase (AST) U/l	27± 2.00	28± 4.06	n.s.	42± 8.12	37± 6.00	n.s.	n.s.	n.s.
Alkaline Phosphatase U/l	154± 5.00	217± 7.80	<0.001	133± 9.78	223± 10.00	<0.001	n.s.	n.s.
α-Amylase U/l	3155± 193.00	2471± 188.33	<0.02	2714± 163.30	2692± 121.00	n.s.	n.s.	n.s.
Glucose mg/dl	125.4± 9.5	152.2± 11.62	n.s.	176.6± 19.36	124.9± 8.9	<0,05	<0,05	n.s.
Ferritin ng/ml	79.4± 6.7	119.3± 9.55	<0.01	79,7± 5.72	135.0± 14.5	<0.01	n.s.	n.s.
Transferrin mg/ml	160.7± 2.7	162.4± 2.41	n.s.	155.4± 1.88	163.3± 3.00	<0.05	n.s.	n.s.

Table 2.5.2: Results of blood parameters, comparison of DnaseX knock-out mice versus controls. Hematological data are shown as mean (\pm SEM); (n.s.=non significant)

	DnaseX -/- and -/0 (A)			Wildtype +/+ and +/0 (B)			A~B male	A~B female
	<u>male</u> (n=15)	<u>female</u> (n=14)	<i>p - value</i>	<u>male</u> (n=14)	<u>female</u> (n=14)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
White blood cell count x 10³/μl	4.20 \pm 0.35	4.34 \pm 0.47	n.s.	3.95 \pm 0.40	4.36 \pm 0.49	n.s.	n.s.	n.s.
Red blood cell count x10³/μl	10.02 \pm 0.67	9.54 \pm 0.31	n.s.	9.92 \pm 0.62	9.57 \pm 0.37	n.s.	n.s.	n.s.
Hemoglobin g/dl	15.22 \pm 0.28	14.12 \pm 0.45	n.s.	15.14 \pm 0.15	14.18 \pm 0.52	n.s.	n.s.	n.s.
Hematocrit %	48.10 \pm 0.91	43.39 \pm 1.35	<0.01	48.06 \pm 0.47	43.42 \pm 1.66	<0.02	n.s.	n.s.
Mean corpuscular volume fl	45.53 \pm 0.56	45.00 \pm 0.25	n.s.	45.6 \pm 0.31	45.36 \pm 0.29	n.s.	n.s.	n.s.
Mean corpuscular hemoglobin Pg	14.37 \pm 0.19	14.81 \pm 0.12	n.s.	14.36 \pm 0.12	14.84 \pm 0.12	<0.01	n.s.	n.s.
Mean corpuscular hemoglobin concentration g/dl	31.65 \pm 0.15	32.57 \pm 0.15	<0.001	31.54 \pm 0.13	32.71 \pm 0.16	<0.001	n.s.	n.s.
Platelet count x 10³/μl	899 \pm 87.95	684 \pm 64.01	n.s.	933 \pm 34.97	728 \pm 66.99	<0.02	n.s.	n.s.

Abbreviations:

ALAT	=	alanine-aminotransferase
AST	=	aspartate-aminotransferase
CK	=	creatine kinase
CK-MB	=	creatine kinase- muscle/brain (heart muscle specific)
CO ₂	=	carbon dioxide
EDTA	=	ethylene diamine tetra acetate (edetate)
KO	=	knock-out
MCH	=	mean corpuscular hemoglobin
MCHC	=	mean corpuscular hemoglobin concentration
MCV	=	mean corpuscular volume

2.6. Immunology Screen

2.6.1. Summary

According to the data summary presented to the phenotyping center, no immunological phenotype was known in DnaseX-deficient mice. Their analysis in the Immunology Screen could also not reveal profound differences between knockouts (ko) and their littermate controls.

2.6.2. Mice

We analyzed 29 ko animals (14 females and 15 males) and the same number of age- and sex-matched littermate controls.

2.6.3. Material and Methods

Peripheral blood leukocytes (PBLs) were isolated from 500 μ l blood (details on the blood taking procedure are described in part 2.5.3.) 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 ($\text{CD}19^+$ clone 1D3), B1 B cells ($\text{CD}19^+\text{CD}5^+$, clone 53-7.3), B2 B cells ($\text{CD}19^+\text{CD}5^-$), T cells ($\text{CD}3^+$, clone 145-2C11), $\text{CD}4^+$ T cells (clone RM4-5), $\text{CD}8^+$ T cells ($\text{CD}8\alpha$, clone 53-6.7; $\text{CD}8\beta$, clone H35-17.2), γ/δ T cells (clone GL3), granulocytes (Gr-1^+ , clone RB6-8C5), and NK cells ($\text{CD}49b^+$, 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 analyzed using FlowJo software (TreeStar Inc, USA). All samples were acquired until a total number of 25000 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, Stein-

heim, 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.

2.6.4. Results

Table 2.6.1.: Basic parameters analyzed in the Immunology Screen. Data are presented as mean \pm standard error of mean.

Parameter	DnaseX ko (-/0, -/-)			DnaseX controls (+/0, +/+)			ko vs controls	
	male (n=15)	female (n=14)	p - value	male (n=15)	female (n=14)	p - value	male p - value	female p - value
CD19 ⁺ (%)	57.3 \pm 8.9	55.4 \pm 9.3	n.s.	63.6 \pm 5.8	58.2 \pm 4.9	<0.02	<0.05	n.s.
CD19 ⁺ CD5 ⁻ (%)	97.6 \pm 1.1	98.8 \pm 0.75	<0.01	98.2 \pm 1.1	98.5 \pm 0.85	n.s.	n.s.	n.s.
CD19 ⁺ CD5 ⁺ (%)	2.4 \pm 1.1	1.2 \pm 0.75	<0.01	1.8 \pm 1.1	1.5 \pm 0.85	n.s.	n.s.	n.s.
CD3 ⁺ (%)	28.7 \pm 7.5	29 \pm 5.4	n.s.	26.7 \pm 6.1	26.8 \pm 5.3	n.s.	n.s.	n.s.
γ/δ TCR ⁺ (%)	1.9 \pm 1.3	0.19 \pm 0.12	<0.001	1.2 \pm 0.5	0.18 \pm 0.01	<0.001	n.s.	n.s.
Gr-1 ⁺ (%)	14 \pm 8	11.7 \pm 9	n.s.	10.5 \pm 4.6	12.6 \pm 2.4	n.s.	n.s.	n.s.
CD49b ⁺ (%)	28.3 \pm 14.2	17.8 \pm 11.3	<0.05	19.3 \pm 8.6	18.6 \pm 11.3	n.s.	n.s.	n.s.
CD4 ⁺ (%)	16.1 \pm 5	18 \pm 1.6	n.s.	15.3 \pm 2.9	16.5 \pm 4.1	n.s.	n.s.	n.s.
CD8 β ⁺ (%)	10.7 \pm 0.9	11.9 \pm 1.5	n.s.	9.6 \pm 0.8	10.5 \pm 2.6	n.s.	n.s.	n.s.
IgG ₁ (μ g/ml)	327.9	113.5	<0.01	285.3	133.3	<0.02	n.s.	n.s.
IgG _{2a} (μ g/ml)	273	101.1	<0.001	177.5	53.9	<0.01	n.s.	n.s.
IgG _{2b} (μ g/ml)	97.3	90.5	n.s.	110.8	129.5	n.s.	n.s.	n.s.
IgG ₃ (μ g/ml)	384.6	260	n.s.	339	378.7	n.s.	n.s.	n.s.
IgM (μ g/ml)	60.4	34.4	n.s.	36.8	62.8	n.s.	n.s.	n.s.
IgA (μ g/ml)	410.1	362.6	n.s.	431.9	356.7	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.

Abbreviation used in the table: *versus* (vs)

The analysis of standard immunological parameters measured in the primary screen (Table 2.6.1.) did not reveal significant differences between DnaseX-deficient mice and their littermate controls. One parameter was different between the analyzed cohorts of mice: the percentage of CD19⁺ cells (B cells) was slightly decreased in

DnaseX ko males. However, since this minor difference was only found for the CD19 antigen, whereas all other B cell markers remained within normal values, it is very unlikely that it indicates a true phenotypical difference in male DnaseX ko mice.

No significant differences could be detected with regard to the other cell surface antigens included in the screen.

2.6.5. Discussion

Under standard screen conditions, DnaseX mice did not show profound changes in immunological parameters. However, because of the assumed function of DnaseX in apoptosis, further immunological investigations in a varied setting could be interesting.

Raw data will be available on demand.

2.7. Allergy Screen

2.7.1. 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.

In the primary Allergy screen of *DnaseX* mice 27 wildtype and 29 knockout animals were screened. The analysis of *DnaseX* mice in Allergy screen did not reveal any profound differences between knockout and wildtype mice.

2.7.2. Mice

An age- and sex-matched group of 27 wildtype (14 females, 13 males) and 29 knockout (14 females, 15 males) mice aged 12 weeks was analysed in Allergy screen. Before taking blood samples all animals were food-restricted overnight.

2.7.3. Material and Methods

12-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 anaesthesia. 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 (BSA) 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, TMB (tetramethylbenzidine) substrate solution was added and after an appropriate incubation time the stop solution (sulphuric acid, 2M) was added and 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.

2.7.4. Results

The analysis of total IgE levels in plasma (mean \pm SE) of *DnaseX*-ko mice and their sex- and age-matched wildtype littermates revealed lower mean IgE levels in k.o. animals. However, this difference was statistically not significant. The plasma IgE concentration of female wildtype and k.o. mice were higher than in males. Again, no

statistical significance of this finding was revealed (Table 7.1.1). Raw data will be available on demand.

	<i>DnaseX</i>							
	Wildtype(A)			Knockout(B)			A~B p-value	A~B p-value
	F n=14	M n=13	p-value	F n=14	M n=15	p-value	F	M
Total IgE (ng/ml)	91± 26.3	74± 23.9	n.s.	62± 13	55± 12.5	n.s.	n.s.	n.s.

Table 7.1.1 : Mean concentration ± SE of total plasma IgE in *DnaseX* mice

2.7.5. Discussion

The mean concentration of total plasma IgE of both *DnaseX*-k.o. and wildtype animals was close to the normal range for total IgE in C57BL/6 mice established in our laboratory (females 87.6 ± 20.12 ng/ml vs. males 30.3 ± 4.41 ng/ml). No statistically significant difference between *DnaseX*-k.o. and wildtype mice was found. In both *DnaseX*-k.o. and wildtype animals, the mean concentration of total IgE in females was higher than in males, but again without statistical significance.

Taken together, under standard screening conditions for primary Allergy screen, *DnaseX*-k.o. mice did not show changes in total plasma IgE levels that would reveal a major allergy phenotype. However, since *DnaseX* has a presumed function in apoptosis, further allergological investigations using an allergen challenge screen should be considered.

2.8. Nociceptive Screen

2.8.1. Summary

In the first screen we tested the responsiveness of the intact somatosensory system to the thermal pain of the DnaseX knock out mice, and of the wildtype animals. The hot plate test was used as the primary screen in the nociception phenotyping module of the GMC. We found no significant difference in pain sensitivity between the knock out and the wildtype mice.

2.8.2. Mice

28 DnaseX knock-out mice (14 male (-/0), 14 female (-/-)), and 29 control animals (14 male (+/0), 15 female (+/+)) were tested in our first screen.

2.8.3. 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). 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 behaviours 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 behaviour. Each mouse was tested only once since repeated testing leads to profound latency changes. The response-latency was measured 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 $p < 0.05$.

2.8.4. Results

We found no difference in thermal nociceptive threshold between different genotypes. For more results see Table 2.8.1.

2.8.5. Discussion

There was no difference in pain sensitivity of DnaseX knock out mice compared to the wild type animals.

They do not need to be tested in a secondary nociception screen.

Raw data will be available on demand

Table 2.8.1. Results from nociceptive screen:

male F(2,25)=0,3357 p=0,71798	DnaseX male			DNaseX female			male/female		male/female	
	(A)-/0 (n=15)	(B)+/0 (n=14)	<i>p - value</i>	(C)-/ (n=14)	(D)+/ (n=14)	<i>p - value</i>	A~C <i>p - value</i>	B~D <i>p - value</i>	A~D <i>p - value</i>	B~C <i>p - value</i>
h.p.licking	21,69 ±2,62	17,2 ±2,71	n.s.	18,43 ±2,86	21,12 ±2,67	n.s.	n.s.	n.s.	n.s.	n.s.
h.p.shaking	10,76 ±1,2	10,12 ±1,31	n.s.	11,37 ±1,31	10,97 ±1,22	n.s.	n.s.	n.s.	n.s.	n.s.
jumping	59,67 ±0,33	60 ±0	n.s.	60 ±0	60 ±0	n.s.	n.s.	n.s.	n.s.	n.s.

2.9. LUNG FUNCTION SCREEN

2.9.1. Summary

Spontaneous breathing pattern was measured in female wildtype and knockout mice by applying whole body plethysmography. Neither measurements made during sleep nor during activity revealed significant differences between groups.

2.9.2. Mice

The workflow of the screen provided female mice (5 $-/-$ and 8 $+/+$) to the LUNG FUNCTION SCREEN at an age of 15 weeks. Body weight of wildtype mice was 24.1 ± 1.9 g, while that of knockout mice was 22.6 ± 0.7 g (mean \pm SEM, ns).

2.9.3. Material and Methods

Whole body plethysmography: The principle described by Drorbaugh and Fenn (1955) was applied for measuring breathing patterns in unrestrained animals. The commercially available system from Buxco[®] Electronics (Sharon, Connecticut) was used. It measured pressure changes due to inspiratory and expiratory temperature and humidity fluctuations during breathing. Calibration of the system allowed transformation of the pressure swings into flow and volume signals so that automatic data analysis provided tidal volume (TV), respiratory rate (f), minute ventilation (MV), inspiratory and expiratory time (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.

Measurements were always performed between 8 am and 11 am to exclude possible 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 supplied to ensure accurate calculations of flow rates and volumes. After placing an animal into the chamber data recording was immediately started and continued for 40 minutes. The mice underwent typical phases during the measuring period. At first, the animals were stressed, leading to a high breathing rate. After 5 minutes the animals usually became calmer, reduced their respiratory rate, and began to explore the chamber and clean themselves – (phase of activity). Later activity was more and more in-

interrupted by phases of sleep, which resulted in further decreases in respiratory rate. The frequency histogram of the respiratory rate was determined for each individual, and breathing was analyzed for the above mentioned parameters during phases of activity and sleep. In addition, mean inspiratory and expiratory flow rates (MEF, MIF) were calculated offline from the ratio of tidal volume and the respective time interval for both phases. The relative duration of inspiration (Ti/TT) was determined from the ratio of inspiratory time to total time required for the breathing cycle. Specific tidal volume and minute ventilation (sTV, sMV) were calculated from the respective absolute values and the body weight of the animals. Furthermore, the mean of the breathing frequencies measured during the 40 minute period was calculated as a rough and ready parameter to assess whether wildtype and knockout mice exhibit similar durations of sleep and activity.

Statistical analysis of data: Statistical analyses were performed using a commercially available statistical package (Statgraphics®, Statistical Graphics Corporation, Rockville, MD). Differences between strains 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).

2.9.4. Results

At the time of measurement the animals were 15 weeks old and did not exhibit any significant differences in body weight. During sleep as well as during activity, all twelve measured parameters characterizing the spontaneous breathing pattern were similar in both groups (Table, Lung function). The means of the breathing frequencies during the entire measuring period was comparable between wildtype and knockout mice ($430 \pm 14 \text{ min}^{-1}$ vs. $439 \pm 11 \text{ min}^{-1}$, ns).

2.9.5. Discussion

These data showed that the breathing pattern in female DnaseX knockout mice was not altered during activity or sleep. The fact that ventilation and specific ventilation was comparable in the two groups suggested that oxygen demand was similar in wildtype and knockout mice and that oxygen uptake in the lungs was not affected by the mutation. The level of activity also appeared to be unaffected in DnaseX mice, since the mean breathing frequency was not altered. However, this estimation of activity should be valued as a rough easily available parameter and compared to findings determined in the behavior screen.

Raw data will be available on demand.

2.9.6. References

Drorbaugh JE, Fenn, WO. A barometric method for measuring ventilation in newborn infants. *Pediatrics* 16:81-87, 1955

Table 2.9.1 Lung function – Spontaneous breathing pattern

Parameter	<u>Wildtype</u> (n=8)	<u>DnaseX</u> (n=5)	<i>p - value</i>
bw	24.1 ± 1.9	22.6 ± 0.7	n.s
sleep			
f	248.5 ± 9.1	270.4 ± 5.2	n.s.
TV	0.28 ± 0.02	0.30 ± 0.02	n.s.
sTV	12.3 ± 1.0	13.3 ± 1.1	n.s.
MV	67.7 ± 5.3	79.3 ± 3.2	n.s.
sMV	3.0 ± 0.2	3.5 ± 0.2	n.s
Ti	72.3 ± 5.6	62.6 ± 2.9	n.s.
Te	171.1 ± 6.6	159.6 ± 3.9	n.s.
Ti/TT	0.30 ± 0.02	0.28 ± 0.01	n.s.
PIF	6.8 ± 0.6	7.8 ± 0.3	n.s.
PEF	4.4 ± 0.4	4.6 ± 0.2	n.s.
MIF	3.9 ± 0.3	4.8 ± 0.2	n.s
MEF	1.6 ± 0.1	1.9 ± 0.1	n.s.
activity			
f	516.0 ± 8.4	485.0 ± 14.7	n.s
TV	0.24 ± 0.01	0.27 ± 0.01	n.s.
sTV	10.1 ± 0.7	12.0 ± 0.9	n.s.
MV	119.0 ± 5.6	127.5 ± 9.0	n.s.
sMV	5.1 ± 0.7	5.7 ± 0.5	n.s
Ti	41.8 ± 0.6	43.2 ± 0.9	n.s.
Te	74.7 ± 2.0	81.0 ± 3.4	n.s
Ti/TT	0.36 ± 0.01	0.35 ± 0.01	n.s
PIF	9.6 ± 0.3	10.4 ± 0.6	n.s.
PEF	7.1 ± 0.4	7.4 ± 0.6	n.s.
MIF	5.6 ± 0.2	6.2 ± 0.4	n.s.
MEF	3.2 ± 0.2	3.3 ± 0.6	n.s.

Abbreviations

bw, body weight (g), **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).

2.10 Expression profiling

The molecular phenotyping screen archives organs of mutant mice for subsequent DNA-chip expression profiling analysis.

Nine male mice of the DnaseX strain 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 am and 12 am by carbon dioxide asphyxiation. The following 17 organs were collected and archived in liquid nitrogen following our established SOPs: 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.

mouse ID	strain	sex	date of birth	genotype	date of collection	Body weight [g]
30006712	DnaseX	m	01.01.2003	-/0	08.04.2003	30
30006714	DnaseX	m	02.01.2003	+/0	08.04.2003	20
30006716	DnaseX	m	02.01.2003	+/0	08.04.2003	23
30006717	DnaseX	m	02.01.2003	+/0	08.04.2003	19
30006718	DnaseX	m	02.01.2003	+/0	08.04.2003	26
30006709	DnaseX	m	01.01.2003	-/0	08.04.2003	20
30006704	DnaseX	m	01.01.2003	-/0	08.04.2003	25
30006710	DnaseX	m	01.01.2003	-/0	08.04.2003	26
30006711	DnaseX	m	01.01.2003	-/0	08.04.2003	30

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.

SOPs: (Standard operating protocols)

2.11. METABOLIC SCREEN

2.11.1. Summary

In the primary metabolic screen 14 (7 female/7 male) wildtype mice and 14 (7 female/7 male) DnaseX-KO mice were analyzed. They were first fed under *ad libitum* conditions for two weeks, followed by one week of food restriction to 60%. The primary metabolic screen investigates the metabolic demands of mice by determining daily body weight, energy uptake, metabolizable energy, body temperature, and adaptive capacity of metabolic processes. While wt mice did not show any adaptation to lowered food supply, KO-males increased food assimilation due to lowered feces production and developed hypothermia during food restriction, indicating a lowered basal metabolic rate.

2.11.2. Mice

Seven adult control males (DnaseX wt) and seven adult mutant males (DnaseX-KO) entered the metabolic screen at the beginning of calendar week 18. The females (7/7) 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% for 7 days to analyze adaptive responses of metabolism.

2.11.3. Material and Methods

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 food assimilation coefficient (F_{ass}) were recorded.

The separation of mice into 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 grinder and squeezed into a pill form for determination of energy content in a bomb calorimeter (IKA Calorimeter C7000) based on the dry measurement principle. E_{up} was determined as the product of food consumed and the caloric value of the food. To obtain a value for metabolizable energy (E_{met}), the energy content of feces and urine (2% of E_{up} , Drozd 1975) were subtracted from energy uptake.

All values are presented as means \pm SEM. The following statistical tests were applied: Two-way-ANOVA (SigmaStat, Jandel Scientific) to test for effects of

the factors strain and gender; the Tukey test for post hoc multiple comparison; and the Mann-Whitney-Test (for paired samples) to analyze the effect of nutritional status on parameters of energy metabolism.

2.11.4. Results

Both KO and wt males were heavier than females and have lower body temperature. The latter was significantly reduced in males, but not in females, during food restriction. E_{up} and E_{met} per unit body weight was higher in KO females than control females. While the E_{fec} was lower in KO females under food restriction compared to ad libitum feeding, the Fec was significantly elevated compared to KO males. This decreased Fec led to a significant increase in F_{ass} in KO males. However, there was no statistical difference between KO and the control animals.

2.11.5. Discussion

No information about metabolic parameters was available prior the metabolic screening of DnaseX-KO mice. Statistically significant differences in the metabolic screen were mostly sex-, rarely strain-specific (Table). Males were heavier than females in both groups, but knock outs did not differ from wild-type mice. Interestingly, both ko and wt males had significantly lower body temperatures than the females. However, females also showed higher food and energy intake than males, with significantly higher values in DnaseX-females than in wildtype females.

Feces production per day was reduced in KO males and females during food restriction, while the males produced significantly less than the females ($p < 0.01$). The caloric value of feces under food restriction was significantly lower in KO –females. In KO males the caloric value did not differ from wt males, but because of the strong reduction of feces production the assimilation coefficient increased in DnaseX-KO males indicating a physiological response to lowered food supply ($p < 0.05$).

The significantly lower energy uptake per gram body weight and the lower body temperature in males of both strains indicate lower basal metabolic rates. The development of hypothermia in response to food restriction is a further indication of lower metabolic rate.

To prove a shift of energy metabolism during food restriction in more details, simultaneous measurements of oxygen consumption, carbon dioxide production, body temperature and activity measurements would be required.

2.11.6. Abbreviation

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 food assimilation coefficient (F_{ass})

2.11.7. References

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

	DnaseX, wt					Dnase-X, KO					BI6~DnaseX	
	ad libitum			food reduction, 7 days to 60%		ad libitum			food reduction, 7 days to 60%		male	fe-male
	male (n=7)	fe-male (n=7)	<i>p</i> -value	male (n=7)	fe-male (n=7)	male (n=7)	fe-male (n=7)	<i>p</i> -value	male (n=7)	fe-male (n=7)	<i>p</i> -value	<i>p</i> -value
Body weight (g)	29.4 ± 0.65	22.8 ± 0.95	<0.001	24.8 ± 0.32	19.8 ± 0.94	32.8 ± 1.96	24.24 ± 0.74	<0.001	27.0 ± 1.64	20.4 ± 0.61	n.s.	n.s.
Rectal body temperature (°C)	35.1 ± 0.15	35.9 ± 0.15	<0.01	33.14 ± 0.55	35.3 ± 0.39	35.2 ± 0.17	35.9 ± 0.08	<0.01	33.6 ± 0.71	35.6 ± 0.3	n.s.	n.s.
Food consumption (g day⁻¹)	3.4 ± 0.15	3.5 ± 0.12	n.s.	60% of <i>ad libitum</i>		3.6 ± 0.17	3.8 ± 0.08	n.s.	60% of <i>ad libitum</i>		n.s.	<0.05
Energy uptake (kJ day⁻¹)	63.2 ± 0.99	65.3 ± 2.26	n.s.	37.9 ± 0.59	39.2 ± 1.35	67.4 ± 3.21	71.7 ± 1.41	n.s.	40.4 ± 1.93	43.0 ± 0.84	n.s.	<0.05
Energy uptake g Bw⁻¹ (kJ g⁻¹ day⁻¹)	2.15 ± 0.06	2.87 ± 0.09	<0.001	1.52 ± 0.03	1.99 ± 0.06	2.09 ± 0.14	2.97 ± 0.08	<0.001	1.52 ± 0.11	2.12 ± 0.06	n.s.	n.s.
Feces production (g day⁻¹)	0.7 ± 0.02	0.7 ± 0.03	n.s.	0.4 ± 0.01	0.39 ± 0.02	0.7 ± 0.02	0.78 ± 0.03	<0.05	0.36 ± 0.02	0.45 ± 0.02	n.s.	n.s.
Energy content feces (kJ g⁻¹)	16.09 ± 0.09	15.89 ± 0.05	n.s.	15.9 ± 0.16	15.9 ± 0.06	16.04 ± 0.05	15.74 ± 0.04	<0.001	16.02 ± 0.1	15.47 ± 0.1	n.s.	<0.05
Metabolized energy(kJ day⁻¹)	52.16 ± 1.0	54.44 ± 1.88	n.s.	31.59 ± 0.52	32.48 ± 1.18	56.4 ± 2.99	56.69 ± 1.37	n.s.	34.4 ± 1.67	36.22 ± 0.81	n.s.	<0.05
Metabolized energy g Bw⁻¹ (kJ g⁻¹ day⁻¹)	1.78 ± 0.05	2.39 ± 0.07	<0.001	1.27 ± 0.03	1.68 ± 0.05	1.75 ± 0.12	2.47 ± 0.08	<0.001	1.3 ± 0.09	1.78 ± 0.06	n.s.	n.s.
Food assimilation coefficient (%)	82.5 ± 0.6	83.4 ± 0.2	n.s.	83.3 ± 0.6	84.5 ± 0.5	83.6 ± 0.5	83.2 ± 0.6	n.s.	85.1 ± 0.3	84.1 ± 0.6	n.s.	n.s.

2.12. Pathology Screen

2.12.1. Summary

The most important histological findings revealed in the Pathology screen of DnaseX mice were in the urinary tract. In eight animals a segmental or total atrophy of the kidney(s) associated with hydronephrosis was identified, however, three of these mice were wild type. In addition, nine animals (six were knockout, and three wild type), all males, showed urinary bladder dilatation. The distribution of the urinary lesions between the control and knockout mice suggests that these lesions are specific for the genetic background and not due to the DnaseX-mutation.

2.12.2. Mice

A total of 59 mice, 28 knockout mice (14 female, 14 male) and 31 control animals (15 females, 16 males) were analyzed. Due to the workflow in the GMC, mice of different ages were received from four different screens (see Table below). The term “other screens” is used when a mouse was received from any other screen not listed in Table 1.

Table 2.12.1: DnaseX and their control littermates.

	DnaseX		Control littermates		Number of animals	Age (days)
	-/0 Males	-/- Females	+/0 Males	+/+ Females		
LUNG SCREEN	0	5	0	5	10	96-102
EXPRESSON PROFILING	5	0	4	0	09	96-97
DYSMORPHOLOGY SCREEN	3	2	2	2	09	121-130
METABOLISMUS SCREEN	6	7	7	7	27	136-149
OTHER SCREENS	0	0	3	1	04	Not specified
Number of animals	14	14	16	15	59	

2.12.3. Materials and Methods

Mice received in the pathology laboratory 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 mu thick sections from skin, heart (longitudinal and cross-sections), muscle, lung, brain, cerebellum, thymus, spleen, lymph nodes, thyroid, parathyroid, adrenal gland, stomach, intestine, liver, pancreas, kidney, reproductive organs, and urinary bladder were cut and stained with haematoxylin and eosin (H&E). When indicated, the femur was also embedded (longitudinal and cross-section). Immunohistochemistry (IHC) was performed with an immunostainer (Ventana Medical Systems, Inc., Tucson AZ). The slides were deparaffined 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: Tdt and CD3 (DAKO, Hamburg, Germany), and B220 (Pharmingen, Germany) The presence of Mouse Hepatitis Virus (MHV) was serologically investigated using an immunofluorescence assay (1:20) by BioDoc, Hannover.

2.12.4. Results

2.12.4.1. Urinary tract

In nine animals (see Table 2), morphological changes of the kidneys, consisting of segmental tubular atrophy and focal renal dysplasia to complete atrophy associated with hydronephrosis were observed. The term hydronephrosis used here signifies the combination of obstructive pelvic dilatation and obstructive renal disease. The alterations ranged from very small foci in one kidney to complete atrophy of both kidneys. The glomerula were well preserved, but the tubuli were atrophic with increased interstitial fibrous tissue (see figure 2.12.1). In three cases a severe inflammatory infiltrate was observed. These changes, in general, are secondary to obstructive urinary tract disease. However, in two mice, a diagnosis of segmental atrophy with renal dysplasia cannot be excluded due to the focal alteration (Figure 2.12.2).

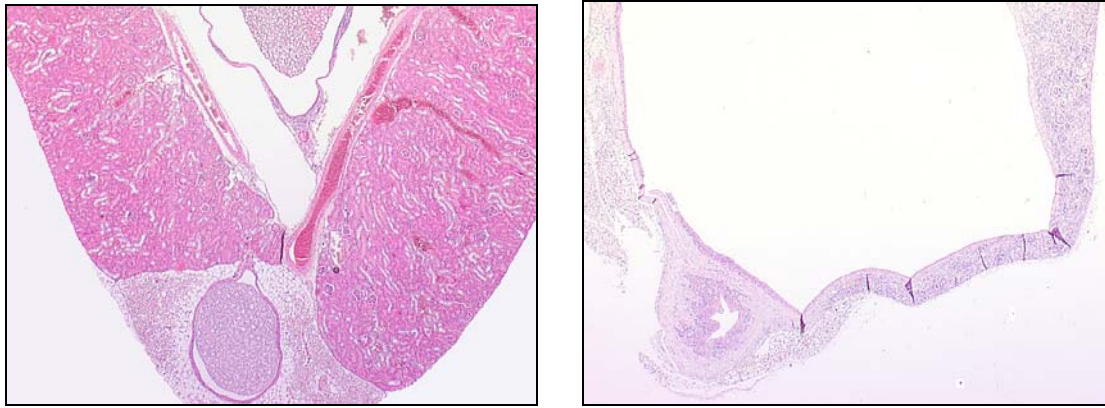


Figure 2.12.1: Hydronephrosis. Left: Normal developed kidney in a wildtype mouse. Right: The renal pelvis is dilated with complete tubuli atrophy in a DnaseX mouse. (H&E, 25x)

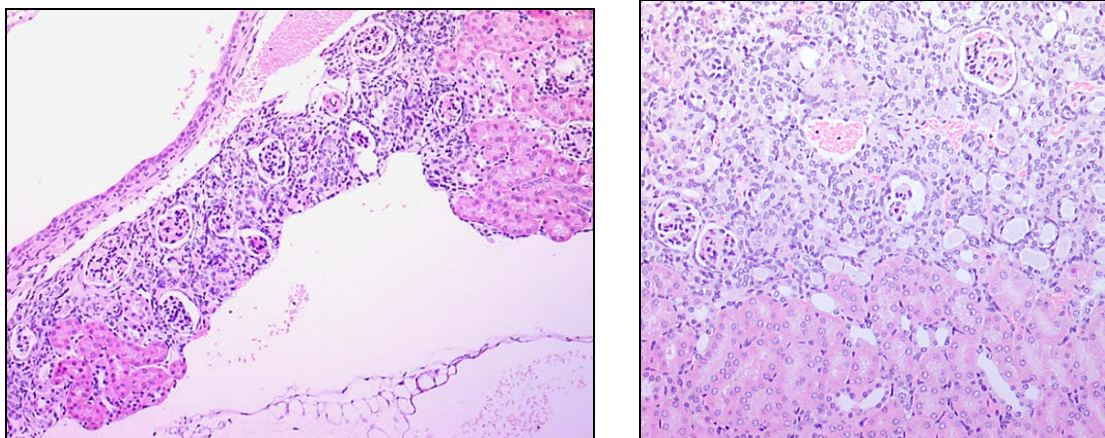


Figure 2.12.2: Segmental atrophy with renal dysplasia. Left picture shows atrophic tubules with increased interstitial fibrous tissue alternating with normal tubuli and glomerula (H&E, 40X). Right picture shows a higher magnification of the border between atrophic tubuli (above) and normal renal tubules and interstitium (lower side). (H&E, 160 X)

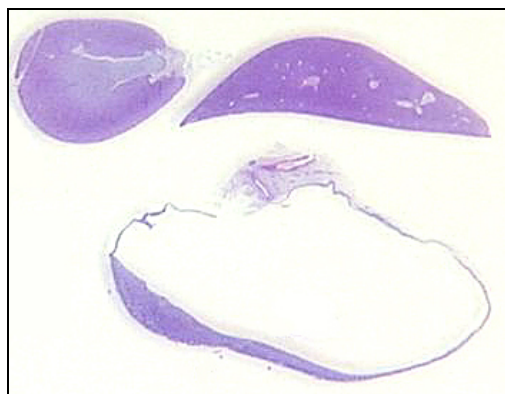


Figure 2.12.3: Macroscopic picture of a slide comparing size and structure of the kidneys. Normal kidney is shown in the upper left corner. The hydronephrotic kidney (below) presents a dilated pelvis with atrophic nephropathy. A normal liver section is shown in the upper right corner. (H&E, 10x)

Table 2.12.2: Mice with segmental or total atrophy and hydronephrosis.

Sex	Age (days)	Genotype	Severity	Urea (Normal range: 60 – 80 mg/dl)
male	97	-/0	segmental (one kidney analyzed)	147 mg/dl
male	142	-/0	bilateral segmental	141 mg/dl
male	136	+/0	unilateral total (with chronic infiltrate)	87,6 mg/dl
male	137	-/0	unilateral segmental (with chronic and acute infiltrate)	102 mg/dl
male	142	+/0	bilateral segmental	93,4 mg/dl
female	102	-/-	unilateral segmental	84,6 mg/dl
female	102	-/-	unilateral total	90,2 mg/dl
female	149	+/+	unilateral focal	65 mg/dl
female	126	-/-	bilateral segmental (with acute infiltrate)	88,2 mg/dl

The right column gives urea values from the clinical-chemical screen. Out of 15 animals with increased urea levels, nine mice also showed a renal phenotype ($p < 0.00000034$). One male only developed dilatation of the bladder.

A second finding was the dilatation of the urinary bladder affecting exclusively male animals (see Table 2.12.3). Due to increased pressure within the bladder, the epithelium flattens and the wall looks thinner (Figure 2.12.4).

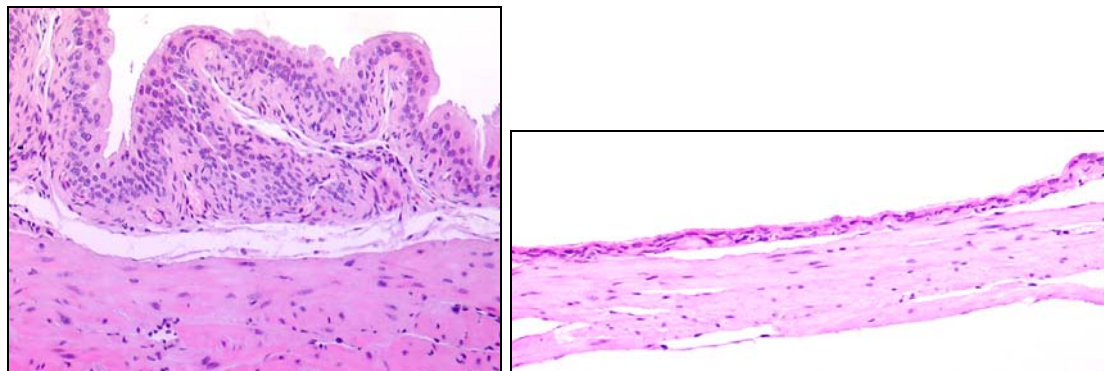


Figure 2.12.4: Left: Normal wall of the urinary bladder: Tunica adventitia (not shown), Tunica muscularis and Tunica mucosa composed of Lamina propria (light band in the middle of the picture) and transitional epithelium (H&E, 100x) Right: Atrophy of Tunica muscularis and Tunica mucosa in an animal with dilatation of the urinary bladder. (H&E, 100x)

Table 2.12.3: Dilatation of the urinary bladder.

Sex	Age (days)	Genotype	Lactate (Normal range 2 - 4 mmol/l)
male	142	+/0	8,67 mmol/l
male	142	-/0	7,51 mmol/l
male	142	-/0	6,97 mmol/l
male	142	-/0	5,86 mmol/l
male	121	-/0	4,42 mmol/l
male	139	-/0	not measured
male	138	+/0	3,38 mmol/l
male	137	-/0	2,07 mmol/l
male	136	+/0	3,99 mmol/l

The right column gives results from the neurology screen. Out of 13 animals (5 females and 8 males) with increased lactate values, 5 males developed a bladder dilatation (Fisher's Exact Test: $p < 0.0918$).

2.12.5. Discussion

An increased susceptibility to “obstructive nephropathy” was identified in this screen, including hydronephrosis with total or segmental tubular atrophy and urinary bladder dilatation. The correlation between the morphological changes and increased urea values were highly statistically significant (Fisher's Exact Test: $p < 0.00000034$), indicating that the renal function was decreased in these animals. However, renal alterations were present in both wildtype and knockout mice. Even though, urinary bladder dilatation tended to occur more often in the knockout mice (six vs. three controls), this was not statistically significant. Nevertheless, this latter finding is gender-specific, since only males were affected. This means that the renal phenotype found here is strain- and not line-specific. The genotype of the affected mice was double-checked confirming the presence of affected wild type animals. Interestingly, such renal alterations were never observed in analysis of about 100 non-manipulated C57BL/6J mice. To our knowledge, the renal alterations observed have not been recognized as part of the normal phenotype in wild type C57BL/6J or 129/Ola mice. In humans, segmental atrophy and dysplasia of renal tubules have been associated with urinary reflux, most probably secondary to obstruction, even though the association cannot be confirmed in all the cases. Furthermore, in the DnaseX line, dilatation of the urinary bladder was not necessarily linked to the hydronephrosis, suggesting alterations at different levels of the urinary tract.

3. Secondary Screen: First Results from Neurology Screen

3.1. Summary

Due to our significant findings in the primary screen, we performed a secondary screen of DnaseX mice to detect further phenotypic differences between mutants and controls. Thirty-seven DnaseX $-/-$ mice (21 males/16 females) and 24 DnaseX $+/+$ mice (10 males/14 females) were analysed in the secondary neurological screen. These animals were tested for motor coordination and motor learning abilities on the Rotarod. Additionally, forelimb grip strength was measured using the motor performance grip test.

Strain and sex differences were observed in grip strength and motor performance. Female DnaseX $-/-$ mice had a significantly lower forelimb grip strength as compared to female DnaseX $+/+$ mice. Male DnaseX $-/-$ mice had also reduced forelimb grip as compared to male DnaseX $+/+$ mice, but the results were not significant. Rotarod screening of females revealed that in contrast to DnaseX $+/+$ mice, DnaseX $-/-$ mice had a significantly decreased motor ability. No significant differences in Rotarod testing were found between male DnaseX $-/-$ and DnaseX $+/+$ mice.

3.2 Mice

Twenty-one male DnaseX $-/-$ mice, 10 male DnaseX $+/+$ mice, 16 female DnaseX $-/-$, and 14 female DnaseX $+/+$ mice entered the neurological screen at the beginning of 39th calendar week (minimum age of all animals – 22 weeks). Male mice were single-caged because of their aggressiveness. All animals were fed *ad libitum* for a period of 4 weeks during their stay in the neurological screen.

3.3 Material and Methods

The grip strength meter system determines the forelimb grip strength i.e. muscle strength of a mouse. The device exploits the tendency of a mouse to grasp a horizontal metal bar while being pulled by its tail. During the trial set-up, the mouse grasps a special adjustable grip (2 mm) mounted on a force sensor. The sensor allows measurements of up to 600 Ponds. Fifteen trials were undertaken for each mouse within two minutes. The mean value of ten maximal force values were used to represent the grip strength of a mouse.

The grip strength was normalized to body weight. All experimental equipment was thoroughly cleaned with Pursept-A and dried prior subsequent tests. Values were presented as means \pm standard error of mean (SEM).

The Rotarod apparatus (Accelerating Model, TSE) was used to measure forelimb and hindlimb motor coordination, balance and motor learning ability. The machine was set up in an environment with minimal stimuli such as noise and movement. The Rotarod device is equipped with a computer controlled motor-driven rotating rod. The Rotarod unit consists of a rotating spindle and 4 individual lanes for each mouse. The software allows pre-programming of session protocols with varying rotational speeds. Infrared beams are used to detect when a mouse falls onto the grids beneath the Rotarod. During a one-day training period, each mouse is habituated to the apparatus. The mouse is placed perpendicular to the axis of rotation, with head facing the direction of the rotation. Additionally, all mice were placed on the Rotarod at a constant speed of 12 and 20 rpm for 180 secs with 20 min between each trial. In performance testing, mice were given two trials at 5 increasing speed levels per day over three consecutive days. The speed range from 20 to 40 rpm in five steps. The mean latency to fall off the Rotarod for the two trials at each speed level was recorded and used in subsequent analysis. Before the start of the first trial, mice were weighed. The Rotarod automatically recorded the length of time that each mouse was able to spend on the rotating rod.

3.4. Results

Grip strength: Female DnaseX^{-/-} mice had a significantly lower forelimb grip strength than DnaseX^{+/+} female mice ($p < 0.0014$). Similar observation was made for male DnaseX^{-/-} mice as compared to controls, but this difference was not significant.

Performance on the Rotarod: The results from the Rotarod experiment are shown in Table 3.4, Fig. 3.5. and Fig. 3.6. We determined Rotarod performance by measuring the active rotation on the rod. This test has been used to measure the coordination of mice as animals are supposed to successfully move their feet in response to the rotation of the rod.

Rotarod testing of female DnaseX^{-/-} and DnaseX^{+/+} mice revealed a reduced latency on the rod with increasing speeds from 20 to 40 rpm for both groups. On day 1, the time on the Rotarod for female DnaseX^{-/-} at 30 and 35 rpm was significantly less than that recorded for DnaseX^{+/+} mice under the same conditions. Similar results were observed on day 2 and day 3. On day 2, female DnaseX^{-/-} mice performed considerably worse at 20, 25, and 30 rpm. On day 3, female DnaseX^{-/-} had a significantly lower latency on the rod at 30 and 35 rpm as compared to female DnaseX^{+/+} mice (see Table 3.3). Similar to the females all male mice had a reduced latency on the Rotarod with increasing speeds from 20 to 40 rpm. During the three day testing male DnaseX^{-/-} showed a slight but statistically insignificant decrease in latency on the rod as compared to male DnaseX^{+/+} mice (see Table 3.4).

Motor learning: To measure motor learning, Rotarod performances on days 1,2 and 3 were compared. Previous experience of a mouse on day 1-2 influencing its performance on day 3, causing it to become more adapted at the Rotarod, would indicate motor learning. No significant improvement in performance over the three testing days could be observed for both DnaseX +/+ and DnaseX -/- female mice (see Fig. 3.4 A+B). However, male DnaseX +/+ and DnaseX -/- mice displayed motor learning. Although, motor learning was increased in control males as compared to male DnaseX -/-, there was no significant correlation between this finding and the genotype of the mice (see Fig.3.4 A+B).

3.5. Discussion

Age and weight have a significant effect on the overall Rotarod performance (Rozas G et al., 1997). Since the tested cohorts of mice were approximately at the same age, the poorer performance of male DnaseX mice could be attributed to higher weight. Several strains display significant correlations between body weight and behavior. For example, rotarod performance is inversely correlated with body weight in the BL6 strain (Brown RE et al., 2002, McFadyen et al., 2003).

We found significantly decreased latencies on the rod for the female DnaseX -/- mice at speeds between 20 and 35 rpm while DnaseX +/+ mice deteriorated at 40 rpm. This shows impaired motor coordination in female DnaseX -/- mice. Grip strength assessment revealed a significant difference between female DnaseX -/- and female DnaseX +/+ mice indicating muscle weakness.

Rotarod performance improved in male, but not in female DnaseX mice, independent from the genotype. Thus, the female DnaseX -/- mice show only a defect in motor coordination.

No data about any defects in motor coordination and muscle function in DnaseX -/- mice were available prior to this neurological screening. Our data may indicate a muscular dysfunction or a defect in central pathways. Motor coordination (Massaquoi and Hallett, 1998) as well as motor learning (Hikosaka et al., 2002, Jueptner et al., 1997) are thought to require functional integration of frontoparietal and motor cortex, cerebellum and striatal circuitry. For further analysis we recommend EMG and muscle biopsies for assessment of muscle function and the staircase test for assessment of extrapyramidal dysfunction.

Raw data will be available on demand.

3.6. References

Brown RE, Stanford L, Williamson M, Luedemann K & Hawken C. Strain and sex differences in Rotarod performance in mice are confounded by body weight. Poster presented at the Fifth Annual Meeting of the International Behavioural and Neural Genetics Society, Tours, France

Hikosaka O, Nakamura K, Sakai K & Nakahara H. Central mechanisms of motor skill learning.
Current Opinion in Neurobiology 2002 (12), 217-222.

Jueptner M, Frith CD, Brooks DJ, Frackowiak RS & Passingham RE. Anatomy of Motor Learning. II. Subcortical Structures and Learning by Trial and Error.
J. Neurophysiol. 1997 (77), 1325-1337.

Massaquoi SG and Hallett M. in Parkinson`s Disease and Movement Disorders, eds. Jankovic J and Tolosa E (Williams & Wilkins, Baltimore), 1998 pp. 623-686.

McFadyen MP, Kusek G, Bolivar VJ and Flaherty L. Differences among eight inbred strains of mice in motor ability and motor learning on the Rotarod.
Gens, Brain and Behavior 2003 (2): 214-219.

Rozas G, Guerra MJ, and Labandeira-Garcia JL. An automated Rotarod method for quantitative drug-free evaluation of overall motor deficits in rat models of parkinsonism.
Brain research Protocols 2. 1997:75-84.

Table 3.1: Recording of body weight (in g)

Data are shown as mean (\pm SEM).Statistical analysis: Kruskal-Wallis test, significance $p < 0.05$

	male			female		
	DnaseX +/+ (n=10)	DnaseX -/- (n=21)	<i>p-value</i>	DnaseX +/+ (n=14)	DnaseX -/- (n=16)	<i>p-value</i>
Body Weight	35.04 \pm 1.1	36.83 \pm 1.06	<i>n.s.</i>	27.12 \pm 0.81	29.48 \pm 0.99	<i>n.s.</i>

Table 3.2: Grip strength (in ponds)

Data shown represent the grip strength from each mouse. Statistical analysis: one-way-analysis of variance; significance $p < 0.05$

	male			female		
	DnaseX +/+ (n=10)	DnaseX -/- (n=21)	<i>p-value</i>	DnaseX +/+ (n=14)	DnaseX -/- (n=16)	<i>p-value</i>
Grip strength	123.32 \pm 4	113.69 \pm 4.9	<i>n.s.</i>	110.73 \pm 2.8	99.98 \pm 2,5	0,0014

Table 3.3: Recording of motor coordination and balance on the Rotarod.

Rotarod data are shown as mean (\pm SEM). Statistical analysis: two-way-analysis of variance, significance $p < 0.05$.

	Latency on the Rotarod for male [in sec]			Latency on the Rotarod for female [in sec]		
	DnaseX +/+ (n=10)	DnaseX -/- (n=21)	<i>p-value</i>	DnaseX +/+ (n=14)	DnaseX -/- (n=16)	<i>p-value</i>
day1 20 rpm	76.06 \pm 19.4	76.8 \pm 12.8	<i>n.s.</i>	175.99 \pm 4	154.4 \pm 12.2	<i>n.s.</i>
25 rpm	85.44 \pm 21.2	68.06 \pm 12.3	<i>n.s.</i>	172.3 \pm 7.6	147.1 \pm 13.2	<i>n.s.</i>
30 rpm	62.5 \pm 18.8	55.27 \pm 11.9	<i>n.s.</i>	157.9 \pm 10.22	114.3 \pm 12.6	0,01
35 rpm	53.67 \pm 18.84	44.19 \pm 11.9	<i>n.s.</i>	136 \pm 12.7	89 \pm 14.9	0,02
40 rpm	45.06 \pm 16.1	41.06 \pm 9.1	<i>n.s.</i>	96.65 \pm 16.4	81.18 \pm 13.4	<i>n.s.</i>
day2 20 rpm	90.24 \pm 25.12	86.37 \pm 15.3	<i>n.s.</i>	180 \pm 0	140.22 \pm 13.5	0,03
25 rpm	83.88 \pm 19.7	83.13 \pm 15.3	<i>n.s.</i>	174.02 \pm 4.7	146.58 \pm 11.3	0,01
30 rpm	75.2 \pm 14.9	68.28 \pm 20.9	<i>n.s.</i>	170.72 \pm 5.3	122.76 \pm 15.6	0,009
35 rpm	75.77 \pm 14.9	51.43 \pm 29.78	<i>n.s.</i>	128 \pm 14.5	100 \pm 16.5	<i>n.s.</i>
40 rpm	52.38 \pm 10.5	46.7 \pm 9.6	<i>n.s.</i>	100.75 \pm 13.2	87.5 \pm 16.5	<i>n.s.</i>
day3 20 rpm	126.14 \pm 17.7	100.7 \pm 15.7	<i>n.s.</i>	176.99 \pm 3	163.6 \pm 8.9	<i>n.s.</i>
25 rpm	120.29 \pm 12.85	99.92 \pm 16.6	<i>n.s.</i>	172.92 \pm 5.7	148.56 \pm 11.06	<i>n.s.</i>
30 rpm	103.7 \pm 19.3	75.09 \pm 14.5	<i>n.s.</i>	157.7 \pm 9.4	105.19 \pm 16.49	0,01
35 rpm	90.65 \pm 17.9	68.99 \pm 14.6	<i>n.s.</i>	135.06 \pm 16.8	83.55 \pm 17.03	0,04
40 rpm	64.41 \pm 15.9	52.89 \pm 12.8	<i>n.s.</i>	81.03 \pm 7.4	64.74 \pm 17.4	<i>n.s.</i>

Fig 3.4 A+B: Recording of motor learning abilities on the Rotarod for female DnaseX mice.
A: Latency time of Rotarod testing of female DnaseX $-/-$ mice revealed no significant improvement in the performance over the three training days.
B: Latency time of Rotarod testing of female DnaseX $+/+$ mice showed no significant increase over the screening period of three days. Rotarod data are shown as mean (\pm SEM).

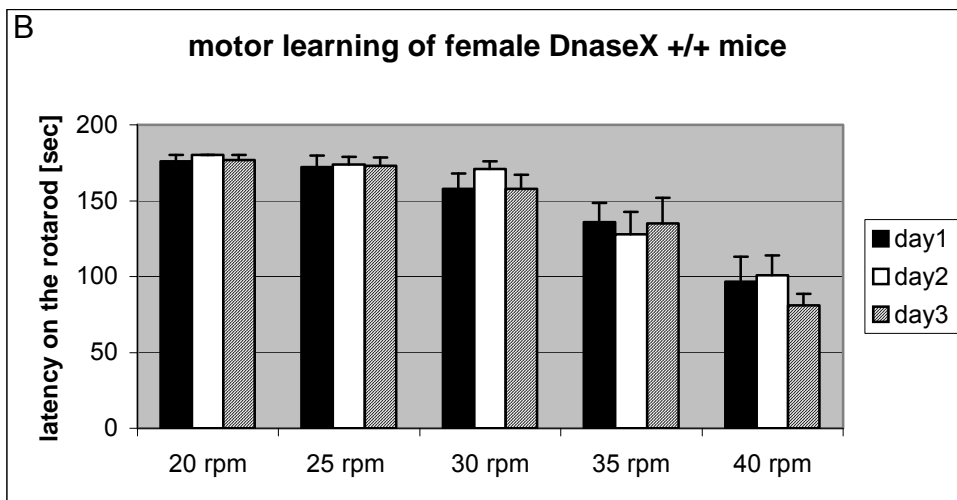
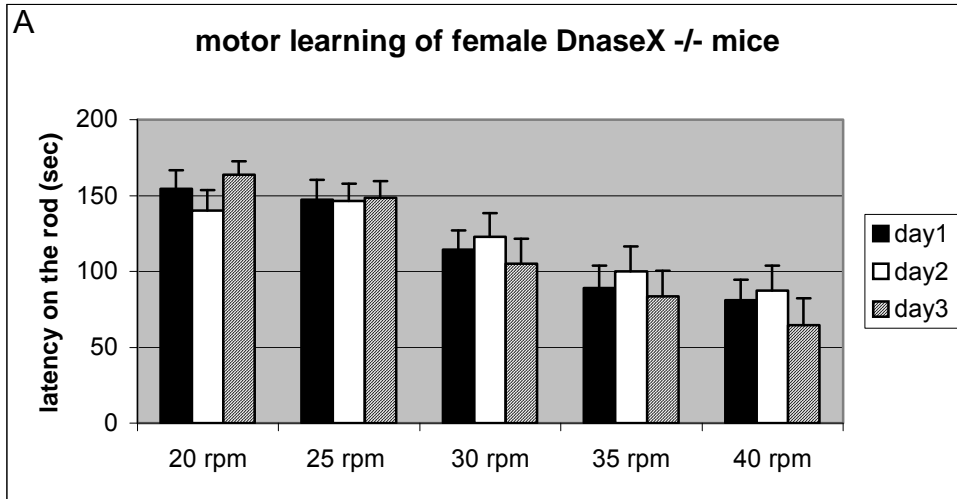
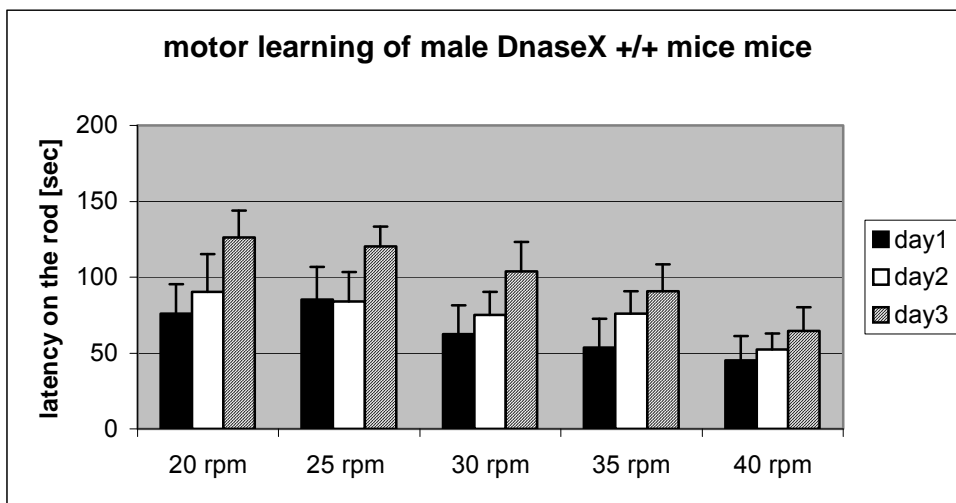
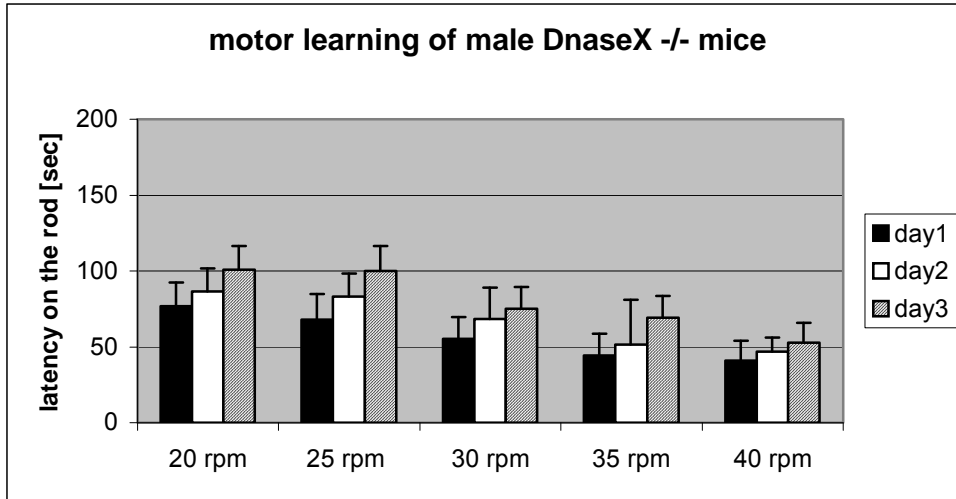


Fig 3.5 A+B: Recording of motor learning abilities on the Rotarod for male DnaseX mice.

A: Latency time of Rotarod testing of male DnaseX $-/-$ mice revealed an observed but no significant improvement in the performance over the three training days.

B: Latency time of Rotarod testing of male DnaseX $+/+$ mice showed an noticeable increase but not statistical difference over the screening period of three days. Rotarod data are shown as mean (\pm SEM).



Acknowledgements

A large team consisting of scientists, technicians and animal caretakers all contribute to the success of the German Mouse Clinic. We want to thank Reinhard Seeliger, Elfi Holupirek, Christine Fürmann, Kerstin Kutzner, Mareike Maurer, Susanne Sommer, Rose Austin, Florian Schleicher, Gregor Pahnke, Susanne Wittich, Martin Taube, Claudia Zeller, Kathrin Seidel, Elenore Samson, Nadine Kink, Jaqueline Müller, Sabine Holthaus for expert technical help and Daniela Kißling, Monika Katzbach, Uwe Drescher, Heiko Engelniederhammer, Manuela Krug, Tina Kohler, Petra Thalmeier, Daniela Gerber and Sven Korb for the care of the mice.

Appendix: Adresses of screeners and modules

Coordinators

Dr. Valerie Gailus-Durner
Dr. Helmut Fuchs
Dr. Christoph Lengger
Prof. Dr. Martin Hrabé de Angelis
Institut for Experimental Genetics
GSF National Research Center for
Environment and Health
Ingolstädter Landstr. 1
85764 Neuherberg
Tel.: 089-3187-3613
Fax: 089-3187-3500
Email: gailus@gsf.de

Behaviour Screen

Dr. Vera Pedersen
Dr. Sabine M. Hölder
GSF
Institut für Entwicklungsgenetik
Ingolstädter Landstr. 1
85764 Neuherberg
Tel.: 089-3187-3674
Fax: 089-3187-3099
hoelter@gsf.de

Dysmorphology Screen,

Dr. Helmut Fuchs
Dr. Elisabeth Grundner-Culemann
Prof. Dr. Martin Hrabé de Angelis
GSF National Research Center for Envi-
ronment and Health
Institute of Experimental Genetics
Ingolstaedter Landstrasse 1
D-85764 Oberschleissheim
PHONE: +49-89-3187-3151
FAX: +49-89-3187-3500
EMAIL: hfuchs@gsf.de

Neurology Screen

Dr. Ilka Schneider
Johannes Tritschler
Dagmar Krüger

Dr. PD Thomas Klopstock
Department of Neurology
Klinikum Großhadern

GSF National Research Center for Environment and Health
Institute of Experimental Genetics
German Mouse Clinic (GMC)/Neurology
PHONE: +49-89-3187-3654
FAX:+49-89-3187-3500
EMAIL: Ilka.Schneider@gsf.de

LMU Ludwig-Maximilians-University
Marchioninstr. 15
81377 Munich
Germany
PHONE:+49-89-7095-5920
FAX:+49-89-7095-3677
EMAIL: Thomas.Klopstock@nro.med.uni-muenchen.de

Eye Screen

Dr. Claudia Dalke
GSF-National Research Center for Environment and Health
Institute of Developmental Genetics
Ingolstädter Landstr. 1
D-85764 Neuherberg, Germany
Tel: 089/3187-2910
Fax:089/3187-2210
e-mail: dalke@gsf.de

Clinical-Chemical Screen

Dr. Martina Klempt
Institute of Experimental Genetics
GMC - German Mouse Clinic
Clinical-Chemical Screen
Institute for Experimental Genetics
GSF - National Research Center for Environment and Health
Ingolstaedter Landstraße 1
D-85764 Oberschleißheim
Tel.: 089/3187-3282
email: klempt@gsf.de

Prof. Dr. Eckhard Wolf
Dr. Birgit Rathkolb
Institute of Molecular Animal Breeding and Biotechnology
Genecenter
LMU Munich
Feodor Lynen-Straße 25
D-81377 Munich
Tel.: 089/21807-6800
email: ewolf@lmb.uni-muenchen.de
email : b.rathkolb@gen.vetmed.uni-muenchen.de

Immunology Screen

Dr. Svetoslav Kalaydjiev
Tobias Franz
Prof. Dirk Busch
German Mouse Clinic - room 7105
Institute for Experimental Genetics
GSF National Research Center for Environment and Health
Ingolstaedter Landstr. 1
85764 Oberschleissheim
tel (089) 3187 3656
fax (089) 3187 3500
e-mail: svetoslav.kalaydjiev@lrz.tum.de
e-mail: franz_tobias@web.de

Prof. Dirk Busch
Institute for Medical Microbiology, Immunology and Hygiene
Technical University-Munich
Trogerstr. 9
81675 Munich
tel (089) 4140 6191
fax (089) 4140 4139
e-mail: dirk.busch@lrz.tum.de

Allergy Screen

Anahita Javaheri, MSc
Prof. Dr. Markus Ollert
Klinik und Poliklinik für Dermatologie
und Allergologie am Biederstein
Technische Universität München (TUM)
Biedersteinerstr. 29
D-80802 München
Tel.: 089-4140-3551 (M.O.)
Tel.: 089-3187-2554 (A.J.)
Fax: 089-4140-3552
E.-mail: ollert@lrz.tum.de

Nociceptive Screen

Dr Ildiko Racz
Laboratory of Molecular Neurobiology,
Department of Psychiatry,
University of Bonn,
Sigmund-Freud-Str. 25,
D-53105 Bonn
Tel.: 0049-228-287-9578
Fax: 0049-228-287-9125

Prof. Dr. Andreas Zimmer
Laboratory of Molecular
Neurobiology, Department
of Psychiatry, University of Bonn,
Sigmund-Freud-Str. 25,
53105 Bonn. Germany
Tel.: 0049-228-287-9124
Fax.: 0049-228-287-9125

Lung Function Screen

PD Dr. Holger Schulz
Institut für Inhalationsbiologie
Ingolstädter Landstraße 1
D-85764 Neuherberg
Tel.: 089-3187-4119
Fax.: 089-3187-2400
E. mail: schulz@gsf.de

Expression Profiling

Tomek Mijalski
Dr. Johannes Beckers
GSF – National Research Center for
Environment and Health
Institute of Experimental Genetics
Ingolstaedter Landstr. 1
85764 Neuherberg
Tel.: 089-3187-3513
Fax: 089-3187-3500
E.-mail: Beckers@gsf.de

Metabolic Screen

Dr. Ralf Elvert
Institute of Experimental Genetics
GMC - German Mouse Clinic
Metabolic Screen
GSF - National Research Center for Environment and Health
Ingolstaedter Landstr. 1
85764 Neuherberg, Germany
Phone: +49 (0)89/3187-3648 or 3151
Fax: +49 (0)89/3187-3500
email: elvert@gsf.de

Pathology Screen

Dr. Julia Calzada-Wack
Sandra Kunder
phone : +49 (089) 3187-2312
 +49 (089) 3187-3241
fax: +49 (089) 3187-3360
e-mail: Calzada@gsf.de,
sandra.kunder@gsf.de GSF - National
Research Center for Environment and
Health
Institute of Pathology
Ingolstaedter Landstr. 1
85764 Neuherberg

PD Dr. Leticia Quintanilla-Fend
phone : +49 (089) 3187-2636
fax: +49 (089) 3187-3360
e-mail: quintanilla-fend@gsf.de
GSF - National Research Center for Environment and Health
Institute of Pathology
Ingolstaedter Landstr. 1
85764 Neuherberg

Steroid Screen

Dr. Jerzy Adamski
Dr. Cornelia Prehn
GSF - National Research Center for Environment and Health
Institute of Experimental Genetics
Ingolstaedter Landstrasse 1
D-85764 Neuherberg
ph.: +49 (0)89/3187-3155 (Adamski),
 -3231 (Prehn)
fax: +49 (0)89/3187-3225
e-mails: adamski@gsf.de, prehn@gsf.de