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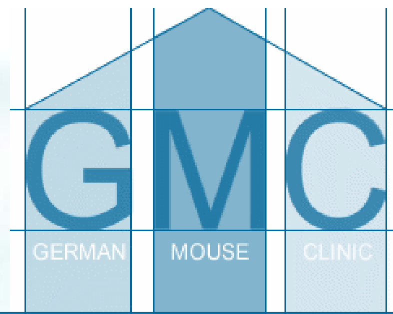

The

GERMAN MOUSE CLINIC

Report for mPtpg

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The German Mouse Clinic

The German Mouse Clinic (GMC) was founded January 2002 at the GSF research center in Munich/Neuherberg to provide an open access platform for standardized mouse phenotyping. The GMC is supported by the National Genome Research Network (NGFN, http://www.ngfn.de/) and is a partner of the EUMORPHIA research program (http://www.eumorphia.org/).

In the GMC, experts from various fields of mouse genetics, physiology and pathology in close collaboration with clinicians work side by side at one location. We offer a primary phenotypic analysis of mouse mutants (more than 240 parameters/mouse) in the areas of allergy, behavior, bone and cartilage, cardiovascular diseases, clinical chemistry, energy metabolism, eye development and vision, immunology, lung function, molecular phenotyping, neurology, nociception, and pathology. Additional screens for host-pathogen interaction can be performed at the GBF Braunschweig. Secondary and tertiary screening for in depth analysis is offered by the different screens and is available on demand.

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1 Summary

1.1 Primary Screening

In a primary screen 55 animals of the mPtpg mutant mouse line (29 mutants, 26 wild-type control littermates) were analyzed in the German Mouse Clinic (GMC) in the screens Behavior, Dysmorphology, Bone and Cartilage, Neurology, Eye, Clinical Chemistry, Immunology, Allergy, Nociception, Lung Function, Metabolism, Expression Profiling, and Pathology. The screening started on November 2, 2004. The results are summarized briefly below.

Behavior Screen: The behavioral observation indicated an effect of the *mPtpg* mutation on activity-related alteration in social affinity in females and in exploration in males. To clarify a potential social phenotype, more specific behavioural tests would need to be performed which would need to be flanked by further neuroanatomical and functional investigations.

Clinical Chemical Screen: Although we detected a significantly elevated Aspartate Aminotransferase (ASAT) activity in two male mutant mice, this finding does not likely indicate genotype-related changes of clinical chemical parameters but most likely is caused by the handling during blood sample collection. Additionally, the elevated Ferritin level of male mutants is judged to be of no importance because the elevated white and red blood cell counts excluded any type of anemia from being the cause of the Ferritin results.

Immunology Screen: We were able to detect some minor, but statistically significant differences affecting NK cell frequencies, which were slightly decreased in the mutants. In addition, we observed changes in the levels of IgM, IgG_{2a} and IgA.

Allergy Screen: The analysis of the *mPtpg* mutant mouse line in the Allergy screen revealed elevated IgE levels in mutant mice.

Lung Function: Male mice were affected by the mutation unlike the females. Despite showing a significantly lower body weight, male mutant mice used larger tidal volumes so that ventilation and specific values for tidal volume and minute ventilation were significantly increased at both levels of activity. A higher oxygen demand but also alterations in the gas exchanging function of the lung can be associated with these findings.

In the screens **Dysmorphology, Neurology, Eye, Nociception, Expression Profiling, Energy Metabolism,** and **Pathology**, no genotype-specific differences could be found.

1.2 Recommendations for Secondary Screening

If the provider is interested in more detailed analysis, we would suggest secondary screening the screens Behavior, Immunology, Allergy, and Lung Function. We would recommend performing the experiments listed below.

Behavior Screen: To clarify the behavioural phenotype, a new batch of mutants and littermate controls of both sexes would need to be analysed for social behaviour, olfaction and social memory (social interaction test, social discrimination test, social odour detection test). Additional tests for anxiety-related behaviour would be helpful to exclude an anxiety phenotype. However, this is a lot of work, which would need to be flanked by further neuroanatomical and functional investigations. Detailed expression pattern analysis of *mPtpg* in the brain with a regional and cellular resolution would be a prerequisite for meaningful further behavioural investigations.

Immunology Screen: We would like to verify our results in an independent experiment using a different batch of mice. If primary findings are confirmed, further secondary screens can be discussed with the provider.

Allergy Screen: We are interested to investigate the mPtpg mutant mouse line further in an allergen challenging system established in our lab.

Lung Function Screen: Lung function studies and morphological analysis of the lung is requested to further illuminate affects of the *mPtpg* mutation and assess possible underlying mechanisms.

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

2 General Part

2.1 The Role of the Gene

The gene codes for a 410 aa protein of unknown function which has high homology to a functionally unknown pfam-domain DUF-803. By sequence analysis nine putative transmembrane domains in the mPtpg protein were identified. A strong expression of *mPtpg* is detectable in brain, hippocampus, brain stem, cortex, kidney, lung, spleen, ovary, uterus, testis, epididymis, and heart, but not in muscle, liver, and skin. Several *Ptpg* transcripts were detected in brain from other mammals and even from frog.

2.2 Known Phenotypes

Homozygous animal do not show any obvious phenotype.

All further findings which will be shown in this report we consider as new.

2.3 Suggested Human Disease Model

So far no human disease has been addressed to a mutation in the human *Ptpg* gene (OMIM).

2.4 Mice

2.4.1 Number and kind of mice

As described by the sender, the *mPtpg* null mutation was generated by replacing of part of exon 2 and all exons up to exon 8 with a neo-cassette.

Table 1: mPtpg mice provided for analysis.
Numbers in brackets indicate animals which were kept in reserve.

Genotype / Sex	Number of Animals	
Mutant female	15	1 died
Mutant male	14	
Control female	15 (+3)	1 died
Control male	11	2 died

Additionally, 23 female and 21 male heterozygous cage mates were provided. As described by the sender, the mice analyzed were a 15th backcross generation on a 129/Sv background.

2.4.2 Housing conditions

In the GMC mice are housed in type II polycarbonate cages in individually ventilated caging (IVC) systems (VentiRack Bioscreen TM, Biozone, Margate,

UK) on wood fibre (Altromin, Lage, Germany). The IVCs operate with positive pressure. Mice are transferred in weekly intervals to new cages with forceps in Laminar Flow Class II changing stations. Mice are fed with irradiated standard rodent high energy breeding diet (Altromin 1314) and given semidemineralized filtered (0.2 µm) 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 (Brielmeier *et al.,* 2002).

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

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

2.5 Workflow

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

Mouse mutants entering the GMC are examined in a primary screen according to the following standard workflow (Fig. 1, Gailus-Durner, Fuchs *et al.,* 2005). Analyzed parameters are listed in Table 2.

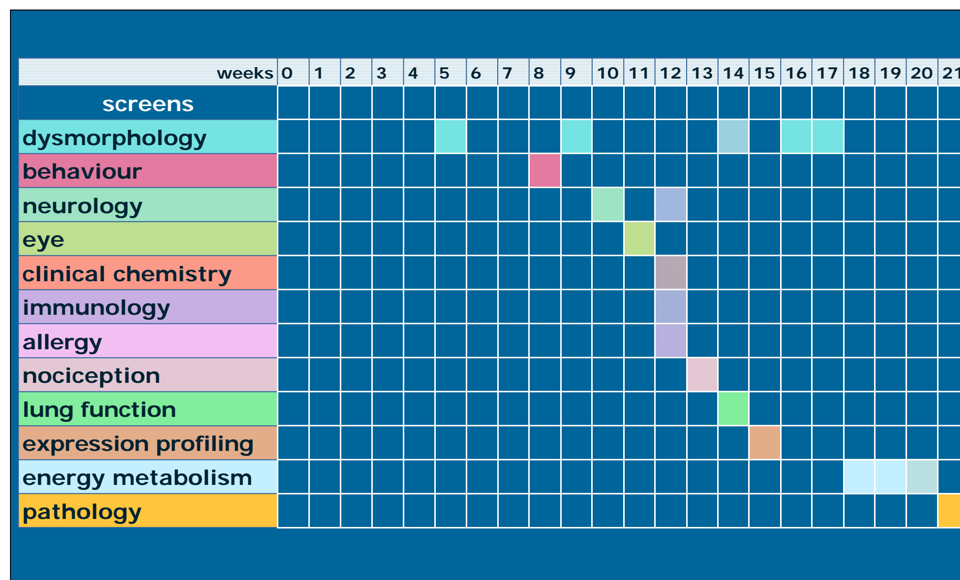

Figure 1: Workflow of the primary screen

Explanation below, ▒ Analysis of blood-based parameters.

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

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

2.5.2 Applied screens

The GMC standard workflow for the primary screen as described above was applied to analyze the mPtpg mutant mouse line. As the demanded number of 60 animals (15 mice per sex per genotype) could not be delivered, the workflow was adapted to the available number of animals. Some parameters measured in the blood based screens could not be determined in all animals, as it was not possible to get the needed amount of blood from these animals. Four animals died during the primary screen (two of them did not recover from anesthesia) and thus could not be analyzed for all parameters (Table 1).

2.5.3 Quality Management

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

2.6 Statistical Analysis of Data

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

2.7 References

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Abbreviations and Wording

mPtpg	murine putative transmembrane protein and mutant mouse line deficient in *mPtpg*
mPtpg	gene
GMC	German Mouse Clinic
IVC	individually ventilated cage
Control	*mPtpg*$^{+/+}$, homozygous wild-type control
Mutant	*mPtpg*$^{-/-}$, homozygous mutant
FELASA	**F**ederation of **E**uropean **L**aboratory **A**nimal **S**cience **A**ssociations, 25 Shaftesbury Avenue, London W1D 7EG, UK, www.felasa.org

Table 2: Primary Screen at GMC		
Screens	**Goal**	**Methods**
Dysmorphology, Bone and Cartilage	morphological analysis of body, skeleton, bone and cartilage	morphological observation, bone densitometry, X-ray, AVL analyzer, micro-computer tomography
Behavior	locomotor, exploratory, emotional and social behavior, object recognition memory	modified hole board
Neurology	assessment of muscle, spinocerebellar, sensory, and autonomic function	modified SHIRPA protocol
Eye	assessment of morphological and functional alterations of the eye	electroretinography, slit lamp biomicroscopy
Clinical Chemistry	determination of clinical-chemical and hematological parameters in blood	blood autoanalyzer, ABC-animal blood counter
Immunology	analysis of peripheral blood samples for immunological parameters	flow cytometry, ELISA
Allergy	analysis of total plasma IgE	ELISA
Nociception	detection of altered pain response	hot plate assay
Lung function	assessment of alterations in breathing patterns	whole body plethysmography (Buxco®)
Expression Profiling	RNA expression profiling	DNA-chip technology
Energy Metabolism	measurement of altered body weight regulation, body temperature and energy balance	bomb calorimetry
Pathology	microscopic and macroscopic examination	histology, immunochemistry

3 Specific part

3.1 Behavior Screen

3.1.1 Summary

Genetic studies in the mouse are important for the elucidation of molecular pathways underlying behavior. The goal of this endeavor is not only the identification of genes that control brain function and influence behavior, but also understanding of genetic factors involved in human psychiatric disorders (Tarantino & Bucan, 2000; Bucan & Abel, 2002). These disorders are associated with quantitative phenotypes called "intermediate traits" or endophenotypes, some of which, in contrast to the full complex disorder, can readily be modeled in mice. These traits are risk factors which are considered to be closer to the genetic etiology than the full syndrome. Examples are anxiety in depression, prepulse inhibition and working memory deficits in schizophrenia, and social interaction deficits in autism and schizophrenia (Seong *et al.,* 2002; Gottesman & Gould, 2003; Inoue & Lupski, 2003).

In the attempt to efficiently screen for candidate endophenotypes within a limited time frame, we use the modified Hole Board (mHB) test as primary screen in the behavioral phenotyping module of the GMC. This test allows the comprehensive analysis of a range of parameters known to be indicative of behavioral dimensions such as locomotor activity, exploratory behavior, arousal, emotionality, memory and social affinity in a single short test (See Ohl *et al*., 2001).

The *mPtpg* mutants demonstrated an effect on activity-related alteration in social affinity in females and in exploration in males.

3.1.2 Mice

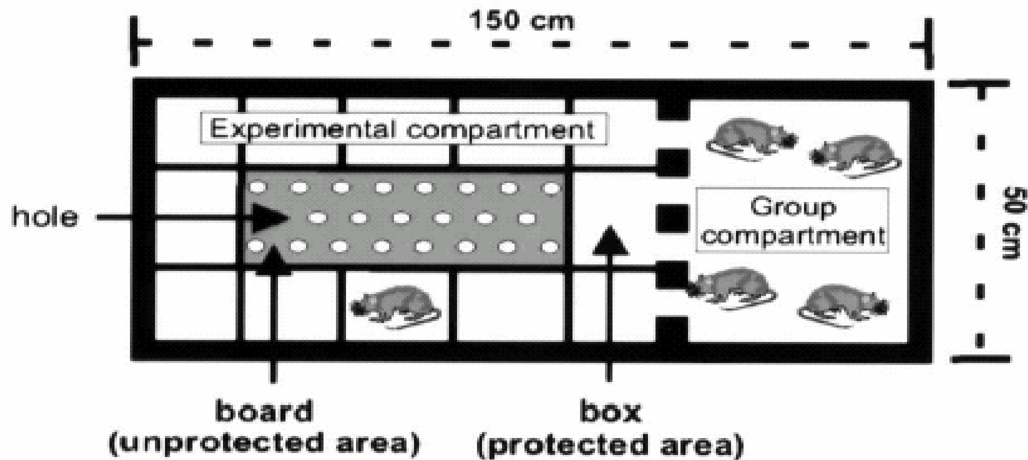
Mice were housed with food and water *ad libitum* under standard laboratory conditions. Animals were separated based on sex, but not genotype. They entered the laboratory at the age of six weeks, were given two weeks for acclimatization and were tested at the age of eight weeks. Three days before testing,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

In this screen, 30 female mice (15 controls, 15 mutants) and 18 male mice (10 controls, 8 mutants) were available for analysis.

3.1.3 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 test arena (100 x 50 cm), in the middle of which a board (60 x 20 x 2 cm) with 23 holes (1.5 x 0.5 cm) staggered in three lines with all holes covered by movable lids was placed, thus representing the central area of the test arena as an open field. The area around the board was divided into 12 similarly sized quadrants by lines taped onto the floor of the box (See 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. The illumination levels were set at approximately 150 lux in the corners and 200 lux in the middle of the test arena.



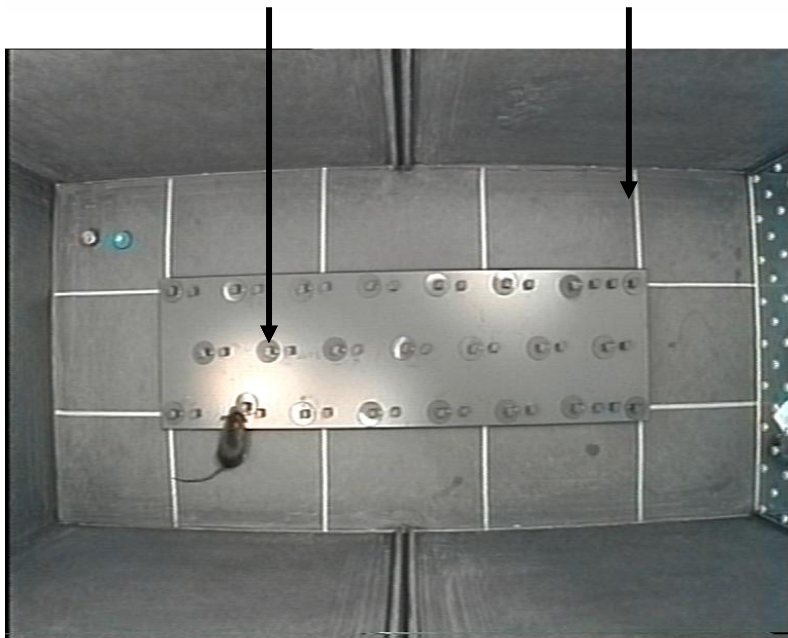


Figure 2: Test arena for modified hole board test.

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

was videotaped and its locomotor path analyzed with a video-tracking system (Ethovision 2.3, Noldus, Wageningen). After each trial, the test arena was cleaned carefully with a disinfectant.

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

3.1.4 Results

Behavioral analysis of spontaneous activity in a novel environment, as measured by the modified Hole Board test, revealed increased frequencies for group contacts (Table 4) and a *tendency* towards reduced latency to first group contact (Table 4) in *mPtpg*-mutant females in comparison to control females. Whereas time spent in social contact - even though higher in mutant females - did not significantly differ from controls (group contact total duration, Table 4). Although mutant males exhibited enhanced values for social contact as compared to control males (group contact frequency and total duration, Table 4) these effects were statistically not significant. Concerning exploration, male mutants started hole exploration earlier than control males (hole exploration latency, Table 4). Frequencies of hole explorations remained unchanged (Table 4). There were no genotype effects in all other observed parameters (Tables 3 and 5).

3.1.5 Discussion

Table 3: Evaluation of the behavioral phenotype
Behaviors which are considered as affected in mutants due to the pattern of significantly altered parameters are marked in red.

Behavior	Measured parameters
Forward locomotor activity	Line crossings, Total distance travelled
Vertical locomotor activity	Rearings in the box , Rearings on the board
Speed of movement	Mean and maximum velocity
Immobility	Time spent immobile
Risk assessment	Stretched attends
Anxiety-related behavior	Latency until first board entry, Time spent on board, Board entries
Exploratory behavior	Directed: Hole exploration (latency), object exploration; Undirected: Rearings, activity levels
Grooming behavior	Latency to grooming, Time spent grooming, Number of groomings
Defecation	Latency to defecation, Number of boli
Social affinity	Group contacts (frequency, latency), Time spent at partition
Familiar object exploration	Latency to obj. expl., Time spent in obj. expl., Number of obj. expl.
Unfamiliar object exploration	Latency to obj. expl., Time spent in obj. expl., Number of obj. expl.

The behavioral observation in the modified Hole Board demonstrated an effect of the *mPtpg* mutation on activity-related alteration in social affinity in mutant females, indicated by the fact that mutant females did more and earlier group contacts without additionally spending more time in social contact as compared to control females (Table 3). Moreover, general locomotor activity was not altered in mutant females.

Whether the alterations in social affinity might be due to alterations in sensory perception or in hormonal regulation would have to be investigated. Although none of the social affinity related parameters of mutant males did statistically differ from control males in this test situation, it can not be excluded that also mutant males are affected. This possibility is indicated by higher values for group contacts and time in group contact for mutant males. Finally, also mutant males showed an activity-related alteration which was evident in direct exploration (hole exploration latency) without any changes in general locomotor activity.

To clarify the behavioural phenotype, a new batch of mutants and littermate controls of both sexes would need to be analyzed for social behaviour, olfaction and social memory (social interaction test, social discrimination test, social odour detection test). Additional tests for anxiety-related behaviour would be helpful to exclude an anxiety phenotype. However, this is a lot of work, which would need to be flanked by further neuroanatomical and functional investigations. Detailed expression pattern analysis of *mPtpg* in the brain with a regional and cellular resolution would be a prerequisite for meaningful further behavioural investigations.

3.1.6 References

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Table 4: Results of behavioral observation in the modified Hole Board Test
Data are presented as mean ± standard error of mean.

Parameter	Control (A)			Mutant (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=10)	(n=15)	*p - value*	(n=8)	(n=15)	*p - value*	*p - value*	*p - value*
Line crossing [frequency]	109.2 ± 6.37	82.13 ± 13.18	N.A.	101.63 ± 7.26	84.93 ± 9.68	N.A.	n.s.	n.s.
Line crossing [latency]	2.02 ± 0.29	3.81 ± 0.97	N.A.	2.24 ± 0.49	4.05 ± 1.83	N.A.	n.s.	n.s.
Rearings in box [frequency]	7.7 ± 1.69	7.53 ± 1.77	N.A.	6.75 ± 1.97	10.67 ± 1.89	N.A.	n.s.	n.s.
Rearings in box [latency]	107.87 ± 18.05	117.89 ± 22.52	N.A.	104.94 ± 10.06	63.39 ± 11.4	N.A.	n.s.	n.s.
Hole exploration [frequency]	25.8 ± 2.9	23.6 ± 3.35	N.A.	19.5 ± 5.1	19 ± 3.39	N.A.	n.s.	n.s.
Hole exploration [latency]	41.29 ± 15.64	24.33 ± 5.06	N.A.	11.29 ± 3.33	75.79 ± 26.18	N.A.	p<0.05	n.s.
Hole visit [frequency]	0 ± 0	0 ± 0	N.A.	0 ± 0	0 ± 0	N.A.	n.s.	n.s.
Hole visit [latency]	300 ± 0	300 ± 0	N.A.	300 ± 0	300 ± 0	N.A.	n.s.	n.s.
Board entry [frequency]	7.2 ± 1	5.73 ± 1.08	N.A.	4.13 ± 1.33	4.07 ± 1.14	N.A.	n.s.	n.s.
Board entry [latency]	81.65 ± 18.52	103.11 ± 27.56	N.A.	135.73 ± 37.48	142.35 ± 28.69	N.A.	n.s.	n.s.
Board entry [total duration %]	9.83 ± 1.64	11.37 ± 2.95	N.A.	7.7 ± 3.06	5.93 ± 1.33	N.A.	n.s.	n.s.
Rearing on board [frequency]	0.1 ± 0.1	0 ± 0	N.A.	0.38 ± 0.26	0 ± 0	N.A.	n.s.	n.s.

Rearing on board [latency]	299.92 ± 0.08	300 ± 0	**N.A.**	273.51 ± 22.27	300 ± 0	**N.A.**	**n.s.**	**n.s.**
Risk assessment [frequency]	0 ± 0	0.93 ± 0.33	**N.A.**	0.13 ± 0.13	0.6 ± 0.25	**N.A.**	**n.s.**	**n.s.**
Risk assessment [latency]	300 ± 0	201.97 ± 32.97	**N.A.**	300 ± 0	209.53 ± 34.23	**N.A.**	**n.s.**	**n.s.**
Group contact [frequency]	12.4 ± 1.24	11.33 ± 1.24	**N.A.**	14 ± 1.51	16.47 ± 1.16	**N.A.**	**n.s.**	**p<0.01**
Group contact [latency]	20.96 ± 5.65	45.45 ± 18.53	**N.A.**	27.31 ± 5.82	19.03 ± 4.66	**N.A.**	**n.s.**	**p=0.05**
Group contact [total duration %]	21.76 ± 3.02	29.81 ± 3.7	**N.A.**	25.36 ± 3.99	38.37 ± 4.1	**N.A.**	**n.s.**	**n.s.**
Grooming [frequency]	1.5 ± 0.43	1.13 ± 0.38	**N.A.**	2.38 ± 0.8	0.8 ± 0.3	**N.A.**	**n.s.**	**n.s.**
Grooming [latency]	212.82 ± 25.53	206.87 ± 26.37	**N.A.**	170.13 ± 0	247.41 ± 15.8	**N.A.**	**n.s.**	**n.s.**
Grooming [total duration %]	1.16 ± 0.46	1.26 ± 0.47	**N.A.**	1.74 ± 0.61	0.74 ± 0.25	**N.A.**	**n.s.**	**n.s.**
Defecation [frequency]	1.2 ± 0.29	1.67 ± 0.37	**N.A.**	1.25 ± 0.31	1.53 ± 0.31	**N.A.**	**n.s.**	**n.s.**
Defecation [latency]	153.3 ± 33.58	90.7 ± 28.77	**N.A.**	136.06 ± 46.57	116.23 ± 29.79	**N.A.**	**n.s.**	**n.s.**
Unfamiliar object exploration [frequency]	4.6 ± 0.43	2.27 ± 0.42	**N.A.**	4.38 ± 0.75	2.33 ± 0.47	**N.A.**	**n.s.**	**n.s.**
Familiar object exploration [frequency]	4.9 ± 0.67	3.13 ± 0.71	**N.A.**	5.38 ± 0.92	2.53 ± 0.72	**N.A.**	**n.s.**	**n.s.**
Unfamiliar object exploration [latency]	29.52 ± 7.76	115.12 ± 28.2	**N.A.**	51.36 ± 30.16	144.02 ± 28.2	**N.A.**	**n.s.**	**n.s.**
Familiar object exploration [latency]	48.41 ± 22.25	121.45 ± 30.36	**N.A.**	31.41 ± 8.05	159.51 ± 34.59	**N.A.**	**n.s.**	**n.s.**

Unfamiliar object exploration [total duration %]	0.84 ± 0.14	0.58 ± 0.1	**N.A.**	1.15 ± 0.27	0.59 ± 0.14	**N.A.**	**n.s.**	**n.s.**
Familiar object exploration [total duration %]	0.91 ± 0.18	0.64 ± 0.17	**N.A.**	0.95 ± 0.14	0.52 ± 0.15	**N.A.**	**n.s.**	**n.s.**
Object Index	-0.02 ± 0.11	0.06 ± 0.13	**N.A.**	0.05 ± 0.07	0.14 ± 0.1	**N.A.**	**n.s.**	**n.s.**

Table 5: Video-tracking results regarding locomotor behavior
Data are presented as mean ± standard error of mean.

Parameter	Control (A)			Mutant (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=10)	(n=15)	*p - value*	(n=8)	(n=15)	*p - value*	*p - value*	*p - value*
Total Distance Moved [cm]	3174.43 ± 155.7	2164.69 ± 267.51	**N.A.**	2989.14 ± 157.33	2276.14 ± 212.69	**N.A.**	**n.s.**	**n.s.**
Mean Velocity [cm/sec])	10.34 ± 0.51	14.83 ± 1.28	**N.A.**	9.73 ± 0.51	15.54 ± 1.05	**N.A.**	**n.s.**	**n.s.**
Maximum velocity [cm/sec]	54.48 ± 2.23	54 ± 3.53	**N.A.**	54.9 ± 2.76	54.58 ± 2.78	**N.A.**	**n.s.**	**n.s.**
Turns [Frequency]	2998.5 ± 29.06	1257.93 ± 124.31	**N.A.**	2946 ± 37.63	1323.33 ± 95.73	**N.A.**	**n.s.**	**n.s.**
Board entry max. duration [sec]	10.06 ± 1.3	14.75 ± 5.75	**N.A.**	11.07 ± 3.88	13.61 ± 2.64	**N.A.**	**n.s.**	**n.s.**
Distance to Wall [cm]	7.38 ± 0.39	7.53 ± 0.63	**N.A.**	6.17 ± 0.7	6.15 ± 0.46	**N.A.**	**n.s.**	**n.s.**
Distance to Board [cm]	8.93 ± 0.36	8.31 ± 0.59	**N.A.**	9.97 ± 0.56	9.58 ± 0.43	**N.A.**	**n.s.**	**n.s.**

3.2 Dysmorphology, Bone and Cartilage

3.2.1 Summary

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

A total of 55 animals of mPtpg mutant mouse line were analyzed in the Dysmorphology, Bone, and Cartilage module of the German Mouse Clinic. In the morphological investigation via visual inspection and X-ray analysis no genotype specific differences were detected between *mPtpg*-mutant mice and wild-type control littermates. In the bone mineral density analysis BMD, pBMD and body weight were increased, but no significant difference was observed when BMD was related to the body weight (sBMD). Therefore differences between mutants and controls in bone mineral density might be due to changes in body weight and thus be secondary effects.

3.2.2 Mice

Twenty-five male (11 controls, 14 mutants) and 30 female (15 controls, 15 mutants) mice were analyzed by morphological inspection at the age of 9 weeks. Blood was taken at the age of 14 weeks for determination of ionic calcium from 19 mutant and 15 control animals, and 16-week-old mutants (18 animals) and controls (15 animals) entered the bone density and X-ray analysis.

3.2.3 Material and Methods

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

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

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

Using a clickbox (supplied by the MRC Institute of Hearing Research, Nottingham, UK) we tested the mice's ability to hear a sound of 20 kHz. The reaction of the animals was classified into six categories (0=no reaction at all, 1=no Preyer reflex, 2= retarded reaction, 3= normal reaction, 4= strong reaction, 5= particularly strong reaction).

Ionized calcium Analysis
Equipment: AVL 9180 Electrolyte Analyzer (distributed by Roche Diagnostics GmbH, Mannheim, Germany)
cleaning solution and conditioning solution (Roche),
ISEtrol Quality Control Solutions (Roche),
lithium-heparin polypropylen tubes,
glass capillary (0.8 mm diameter, 32 mm length, without heparin; special product of Laborteam K+K, Munich).
Quality control: Calibration of the system and quality control were performed at intervals recommended and with solutions provided by the manufacturer. The results from the quality control were recorded by the system. Before blood measurement, daily cleaning, conditioning and calibration of the analyzer were performed.
Procedure: Blood (100 µl) was collected from anesthetized mice in lithium heparin tubes and transferred directly to the analyzer. Values were transferred directly to the database.

X-ray Images
Equipment: Faxitron X-ray Model MX-20 (Specimen Radiography System, Illinois, USA),
NTB Digital X-ray Scanner EZ 40 (NTB GmbH, Diepholz, Germany),
Quality control: Calibration of the system is done in monthly intervals,
Settings: Voltage 25 kV, integration time 40 ms,
Procedure: The anesthetized mouse was fixed on an X-ray-permeable plate and placed in the machine. Using iX-Pect software supplied by the manufacturer of the X-ray scanner, the image was taken and analyzed. Analysis was done qualitatively by visual inspection of the images as well as quantitatively by using the ruler tool of iX-Pect software.

Bone density analysis
Equipment: pDEXA Sabre X-ray Bone Densitometer (Norland Medical Systems. Inc., Basingstoke, Hampshire, UK; distributed by Stratec Medizintechnik GmbH, Pforzheim, Germany),
Quality control: Calibration of the system was done in daily intervals using the QC and the QA phantoms delivered by the manufacturer. Results from the quality control were recorded by the system.
Settings: Scan speed 20 mm/s, Resolution 0.5 mm x 1.0 mm, HAW 0.020

Procedure: After anesthesia, the weight and length of the mouse were recorded, and the mouse was placed in the analyzer. After a scout scan, the area of interest was optimized and the measure scan started.
Data-analysis: For analysis of the data, regions have to be defined. The standard analysis comprises a whole body analysis as well as a whole body analysis excluding the skull.

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

3.2.4 Results and Discussion

Fifty-five animals of mPtpg mutant mouse line were analyzed in the Dysmorphology, Bone, and Cartilage module of the German Mouse Clinic. In the morphological investigation via visual inspection and X-ray analysis no genotype-specific differences were detected between *mPtpg*-mutant mice and controls (Tables 7 and 8). In the bone densitometry using DEXA analysis (Table 9), BMD, pBMD, and body weight were increased, but no significant difference was observed when BMD was related to the body weight (sBMD). Therefore differences between mutants and controls in bone mineral density might be due to changes in body weight and thus be secondary effects. In addition the measured values were in the range of our 129/Sv baseline data (in BMD and pBMD the mutants fit better than wild-type littermate controls). The sex differences we observed are common in many mouse strains, and thus are not abnormal.

3.2.5 References

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Abbreviations

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

Table 6: Results from clickbox test (hearing test)				
Phenotype	**Male**		**Female**	
	Control	**Mutant**	**Control**	**Mutant**
0	-	-	-	-
1	-	-	-	-
2	-	1	1	1
3	11	13	14	14
4	-	-	-	-
Mean Score	***3.00***	***2.93***	***2.93***	***2.93***
Kruskal-Wallis Anova on Ranks: n.s.				

0: no reaction at all,
1: very slow reaction,
2: retarded reaction,
3: normal reaction,
4 strong reaction

Table 7: Results from the morphological inspection				
Parameter	**Male**		**Female**	
	Control	**Mutant**	**Control**	**Mutant**
Growth				
normal	10	14	15	15
smaller	1	-	-	-
Weight				
normal	11	14	15	15
Body size				
normal	11	14	15	15
Eye				
normal	11	14	15	15
Coat hair growth				
normal	11	14	15	15
Coat hair texture				
normal	11	14	15	15
Coat color				
agouti unicolored	11	14	15	14
blond tuft on the head	-	-	-	1
Hair follicle structure / orientation				
normal	11	14	15	15
Skin pigmentation				
normal	11	14	15	15
Skin texture / condition				
normal	11	14	15	15

Vibrissae				
normal	11	14	15	15
Limbs				
normal	11	14	15	15
Digits				
normal	11	14	14	15
Absent toe	-	-	1	-
Tail				
normal	11	14	15	15
Teeth				
normal	11	14	15	15
Ear morphology				
normal	11	14	15	15
Musculature				
normal	11	14	15	15
Seizures / epilepsy				
no	11	14	15	15
Motor capabilities / coordination				
normal	11	14	15	15
Movement				
normal	11	14	15	15
Feeding / drinking behavior				
normal	11	14	15	15
Respiratory system				
normal	11	14	15	15
Reproductive system				
normal	11	14	15	15
Other abnormalities				
no	11	13	15	15
shivers	-	1	-	-
Animals analyzed	**11**	**14**	**15**	**15**

Table 8: Results from the X-ray analysis				
Parameter	**Male**		**Female**	
	control	**mutant**	**control**	**mutant**
Skull shape				
normal	4	9	10	9
abnormal snout shape	1	-	-	-
Mandibles				
normal	5	9	10	9
Maxilla				
normal	5	9	10	9
Teeth				
normal	5	9	10	9
Orbit				
normal	5	9	10	9
Number of cervical vertebrae				
normal	5	9	10	9
Number of thoracic vertebrae				
normal	5	9	10	9
Number of lumbar vertebrae				
normal	5	9	10	9
Number of pelvic vertebrae				
normal	5	9	10	9
Number of sacral vertebrae				
normal	5	9	10	9
Vertebrae shape				
normal	5	9	10	9
Number of ribs				
26	5	9	10	9
Rib shape				
normal	5	9	10	9
Scapulas				
normal	5	9	10	9
Clavicle				
normal	5	9	10	9
Pelvis				
normal	5	9	10	9
Femur shape				
normal	5	9	10	9
Tibia				
normal	5	9	10	9
Fibula				
normal	5	9	10	9
Humerus				
normal	5	9	10	9

Ulna				
normal	5	9	10	9
Radius				
normal	5	9	10	9
Number of digits				
normal	5	9	9	9
absent toe	-	-	1	-
Completeness of digits				
yes	5	9	10	9
Joints				
normal	5	9	10	9
Body fat				
normal	5	9	10	9
Growth				
normal	5	9	8	8
small	-	-	2	1
Body features				
slender	1	3	2	2
normal	4	6	8	7
Animals analyzed	***5***	***9***	***10***	***9***

Table 9: Bone- and weight-related quantitative parameters
(Data presented as mean ± standard error of mean)

Parameter	Control (A)		Mutant (B)		A ~ B	A ~ B	ANOVA		
	Male	Female	Male	Female	Male	Female	*p – value genotype*	*p – value sex*	*p – value interaction*
	(n=5)	(n=10)	(n=9)	(n=9)	*p – value*	*p – value*			
BMD [mg/cm^2]	62 ± 1	65 ± 1	67 ± 2	69 ± 2	n.s.	n.s.	< 0.05	n.s.	n.s.
pBMD [mg/cm^2]	52 ± 1	52 ± 1	56 ± 2	56 ± 2	n.s.	n.s.	< 0.05	n.s.	n.s.
sBMD [10^{-3} x cm^{-2}]	2.23 ± 0.01	2.87 ± 0.01	2.25 ± 0.01	2.95 ± 0.11	n.s.	n.s.	n.s.	< 0.0001	n.s.
BMC [mg]	745 ± 14	621 ± 37	785 ± 52	617 ± 47	n.s.	n.s.	n.s.	< 0.01	n.s
Body Length [cm]	10.00 ± 0.00	9.39 ± 0.07	10.00 ± 0.08	9.44 ± 0.06	n.s.	n.s.	n.s.	< 0.0001	n.s.
Body Weight [g]	27.94 ± 0.56	22.67 ± 0.38	30.07 ± 0.78	23.29 ± 0.43	n.s.	n.s.	< 0.05	< 0.0001	n.s.
Lean mass [units]	20.96 ± 1.23	16.13 ± 0.58	21.01 ± 1.14	17.54 ± 0.62	n.s.	n.s.	n.s.	< 0.0001	n.s.
Fat mass [units]	3.83 ± 0.83	3.60 ± 0.63	5.89 ± 0.84	2.90 ± 0.64	n.s.	n.s.	n.s.	< 0.05	n.s.
Bone Content [%]	2.67 ± 0.06	2.73 ± 0.14	2.61 ± 0.16	2.64 ± 0.18	n.s.	n.s.	n.s.	n.s.	n.s
Lean Content [units x 100/g]	74.85 ± 3.37	71.36 ± 3.06	69.76 ± 3.07	75.45 ± 2.84	n.s.	n.s.	n.s.	n.s.	n.s
Fat Content [units x 100/g]	13.86 ± 3.20	15.69 ± 2.67	19.60 ± 2.87	12.28 ± 2.66	n.s.	n.s.	n.s.	n.s.	n.s
Femur span[1] [mm]	1.36 ± 0.04	1.17 ± 0.03	1.40 ± 0.03	1.20 ± 0.02	n.s.	n.s.	n.s.	< 0.0001	n.s.
Subcutaneous fat[1] [mm]	3.68 ± 0.1	4.27 ± 0.1	3.96 ± 0.1	4.23 ± 0.1	n.s.	n.s.	n.s.	< 0.01	n.s.
Vertebrae hight[2] [mm]	3.22 ± 0.05	3.12 ± 0.02	3.33 ± 0.07	3.17 ± 0.05	n.s.	n.s.	n.s.	< 0.05	n.s.
	Male	Female	Male	Female	Male A ~ B	Female A ~ B	ANOVA		
	(n=5)	(n=10)	(n=9)	(n=10)	*p – value*	*p – value*	*p – value genotype*	*p – value sex*	*p – value interaction*
Ionized Calcium [mmol/l]	1.33 ± 0.01	1.28 ± 0.05	1.31 ± 0.02	1.33 ± 0.02	n.s.	n.s.	n.s.	n.s.	n.s.

1: mean value of the two hind limbs
2: third lumbar vertebra

3.3 Neurology Screen

3.3.1 Summary

In the primary neurological screen 29 mutant (14 males/ 15 females) and 26 control mice (11 males/15 females) were screened. Animals were analyzed according to our modified SHIRPA protocol where a battery of behavioral tests is carried out. This primary observation screen is a modification of the Irwin procedure (Irwin, 1968) and was proposed as a rapid, comprehensive and semi-quantitative screening method for qualitative analysis of abnormal phenotypes in a mouse strain (Rogers *et al.,* 1994). We carried out 23 designed test parameters (See web page: http://www.mgu.har.mrc.ac.uk/facilities/mutagenesis/mutabase/shirpa_summary.html) to detect phenotypic differences between knockout and control mice. The test parameters contribute to an overall assessment of muscle, motor neuron, spinocerebellar, sensory and autonomic functions. The primary neurological screen is focused on investigating neurological signs to determine the neurological functioning of a mouse. We also examine lactate levels in the blood of mice to draw conclusions about energy metabolism. Moreover, we measured forelimb grip strength to evaluate muscle function.

The comparison of mutant mice to controls revealed no significant genotype-related differences. All SHIRPA test parameters were without pathological findings. Measurement of grip strength also revealed no significant differences between mutant mice and wild-type control animals.

3.3.2 Mice

Fourteen 10-week-old male mutant and 11 control mice entered the neurological screen at the beginning of the 48th calendar week. Fifteen female mutant and control mice entered the neurological laboratory one week later. All animals were fed *ad libitum* for a period of one week during their stay in the neurological screen.

3.3.3 Material and Methods

Primary screening: modified SHIRPA protocol

Assessment of each animal at age 10 weeks began with observation of undisturbed behavior (*Viewing Jar Behavior*) in a glass cylinder (11 cm in diameter). The mice were then transferred to an arena consisting of a clear Perspex box (420 x 260 x 180 mm) in which a Perspex sheet on the floor is marked with 15 squares. Locomotor activity and motor behavior was observed within this area (*Behavior recorded in the Arena*). This was followed by a sequence of manipulations testing reflexes (*Behavior recorded on or above the arena*). Measurements were completed with the recording of body weight. The last part of the primary screen also involved the analysis of righting reflex and contact righting reflex. A glass cylinder (35 mm diameter, 135 mm length) was used for testing of the contact righting reflex. Throughout the entire procedure, abnormal behavior, biting, defecation and vocalization were recorded.

Between testing of each mouse, fecal pellets and urination were removed from the viewing jar and arena. All experimental equipment was thoroughly cleaned with Pursept-A and dried prior to testing.

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

Further screening: grip strength

The grip strength meter system determines the fore limb 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. Five trials were undertaken for each mouse within one minute. The mean value is used to represent the grip strength of a mouse.

All experimental equipment was thoroughly cleaned with Pursept-A and dried prior subsequent tests. Values were presented as means ± standard error of mean (SEM).

Statistical analysis of the grip strength trial results. Grip strength trial results are compared between genotypes, controlling for the effects of sex and weight, by fitting linear mixed effect models (Pinheiro and Bates, 2000). A linear mixed effect model is a modified analysis of variance/covariance approach allowing for dependecies in the data. In our case, dependencies arise from repeated trials within each mouse. Genotype, sex and weight are modelled as fixed effects; mouse-specific intercepts are modelled by including the intercept as random effect. Interaction effects are tested for and included in the model if they show a significant contribution. A serial dependency on the trial number can be tested by including the trial number as random effect with an autoregressive correlation structure. Model fitting is performed by the nlme-Package in the open-source statistical software R, a close relative of S-PLUS (The R Project for Statistical Computing, 2004).

3.3.4 Parameters

Muscle/lower motor neuron function
Body position, gait, Positional passivity, tail elevation, grip strength, defecation
Spinocerebellar function
Body position, gait, righting reflex, tail elevation, grip strength
Sensory function
Transfer arousal, touch escape, gait, pinna reflex, righting reflex
Autonomic function
Palpebral closure, defecation, lacrimation
Neurological reflexes
Righting reflex (pons), contact righting reflex, pinna reflex
General appearance
Body weight, body position, transfer arousal, touch escape, vocalization, positional passivity, aggression, spontaneous activity, locomotor activity, skin color

3.3.5 Results

The comparison of *mPtpg*-mutant mice to controls revealed no genotype-related differences in the primary neurological screen. All SHIRPA test parameters were without pathological findings (Tables 10-14). Measurement of grip strength revealed no differences between mutant mice and wild-type controls as well (Fig. 3).

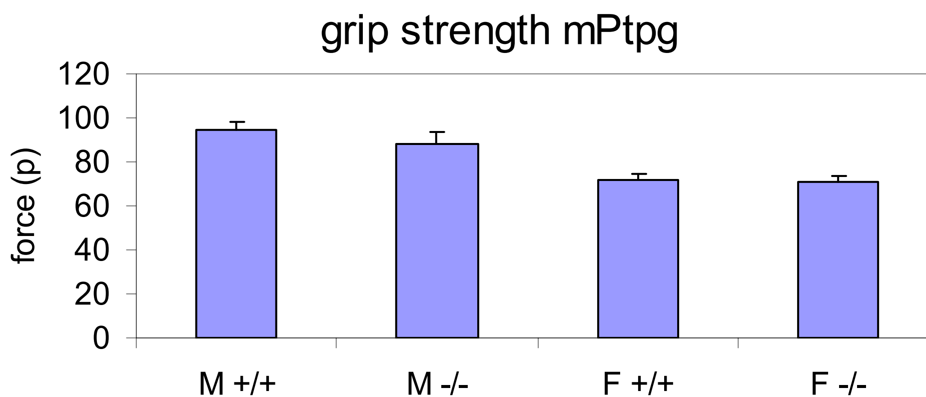

Figure 3: Results from grip strength testing

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

3.3.6 Discussion

In our neurological screening male and female *mPtpg*-mutant mice did not show any neurological abnormalities. All parameters tested were without pathological findings.

Since the function of the gene is still unknown and there is expression - among other tissues - in CNS, there could have been neurological alterations in the mutant mice. However, none were found in the primary neurological screen. To evaluate further a possible neurological defect the expression profile of *mPtpg* should be analyzed in more detail to get a hint of possible mechanisms involved.

3.3.7 References

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

The R Project for Statistical Computing, 2004; http://www.r-project.org/

Abbreviations

SHIRPA **S**mithKline Beecham Pharmaceuticals; **H**arwell, MRC Mouse Genome Centre and Mammalian Genetics Unit; **I**mperial College School of Medicine at St. Mary's **R**oyal London Hospital, **P**henotype **A**ssessment
http://www.mgu.har.mrc.ac.uk/mutabase/shirpa_summary.html

Table 10: Recording of body weight
Data are presented as mean ± standard error of mean.

Parameter	Male			Female		
	Control (n=11)	Mutant (n=14)	*p-value*	Control (n=15)	Mutant (n=15)	*p-value*
Body Weight [g]	25.5±0.5	26.6±0.6	***n.s.***	21.6±0.4	21.6±0.3	***n.s.***

Table 11: Behavior recorded in viewing jar
Statistical analysis: chi-squared test; significance p<0.05

Parameter	Male			Female		
	Control (n=11)	Mutant (n=14)	*p-value*	Control (n=15)	Mutant (n=15)	*p-value*
Body Position						
Inactive Active Excessive Activity	0 11 0	0 14 0	***n.s.***	0 15 0	0 15 0	***n.s.***
Tremor						
Absent Present	11 0	14 0	***n.s.***	15 0	15 0	***n.s.***
Palpebral closure						
Eyes open Eyes closed	11 0	14 0	***n.s.***	15 0	15 0	***n.s.***
Coat Appearance						
Tidy and well groomed Irregularities	11 0	14 0	***n.s.***	15 0	15 0	***n.s.***
Whiskers						
Present Absent	11 0	14 0	***n.s.***	15 0	14 1	***n.s.***
Lacrimation						
Absent Present	11 0	14 0	***n.s.***	15 0	15 0	***n.s.***
Defecation						
Present Absent	10 1	14 0	***n.s.***	15 0	15 0	***n.s.***

Table 12: Recording of locomotor activity and behavior in the arena
Statistical analysis: chi-squared test; significance $p < 0.05$. Locomotor activity data are shown as mean (± SEM)

Parameter	**Male**			**Female**		
	Control (n=11)	**Mutant (n=14)**	***p-value***	**Control (n=15)**	**Mutant (n=15)**	***p-value***
Transfer arousal						
Extended freeze Brief freeze Immediate movement	0 11 0	1 13 0	***n.s.***	1 14 0	0 15 0	***n.s.***
Locomotor activity	18.1±0.9	15.4±1.2	***n.s.***	17.9±1.1	16.9±1.4	***n.s.***
Gait						
Fluid movement Lack Fluidity	10 1	14 0	***n.s.***	15 0	15 0	***n.s.***
Tail Elevation						
Dragging Horizontally extension Elevated/Straub tail	0 11 0	0 14 0	***n.s.***	1 14 0	0 15 0	***n.s.***
Touch Escape						
No response Response to touch Flees prior to touch	0 11 0	1 13 0	***n.s.***	0 15 0	0 15 0	***n.s.***
Positional Passivity						
Struggles when held by tail No struggle	11 0	14 0	***n.s.***	15 0	15 0	***n.s.***

Table 13: Behavior recorded in or above the arena
Statistical analysis: chi-squared test; significance $p < 0.05$

Parameter	Male			Female		
	Control (n=11)	Mutant (n=14)	*p-value*	Control (n=15)	Mutant (n=15)	*p-value*
Skin Color						
Blanched	0	0		2	0	
Pink	11	14		13	15	
Bright deep red	0	0	***n.s.***	0	0	***n.s.***
Trunk curl						
Absent	8	8		15	13	
Present	3	6	***n.s.***	0	2	***n.s.***
Limb Grasping						
Absent	11	13		15	15	
Present	0	1	***n.s.***	0	0	***n.s***
Pinna Reflex						
Present	10	13		14	12	
Absent	1	1	***n.s.***	1	3	***n.s.***
Corneal Reflex						
Present	11	14		14	15	
Absent	0	0	***n.s.***	1	0	***n.s.***
Righting Reflex						
Rights itself	11	14		14	14	
Fails to right when released	0	0	***n.s.***	1	1	***n.s.***
Contact Righting						
Present	6	11		6	9	
Absent	5	3	***n.s.***	9	6	***n.s.***
Evidence of biting						
None	11	14		15	15	
Biting in response to handling	0	0	***n.s.***	0	0	***n.s.***
Vocalization						
None	9	14		15	15	
Vocal	2	0	***n.s.***	0	0	***n.s.***

Table 14: Lactate levels
Data shown represent the results of the mean blood lactate concentrations, value (± SEM)

	Male			Female		
	Control (n=11)	**Mutant (n=14)**	***p-value***	**Control (n=15)**	**Mutant (n=15)**	***p-value***
Lactate [mmo/l]	6.6±0.4	5.8± 0.3	***n.s.***	5.5± 0.2	5.0± 0.2	***n.s.***

3.4 Eye Screen

3.4.1 Summary

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

In humans blindness is caused by several different ocular diseases. Among these, the cataracts are responsible for half of all cases (Johnson and Foster, 2003). The retinal disorders cover a broad variety of clinical symptoms and many different genes are involved in the corresponding pathological conditions in humans. The two most important groups are retinitis pigmentosa (RP) and age-related-macular-degeneration (ARMD; for recent reviews, see Rivolta et al., 2002 and Stone et al., 2001). Mouse models are appropriate tools to understand the genetic and biochemical mechanisms of ocular disorders. There is a rapid increasing number of mouse mutants available suffering from various types of eye diseases (for recent reviews see Graw, 2003 and Dalke & Graw, 2005).

No genotype-specific differences between mutant mice and their control littermates were detected.

3.4.2 Mice

Twenty-five control (11 male. 14 female) and 30 mutant mice (15 male. 15 female) 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*.

3.4.3 Materials and Methods

Electroretinography (ERG) was used to examine the retinal function as described (Dalke *et al.,* 2004). Mice were dark-adapted for at least 12 hours and anaesthetized with 137 mg Ketamine and 6.6 mg Xylazine per kg body weight. After pupil dilation (1 drop Atropine 1%), individual mice were fixed on a sled with Velcro straps. Gold wires (as active electrodes) were placed on the cornea; care was taken not to obstruct the pupillary opening. The ground electrode was a subcutaneous needle in the tail; a reference electrode was placed subcutaneously between the eyes. The mice were introduced into an ESPION ColorBurst Handheld Ganzfeld LED stimulator (Diagnosys LLC, Littleton, MA, USA) on a rail to guide the sled (High-Throughput Mouse-ERG, STZ for Biomedical Optics and Function Testing, Tübingen, Germany). To minimize temperature influences on the ERG, body temperature was kept at $37^{o}C$ using a warming plate. 10 ms light pulses were delivered at a frequency of 0.48 Hz in two steps at 500 and 12,500 cd/m^2. Bandpass filter was set ranging from 0.15 to 1000 Hz. Responses were recorded simultaneously from both eyes with an ESPION Console (Diagnosys LLC, Littleton, MA, USA) and stored for offline analysis after averaging 10-40 individual measurements at each step.

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

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

3.4.4 Parameters

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

3.4.5 Results and Discussion

ERG responses were recorded from the groups of mPtpg (wild type control – mutant) mice with light pulses at two different light intensities. These two luminance levels were chosen because at 500 cd/m^2 a well discernable b-wave amplitude (nearly no a-wave) mainly stemming from the rod system is induced, while light pulses at 12,500 cd/m^2 induce a maximally developed b-wave response and an a-wave, coming presumably from rods and cones (Dalke et al., 2004).

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 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, wild type and mutant (Table 15). The comparison of a- and b-wave amplitudes of males and females revealed no consistent differences. Between the groups of mutant and control mice no significant differences were found, neither in the male nor in the female group. Histological analysis of the retinal structure revealed no gross abnormalities (Fig. 4).

A total of 55 mice were examined ophthalmologically by **slit lamp biomicroscopy**. No eye phenotype was shown to be associated with the *mPtpg* muta-

tion (Table 16). However, all animals expressed a nuclear-zonular opacity, suggesting a genetic background effect on eye phenotype.

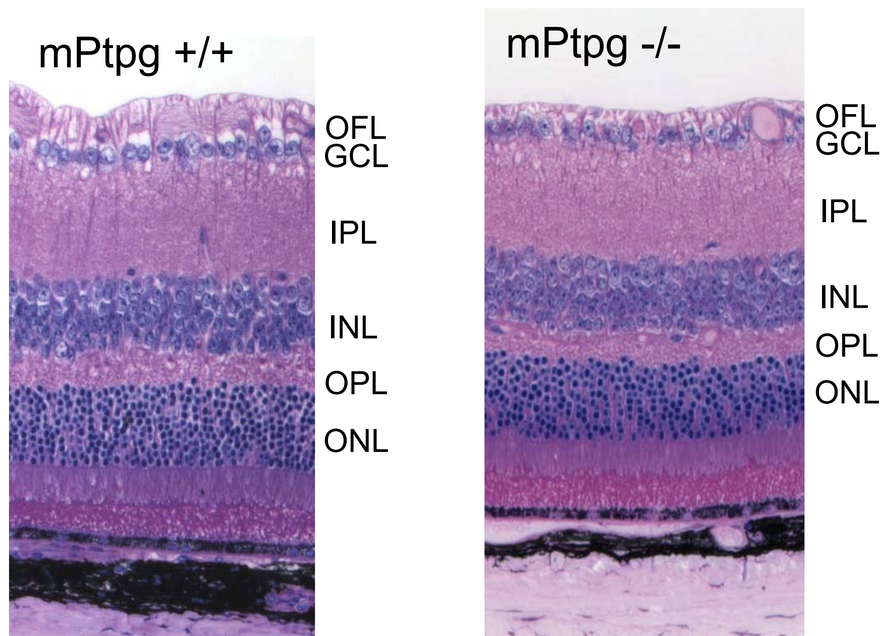

Figure 4: Histological analysis of the retinal structure.
No gross abnormalities.

OFL: outer fibre layer
GCL: ganglion cell layer
IPL: inner plexiforme layer
INL: inner nuclear layer
OPL: outer plexiforme layer
ONL: outer nuclear layer

3.4.6 References

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Abbreviations

cd/m^2	candela per square meter
ERG	electroretinography
Hz	hertz
n.s.	not significant
NAD	no abnormality detected

Table 15: Comparison of ERG-responses at illumination levels of 500 and 12,500 cd/m^2.
Mean ± standard error is calculated for a- and b-wave amplitudes.

Parameter	Control (A)			Mutant (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=11)	(n=14)	***p - value***	(n=15)	(n=15)	***p - value***	***p - value***	***p - value***
a-wave [µV] 500 cd/m²	-8 ± 1.7	-8 ± 1.2	n.s.	-9 ± 1.5	-9 ± 1.0	n.s.	n.s.	n.s.
b-wave [µV] 500 cd/m²	168 ± 9.1	157 ± 5.2	n.s.	165 ± 7.3	148 ± 5.1	n.s.	n.s.	n.s.
a-wave [µV] 12,500 cd/m²	-29 ± 2.8	-21 ± 2.2	<0.05	-24 ± 2.1	-23 ± 1.4	n.s.	n.s.	n.s.
b-wave [µV] 12,500 cd/m²	219 ± 14.7	227 ± 12.7	n.s.	208 ± 7.3	198 ± 9.7	n.s.	n.s.	n.s.

Table 16: Results from slit lamp biomicroscopy

Genotype	NAD	Nuclear-zonular Opacity	Corneal Erosions	Microphthalmia
mPtpg$^{-/-}$	-	29	-	-
mPtpg$^{+/+}$	-	26	-	-

3.5 Clinical-Chemical Screen

3.5.1 Summary

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

In the primary clinical chemical screen, twenty-nine (14 males/15 females) mutant and twenty-four (10 males/ 14 females) control mice were analyzed. Twenty different clinical-chemical parameters were measured including various enzyme activities, as well as plasma concentrations of specific substrates and electrolytes. Additionally, we measured eight basic hematological parameters. All parameters of both mutant and control mice were within the normal ranges usually found in 129/Sv mice. All hematological parameters were without pathological findings. Although we detected a few significant differences it seems as if clinical chemical and hematological parameters are not influenced by the genotype of *mPtpg*-mutant mice. The differences detected are most likely a result of physiologic variation.

3.5.2 Mice

Ten 12-week-old wild-type littermate controls and 14 mutant males entered the clinical-chemical screen at the beginning of the 50th calendar week in 2004. Twenty-nine females (14 control and 15 mutant mice) entered the screen at the beginning of the 51st calendar week.

3.5.3 Materials and Methods

Blood Withdrawal and Storage

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

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

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

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

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

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

3.5.4 Parameters

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

3.5.5 Results

Clinical Chemistry

Most values obtained for the clinical chemical parameters were within the normal ranges usually found in 129/Sv mice at the age of three months as supported by previously published data (Hough *et al.*, 2002; Quimby, 1999; Table 17).

Sex differences were detected for many clinical chemical parameters in the control animals as well as in the mutant mice reflecting the physiological differences usually found in 129/Sv mice. Concerning the clinical chemical parameters we found significant differences between mutant males and controls for Aspartate-Aminotransferase (GOT) activity and ferritin concentrations. In the female group, the mean GOT activity of the mutant animals was also higher than the mean of the control mice, but the difference was not significant. Concerning the ferritin concentration, we saw contrariwise results in the female group: Herein control females had a higher mean ferritin level than the mutant mice. The most striking result for ferritin is the loss of sex differences in the mutant mice and an elevated ferritin concentration for mutant males.

Hematology

The most striking findings in the haematological parameters were exceptionally high white and red blood cell counts in all animals and no sex-related differences in both genotypes (Table 18). Dependent on these results haemoglobin and hematocrit were also elevated compared to our baseline data (unpublished results). There were no significant differences between mutant and control animals.

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

3.5.6 Discussion

The slightly significant difference for GOT activity in male mice is caused by elevated activity levels of muscle enzymes in two of the mutant male mice, most likely due to the handling during the blood sample collection. The absence of sex differences in ferritin measurements and the elevated ferritin concentration in mutant males are unusual. Serum ferritin is a sensitive indicator of body iron stores and is especially useful in distinguishing iron deficiency from the anemia of chronic disorders because in the latter ferritin levels are increased. Serum ferritin is also increased in other anemias including aplastic anemia, sideroblastic anemia, chronic haemolytic anemias and inherited iron overload (hemochromatosis). For female animals there were reversed results for ferritin concentrations detectable. However, the elevated white and red blood cell counts we found in all animals excluded any type of anemia from being these cause of the ferritin results. Since there were not genotype- or sex-specific differences in the hematological parameters, the finding most likely reflects an unusual background specific characteristic.

Most values of mutant and control animals for all parameters were within the normal ranges typical for baseline 129/Sv mice. All clinical chemical and hematological parameters were without pathological findings. In conclusion of these results we were not able to detect clearly genotype-related changes of

clinical chemical or hematological parameters for the mPtpg mutant mouse line.

3.5.7 References

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Table 17: Clinical-chemical parameters.
Data are presented as mean ± standard error of mean.

Parameter	Mutant (A)			Control (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=14)	(n=15)	*p- value*	(n=10)	(n=14)	*p-value*	*p-value*	*p-value*
Sodium [mmol/l]	154 ±0.59	153 ±0.43	n.s.	153 ±0.85	153 ±0.45	n.s.	n.s.	n.s.
Potassium [mmol/l]	4.5 ±0.05	4.5 ±0.09	n.s.	4.6 ±0.11	4.4 ±0.07	n.s.	n.s.	n.s.
Calcium [mmol/l]	2.0 ±0.02	2.1 ±0.02	n.s.	2.1 ±0.03	2.1 ±0.03	n.s.	n.s.	n.s.
Chloride [mmol/l]	110.1 ±0.50	113.3 ±0.30	<0.001	110.0 ±0.62	112.7 ±0.50	<0.01	n.s.	n.s.
Inorganic Phosphate [mmol/l]	1.2 ±0.07	0.9 ±0.05	<0.01	1.2 ±0.08	0.9 ±0.05	<0.01	n.s.	n.s.
Total Protein [g/dl]	5.5 ±0.09	5.4 ±0.08	n.s.	5.4 ±0.10	5.4 ±0.12	n.s.	n.s.	n.s.
Creatinine [mg/dl]	0.340 ±0.00	0.333 ±0.01	n.s.	0.356 ±0.01	0.331 ±0.00	<0.01	n.s.	n.s.
Urea [mg/dl]	58.3 ±2.06	47.7 ±1.36	<0.001	59.6 ±4.42	46.2 ±1.73	<0.02	n.s.	n.s.
Uric acid [mg/dl]	1.4 ±0.12	2.3 ±0.21	<0.001	1.0 ±0.18	1.9 ±0.17	<0.01	n.s.	n.s.
Cholesterol [mg/dl]	125.8 ±3.98	111.8 ±2.33	<0.01	122.5 ±2.94	111.2 ±3.22	<0.02	n.s.	n.s.
Triglyceride [mg/dl]	145.5 ±8.89	108.0 ±5.27	<0.01	129.8 ±9.65	101.6 ±5.41	<0.05	n.s.	n.s.
Creatine Kinase [U/l]	158 ±32.01	117 ±19.29	n.s.	137 ±37.18	96 ±28.30	n.s.	n.s.	n.s.
Alanine-Aminotransferase (ALAT,GPT) [U/l]	19 ±2.02	17 ±2.12	n.s.	21 ±2.46	16 ±1.31	n.s.	n.s.	n.s.
Aspartate-Aminotransferase (AST,GOT) [U/l]	28 ±1.94	35 ±2.93	n.s.	23 ±1.31	28 ±1.64	<0.05	<0.05	n.s.
Alkaline Phosphatase [U/l]	72 ±2.55	85 ±1.92	<0.001	72 ±2.03	85 ±3.72	<0.01	n.s.	n.s.
α-Amylase [U/l]	2740 ±48.85	2620 ±45.58	n.s.	2746 ±83.77	2691 ±67.65	n.s.	n.s.	n.s.
Glucose [mg/dl]	133.4 ±6.81	132.2 ±6.80	n.s.	150.8 ±8.44	131.6 ±5.28	n.s.	n.s.	n.s.
Ferritin [ng/ml]	40.4 ±1.88	40.9 ±2.02	n.s.	35.5 ±0.87	43.8 ±1.44	<0.001	<0.05	n.s.
Transferrin [mg/dl]	153.3 ±1.01	153.3 ±1.05	n.s.	153.9 ±1.70	153.1 ±1.34	n.s.	n.s.	n.s.
Lipase [U/l]	60.4 ±2.09	46.7 ±1.66	<0.001	59.7 ±2.02	47.6 ±5.52	n.s.	n.s.	n.s.

Table 18: Hematological parameters.
Data are presented as mean ± standard error of mean.

Parameter	Mutant (A)			Control (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=14)	(n=15)	*p - value*	(n=10)	(n=14)	*p - value*	*p - value*	*p - value*
White blood cell count [10³/µl]	10.77 ±0.39	10.56 ±0.63	n.s.	10.03 ±0.51	9.25 ±0.59	n.s.	n.s.	n.s.
Red blood cell count [10³/µl]	12.17 ±0.18	11.92 ±0.24	n.s.	11.76 ±0.24	11.62 ±0.24	n.s.	n.s.	n.s.
Hemoglobin [g/dl]	18.47 ±0.30	18.28 ±0.35	n.s.	17.82 ±0.36	17.76 ±0.34	n.s.	n.s.	n.s.
Hematocrit [%]	59 ±1.05	58 ±1.22	n.s.	57 ±1.11	56 ±1.17	n.s.	n.s.	n.s.
Mean corpuscular volume [fl]	48.29 ±0.22	48.47 ±0.24	n.s.	48.10 ±0.31	48.67 ±0.23	n.s.	n.s.	n.s.
Mean corpuscular hemoglobin [pg]	15.19 ±0.07	15.34 ±0.09	n.s.	15.16 ±0.12	15.29 ±0.09	n.s.	n.s.	n.s.
Mean corpuscular hemoglobin con-centration [g/dl]	31.52 ±0.10	31.59 ±0.11	n.s.	31.48 ±0.12	31.47 ±0.15	n.s.	n.s.	n.s.
Platelet count [10³/µl]	473 ±18.74	504 ±17.53	n.s.	488 ±19.06	481 ±16.46	n.s.	n.s.	n.s.

3.6 Immunology Screen

3.6.1 Summary

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

According to the data summary of what is already known about the mutant mouse line presented to the GMC by the mouse provider, no immunological phenotype was known for the mPtpg mutant mouse line. Their analysis in the Immunology Screen revealed minor differences between the mutants and their littermate controls, namely slightly decreased NK cell frequencies in the mutants, and changes in the levels of IgM, IgG_{2a} and IgA.

3.6.2 Mice

We analyzed 29 *mPtpg*-mutant animals (15 females and 14 males) and 25 age- and sex-matched littermate controls (15 females and 10 males).

3.6.3 Material and Methods

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

The following main cell populations were analyzed: B cells ($CD19^+$ clone 1D3), B1 B cells ($CD19^+CD5^+$, clone 53-7.3), B2 B cells ($CD19^+CD5^-$), T cells ($CD3^+$, clone 145-2C11), $CD4^+$ T cells (clone RM4-5), $CD8^+$ T cells (CD8α, clone 53-6.7; CD8β, clone H35-17.2), γ/δT cells (clone GL3), granulocytes ($Gr\text{-}1^+$, clone RB6-8C5), and NK cells ($CD49b^+$, clone DX5). We also analyzed additional subpopulations based on the following surface antigens: IgD (clone 11-26c.2a), B220 (clone RA3-6B2), CD11b (clone M1/70), CD103 (clone 2E7), CD25 (clone PC61), CD62L (clone MEL-14), CD45RA (clone 14.8), Ly-6C (clone AL-21), and CD44 (clone IM7). Data were acquired on a FACS Calibur (BectonDickinson, San Diego, USA) and were analyzed using FlowJo software (TreeStar Inc, USA). All samples were acquired until a total number of 25,000 cells was reached.

The plasma levels of IgM, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, and IgA were determined by standard sandwich ELISAs using goat anti-mouse immunoglobulin

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

3.6.4 Parameters

Flow cytometry
B cells ($CD19^+$), B1 B cells ($CD19^+CD5^+$), B2 B cells ($CD19^+CD5^-$), T cells ($CD3^+$), $CD4^+$ T cells, $CD8^+$ T cells, γ/δT cells, granulocytes ($Gr\text{-}1^+$), and NK cells ($CD49b^+$). Furthermore, all potential subpopulations which can be identified by co-staining for other surface markers (IgD, B220, CD11b, MHC II, I-A^k, CD25, CD8β, CD62L, CD45RA, Ly-6C, CD44) using 6 parameter/5 color flow cytometry were analyzed.
ELISA
IgM, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgA; anti-DNA antibodies, rheumatoid factor

3.6.5 Results and Discussion

The analysis of the mPtpg mutant mouse line in the primary Immunology Screen did not reveal profound alterations in the tested parameters (Table 19). However, we were able to detect some minor, but statistically significant differences affecting NK cell (CD49b+) frequencies, which were slightly decreased in the mutants. In addition, we observed changes in the levels of IgM, IgG_{2a} and IgA.

Although the detected alterations affecting the immune system are not particularly striking, keeping in mind the unknown function of the knocked-out gene, one cannot exclude that the subtle changes in mutant mice are attributed to *mPtpg* function. If the provider is interested in its immunological function, a repetition of the primary screen and eventual secondary screening would help elucidate this issue.

3.6.6 References

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Table 19: Basic parameters analyzed in the Immunology Screen.
Data are presented as mean ± standard error of mean.

Parameter	Mutants (A)			Control (B)			A ~ B	
	Male (n=14)	Female (n=15)	*p - value*	Male (n=10)	Female (n=15)	*p - value*	Male *p - value*	Female *p - value*
$CD19^+$ [%]	8.9±0.5	22.7±0.8	<0.001	8.9±0.7	23.3±1.0	<0.001	n.s.	n.s.
$CD19^+CD5^-$ [%]	98.1±0.3	88.5±0.8	<0.001	97.9±0.3	89.8±0.7	<0.001	n.s.	n.s.
$CD19^+CD5^+$ [%]	1.9±0.3	11.5±0.8	<0.001	2.1±0.3	10.2±0.7	<0.001	n.s.	n.s.
$CD3^+$ [%]	47.6±0.9	59.7±0.8	<0.001	45.6±1.2	59.6±1.0	<0.001	n.s.	n.s.
γ/δ TCR^+ [%]	0.04±0.01	NA	NA	0.03±0.01	NA	NA	n.s.	NA
$Gr\text{-}1^+$ [%]	13.5±1.0	7.2±0.4	<0.001	15.2±1.1	8.0±0.6	<0.001	n.s.	n.s.
$CD49b^+$ [%]	8.5±0.7	6.5±0.7	<0.05	11.3±0.8	10.3±1.0	n.s.	<0.02	<0.01
$CD4^+$ [%]	39.6±0.7	45.3±0.7	<0.001	38.1±1.1	45.8±0.8	<0.001	n.s.	n.s.
$CD8\beta^+$ [%]	12.3±0.3	14.3±0.3	<0.001	11.9±0.3	13.9±0.3	<0.001	n.s.	n.s.
IgG_1 [μg/ml]	62.6±5.6	289.6±27.4	<0.001	78.2±19.0	272.1±16.0	<0.001	n.s.	n.s.
IgG_{2a} [μg/ml]	NA	1603.5±173	NA	NA	995.4±126	NA	NA	<0.05
IgG_{2b} [μg/ml]	128.6±5.9	215.9±10.4	<0.001	145.6±14.6	202.1±10.8	<0.01	n.s.	n.s.
IgG_3 [μg/ml]	NA	66.9±4.6	NA	NA	75.4±7.2	NA	NA	n.s.
IgM [μg/ml]	492.3±66.2	657.7±47.3	n.s.	692.8±67.1	515.1±39.0	<0.05	<0.05	<0.05
IgA [μg/ml]	55.7±9.7	117.2±18.5	<0.01	30.7±6.0	166.1±28.2	<0.001	<0.05	n.s.
Anti-DNA Ab [%]	0	0	n.s.	0	0	n.s.	n.s.	n.s.
Rheuma-toid factor [%]	0	0	n.s.	0	0	n.s.	n.s.	n.s.

Raw data will be available on demand.

3.7 Allergy Screen

3.7.1 Summary

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

In the primary Allergy screen of the mPtpg mutant mouse line, 24 control and 29 mutant animals were screened. Their analysis revealed profound differences between mutant and control male mice, namely statistically significant higher mean IgE concentrations in male mutant mice compared to male controls.

3.7.2 Mice

An age- and sex-matched group of 24 controls (14 females, 10 males) and 29 mutants (15 females, 14 males) mice aged 12 weeks was analysed in Allergy screen.

3.7.3 Material and Methods

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

3.7.4 Results and Discussion

The analysis of total IgE levels in mPtpg mice revealed statistically significant higher mean IgE concentrations in male mutant mice compared to male controls. In addition, IgE levels were higher in female animals (Table 20). This

sex difference is a common finding in inbred lines (Allessandrini *et al.,* 2000; Corteling *et al.,* 2004; Seymour *et al.,* 2002).

Table 20: Total plasma IgE
Data are presented as mean ± standard error of mean.

	Control (A)			Mutant (B)			A~B	A~B
	Female	Male		Female	Male		Female	Male
	(n=10)	(n=11)	*p - value*	(n=15)	(n=14)	*p - value*	*p - value*	*p - value*
Total IgE [ng/ml]	380± 58.2	168± 12.7	<0.001	394± 64.4	298± 39	n.s.	n.s.	<0.02

Taken together, under standard screening conditions for primary Allergy screen, *mPtpg*-mutant mice, especially male animals showed changes in total plasma IgE levels that could reveal a major allergy phenotype. If the provider is interested in further experiments, the Allergy Screen could investigate the *mPtpg*-mutant mice and their control littermates in a secondary screening in an allergen challenging system established in our lab.

Raw data will be available on demand.

3.7.5 References

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3.8 Nociceptive Screen

3.8.1 Summary

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

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

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

In the Primary Screen the responsiveness of the intact somatosensory system to thermal pain was tested in the mPtpg mutant mouse line by means of the hot plate test (nociceptive pain). We found no significant differences in pain reactivity, neither between the mutant and control animals nor between the sexes. We do not recommend performing further pain related studies in this mutant mouse line.

3.8.2 Mice

Twenty-nine *mPtpg* knockout mice (14 male, 15 female), and 24 control animals (nine male, 15 female) were tested in our first screen.

3.8.3 Material and Methods

Hot plate test

The mice were placed on a metal surface maintained at 52±0.2°C (Hot plate system was made by TSE GMBH, Germany; Eddy and Leimbach, 1953). Locomotion of the mouse on the hot plate was constrained by 20 cm high plexiglas wall to a circular area with a diameter of 28 cm (Fig. 5). Mice remained on the plate until they performed one of three behaviors regarded as indicative of nociception: hind paw lick (h.p. licking), hind paw shake/flutter (h.p. shaking) or jumping.

We evaluated only hind paw but not the front paw responses, because fore paw licking and lifting are components of normal grooming behavior. Each mouse was tested only once since repeated testing leads to profound changes in response latencies. The latency was recorded to the nearest 0.1 s.

To avoid tissue injury 60 s cut-off time was used. The data values are given in seconds.

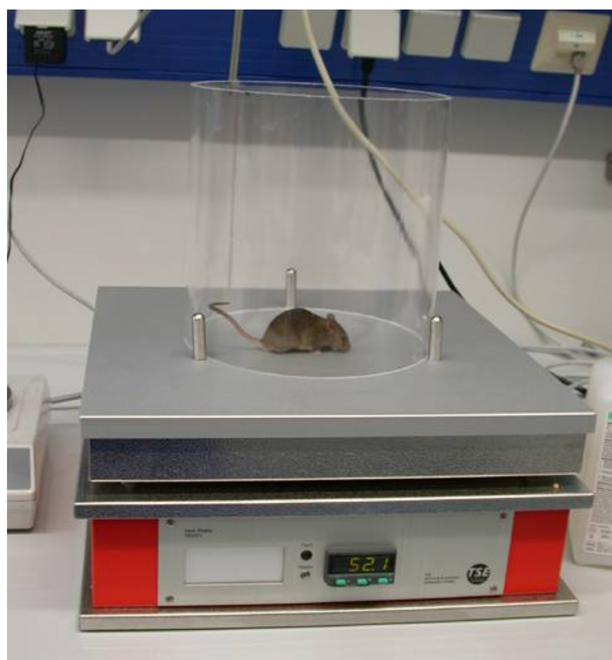

Figure 5: Hot plate system

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

3.8.4 Parameters

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

3.8.5 Results and Discussion
The first nociceptive response observed in these mice was hind paw shaking, followed by hind paw licking. The latencies did not differ significantly, either between the genotypes or between the sexes. The third examined response was the jumping of animals. Again the latencies did not differ (Table 21).

There were no significant differences in the pain reactivity, neither between the control and mutant mice nor between the sexes. Therefore we do not recommend making further pain related studies in this mutant mouse line.

3.8.6 References

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Abbreviations

h.p. hind paw

Table 21: Nociceptive Screen
Data are presented as mean ± standard error of mean.

							ANOVA		
							genotype		sex*genotype
Parameter Latency [s]	**Mutant (A)**			**Control (B)**			**A~B**	**A~B**	**ANOVA**
	Female	**Male**		**Female**	**Male**		**Female**	**Male**	
	(n=15)	(n=14)	***p - value***	(n=15)	(n=9)	***p - value***	***p - value***	***p - value***	***p - value***
H.p. licking	36.0± 2.7	33.1± 2.8	n.s.	41.9± 2.7	33.2± 3.5	n.s.	n.s.	n.s.	n.s.
H.p. shaking	25.6± 2.25	19.9± 2.3	n.s.	28.6± 2.25	22.7± 2.9	n.s.	n.s.	n.s.	n.s.
Jumping	60	58.3± 1	n.s.	59.4± 0.95	59.1± 1.2	n.s.	n.s.	n.s.	n.s.

3.9 Lung Function Screen

3.9.1 Summary

Neural and mechanical processes that control breathing frequency have been investigated in man for a long time (Mead, 1960; Otis *et al.*, 1959), but only with the availability of mouse inbred strains the contribution of genetic determinants to differential baseline breathing patterns could be elucidated (Tankersley *et al.*, 1997; Tankersley, 1999; Reinhard *et al.,* 2002; Reinhard *et al.,* 2005). By use of genetically engineered mice, candidate genes for human developmental disorders of breathing have been identified (Katz, 2003).

Spontaneous breathing patterns during rest and activity were studied in 16-week-old male and female *mPtpg*-mutant and control mice. Typical sex differences were observed in control animals at rest and during activity, although not all differences reached statistical significance. Absolute values of tidal volume and minute ventilation were comparable between sexes, but body weight related values for minute ventilation and tidal volume were higher in females. This is due to the about 20% lower body weight of females.

Despite showing a significantly lower body weight, male mutant mice used larger tidal volumes so that ventilation and specific values for tidal volume and minute ventilation were significantly increased at both levels of activity. A higher oxygen demand but also alterations in the gas exchanging function of the lung can be associated with these findings. Females appear to be not affected by the mutation. Lung function studies and morphological analysis of the lung is requested to further illuminate affects of the *mPtpg* mutation and assess possible underlying mechanisms.

3.9.2 Mice

Male and female control and mutant mice were studied at the age of 16 weeks. Body weight differed significantly between sexes and between male mutant and control mice (Table 22).

3.9.3 Material and Methods

Whole Body Plethysmography

A commercially available system from Buxco® Electronics (Sharon, Connecticut) was used to assess breathing patterns in unrestrained animals according to the principle described by Drorbaugh and Fenn (1955). It measures the pressure changes which arise from inspiratory and expiratory temperature and humidity fluctuations during breathing (Figs. 6 and 7).

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

Figure 6: System used at GMC to assess breathing patterns.

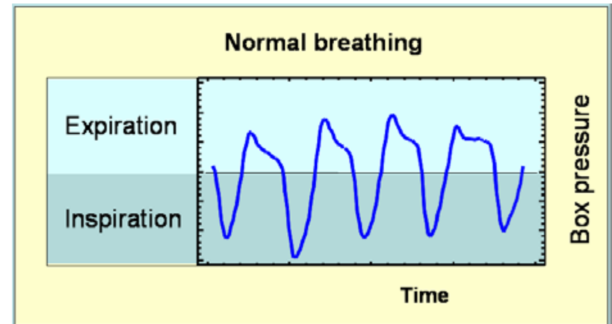

Figure 7: Recorded data used to calculate the breathing parameters.

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

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

3.9.4 Parameters

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

3.9.5 Results

Table 23 summarizes the results obtained for spontaneous breathing under resting and active conditions. Raw data are available on demand.

Typical **sex-related** differences were observed in control animals at rest and during activity, although not all differences reached statistical significance. Absolute values of tidal volume and minute ventilation were comparable between sexes, but females had somewhat higher respiratory rates. Accordingly, body weight related values for minute ventilation and tidal volume were higher in females. The slightly higher breathing rates in females resulted in shorter inspiratory and expiratory times in controls.

The obvious finding **associated with the mutation** is that male mutants were affected but females do not appear to be - none of the parameters measured at rest or during activity was significantly altered in females, but several parameters in males. Therefore, sex-specific differences in mutant animals must be valued with precaution and cannot be related to those observed in control animals. Despite showing a significantly lower body weight, male mutant mice used larger tidal volumes and a little elevated respiratory rates during rest and activity so that ventilation as well as specific tidal volumes and minute ventilations were significantly increased. The difference in flow rates – PEF and MEF during rest and MEF during activity - correlate to the differences in tidal volume.

3.9.6 Discussion

While control animals exhibited typical breathing patterns in terms of sex differences and changes from rest to activity similar differences were not de-

tected in *mPtpg*-mutant mice. This is due to the fact that the mutation appears to act sex-specific, i.e. only males were affected. The significantly elevated ventilation detected during rest and activity is primarily related to the higher tidal volume used by mutant males. Effects of body weight can be excluded because specific values show similar differences. That lung function is not at its limit can be inferred from the fact that ventilation can still be substantially increased during activity. A higher oxygen demand but also alterations in the gas exchanging function of the lung can be associated with these findings. Lung function studies and morphological analysis of the lung is requested to further illuminate affects of the *mPtpg* mutation and assess possible underlying mechanisms.

3.9.7 References

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Abbreviations

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

Table 22: Characterization of studied mice
Data are presented as mean ± standard error of mean.

Parameter	**Control (A)**			**Mutant (B)**			**A~B**	**A~B**
	Male	**Female**		**Male**	**Female**		**Male**	**Female**
	(n=4)	(n=5)	***p - value***	(n=5)	(n=5)	***p - value***	***p - value***	***p - value***
Bw [g]	29.4 ± 0.9	22.7 ± 0.4	< 0.001	26.1 ± 1.0	22.5 ± 0.9	< 0.05	< 0.05	n.s.
Mean_f [1/min]	287.0 ± 11.2	339.2 ± 9.1	< 0.01	330.1 ± 7.9	294.9 ± 15.3	n.s.	< 0.02	< 0.05

Table 23: Spontaneous breathing pattern of *mPtpg*-mice during rest and activity
Data are presented as mean ± standard error of mean.

Parameter	Control (A)			Mutant (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=4)	(n=5)	*p - value*	(n=5)	(n=5)	*p - value*	*p - value*	*p - value*
Rest								
f [1/min]	265.7 ± 12.9	329.4 ± 8.5	< 0.01	313.3 ± 22.3	300.5 ± 10.5	n.s.	n.s	n.s
TV [ml]	0.27 ± 0.01	0.25 ± 0.02	n.s.	0.30 ± 0.01	0.24 ± 0.01	< 0.001	n.s.	n.s.
sTV [µl/g]	9.3 ± 0.3	11.2 ± 0.7	n.s.	11.6 ± 0.3	10.7 ± 0.2	n.s.	< 0.01	n.s.
MV [ml/min]	70.5 ± 4.0	81.3 ± 4.7	n.s.	91.1 ± 4.0	71.1 ± 3.2	< 0.01	< 0.01	n.s
sMV [ml/min/g]	2.4 ± 0.1	3.6 ± 0.2	< 0.01	3.5 ± 0.3	3.2 ± 0.1	n.s.	< 0.01	n.s.
Ti [ms]	75.6 ± 4.3	56.2 ± 1.7	< 0.01	69.3 ± 5.1	63.9 ± 4.2	n.s.	n.s.	n.s.
Te [ms]	151.8 ± 8.0	126.4 ± 4.4	< 0.05	126.3 ± 9.3	136.7± 4.0	n.s.	n.s.	n.s
Ti/TT	0.33 ± 0.01	0.31 ± 0.01	n.s.	0.35 ± 0.01	0.32 ± 0.01	< 0.02	n.s.	n.s.
PIF [ml/s]	6.2 ± 0.3	7.7 ± 0.5	n.s.	7.4 ± 0.4	6.5 ± 0.4	n.s.	n.s.	n.s.
PEF [ml/s]	3.5 ± 0.3	3.9 ± 0.3	n.s.	4.5 ± 0.2	3.2 ± 0.3	< 0.02	< 0.02	n.s.
MIF [ml/s]	3.6 ± 0.2	4.5 ± 0.3	n.s.	4.4 ± 0.2	3.8 ± 0.2	n.s.	n.s.	n.s.
MEF [ml/s]	1.8 ± 0.1	2.0 ± 0.1	n.s.	2.4 ± 0.1	1.8 ± 0.1	< 0.01	< 0.02	n.s.
Activity								
f [1/min]	412.5 ± 6.8	445.9 ± 8.4	n.s.	433.5 ± 18.1	427.9 ± 6.9	n.s.	n.s.	n.s.
TV [ml]	0.25 ± 0.01	0.25 ± 0.01	n.s.	0.29 ± 0.01	0.24 ± 0.01	< 0.01	< 0.02	n.s.
sTV [µl/g]	8.5 ± 0.2	10.9 ± 0.6	< 0.01	11.2 ± 0.4	10.5 ± 0.3	n.s.	< 0.01	n.s.
MV [ml/min]	101.0 ± 2.6	109.3 ± 6.0	n.s.	123.5 ± 5.5	101.1 ± 5.7	< 0.05	< 0.02	n.s
sMV [ml/min/g]	3.4 ± 0.1	4.8 ± 0.3	< 0.01	4.8 ± 0.3	4.5 ± 0.2	n.s.	< 0.01	n.s.
Ti [ms]	51.0 ± 0.4	45.5 ± 1.1	< 0.01	50.7 ± 2.0	47.9 ± 1.7	n.s.	n.s.	n.s.
Te [ms]	94.6 ± 2.5	89.2 ± 2.1	n.s.	88.7 ± 3.9	92.5 ± 0.9	n.s.	n.s.	n.s
Ti/TT	0.35 ± 0.01	0.34 ± 0.01	n.s.	0.36 ± 0.01	0.34 ± 0.01	< 0.02	n.s.	n.s.
PIF [ml/s]	8.3 ± 0.2	9.2 ± 0.6	n.s.	9.7 ± 0.4	8.3 ± 0.4	n.s.	n.s.	n.s.
PEF [ml/s]	5.1 ± 0.3	5.3 ± 0.4	n.s.	6.3 ± 0.4	4.7 ± 0.4	< 0.05	n.s.	n.s.
MIF [ml/s]	4.9 ± 0.1	5.4 ± 0.4	n.s.	5.8 ± 0.2	4.9 ± 0.2	n.s.	n.s.	n.s.
MEF [ml/s]	2.6 ± 0.1	2.8 ± 0.1	n.s.	3.3 ± 0.1	2.6 ± 0.1	< 0.01	< 0.01	n.s.

3.10 Expression Profiling

3.10.1 Summary

In this report, we describe the results of the RNA expression profiling of **brain** of male animals of the mPtpg mutant mouse line. In total 10 chip hybridizations were performed. The data analysis and various statistical methods could not detect differential gene expression in wild-type control and mutant tissues.

3.10.2 Mice

The molecular phenotyping screen archives organs of mutant and wild-type mice for subsequent DNA-chip expression profiling analysis. Nine male mice (five mutants and four controls) of the mPtpg mutant mouse line were provided to the molecular phenotyping screen (Table 24).

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 organs were collected and archived in liquid nitrogen following our established SOPs (Standard operation protocols): bulbourethral gland, spleen, kidney, seminal vesicles, testis, liver, heart, lung, thymus, skin/cartilage (outer ear), skeletal muscle, salivary gland and brain. Organs were immediately frozen and stored in liquid nitrogen until isolation of total RNA.

Table 24: Organs of *mPtpg*-mutant and control mice stored for expression profiling.

Mouse ID	Strain	Sex	Genotype	Date of Collection
30025477	mPtpg	m	+/+	11.01.2005
30025476	mPtpg	m	-/-	11.01.2005
30025429	mPtpg	m	+/+	11.01.2005
30025433	mPtpg	m	+/+	11.01.2005
30025434	mPtpg	m	+/+	11.01.2005
30025495	mPtpg	m	-/-	11.01.2005
30025494	mPtpg	m	-/-	11.01.2005
30025492	mPtpg	m	-/-	11.01.2005
30025479	mPtpg	m	-/-	11.01.2005

3.10.3 Material and Methods

Isolation of total RNA

Total RNA was isolated just before processing for expression profiling. For preparation of total RNA individual organs were thawed in buffer containing chaotropic salt (RLT buffer, Qiagen) and homogenized using a Polytron homogenizer. Total RNA from individual samples was obtained according to manufacturer's protocols using RNeasy Midi kits (Qiagen). 2 µg RNA aliquots were run on a formaldehyde agarose gel to check for RNA integrity and the

concentration was calculated from $OD_{260/280}$ measurement. The RNA was stored at –80°C in RNase free water (Qiagen).

Chip design

We use a glass-surface DNA-chip containing ≈ 21,000 probes. About 20,200 of these probes are from the commercial Lion mouse array-TAG clone set, which is mostly derived from 3'UTRs. All Lion probes have been sequenced. The remaining probes are genes associated with immune response. Mouse array-TAG clones have the general ID MG-VW-XYZ (e.g. MG-3-1a5, MG-12-190m5,…) and the other probes are named s0-geneID (e.g. s0-birk, s0-mark1…).

DNA Microarrays

PCR products with 5'-aminogroup were amplified from the mouse arrayTAG library from Lion Bioscience comprising approximately 20,200 clones (Heidelberg, Germany). PCR products were dissolved in 3x SSC buffer and spotted on aldehyde-coated slides (Telechem, USA) using a Microgrid TAS II spotter (Biorobotics) with 48 Stealth™ SMP3 pins (Telechem). Spotted slides were rehydrated overnight in a humid chamber containing 50-70% aqueous solution of glycerol. Rehydrated slides were immersed in blocking solution (0.1 M sodium borohydride in 0.75x PBS with 25% ethanol) for 5 minutes, boiled in water for 2 minutes, briefly immersed in 100% ethanol and air-dried. Slides were pre-hybridized for 1 hour in pre-hybridization buffer (6x SSC, 1% BSA, 0.5% SDS) rinsed in water, dried and hybridized the same day (Seltmann *et al*., 2005).

Reverse Transcription and Fluorescent Labelling

For labeling 20 µg of total RNA were used for reverse transcription and indirectly labeled with Cy3 or Cy5 fluorescent dye according the TIGR protocol (Hedge et al 2000). Labeled cDNA was dissolved in 30 µl hybridization buffer (6x SSC, 0.5% SDS, 5x Denhardt's solution and 50% formamide) and mixed with 30 µl of reference cDNA solution (pool from five control animals) labeled with the second dye. This hybridization mixture was placed on a pre-hybridized microarray, under a cover slip, placed into a hybridization chamber (Genetix) and immersed in a thermostatic bath at 42°C for at least 16 hours. After hybridization slides were washed in 40 ml of 3x SSC, 40 ml of 1x SSC and 40 ml of 0.25x SSC at room temperature. For drying slides were placed in an empty 50 ml Falcon tube (Becton Dickinson, USA) and centrifuged at 4000 m/s^2. Dried slides were scanned with a GenePix 4000A microarray scanner and the images were analyzed using the GenePix Pro3.0 image processing software (Axon Instruments, USA). All data were normalized by adjusting the median of log-ratios of Cy5 to Cy3 intensities to 0. For data analysis Pattern Analysis of Microarrays (PAM; http://www.gsf.de/ieg/groups/exppro_cpt.html#PAM) was used.

Chip Hybridization

In general two chip hybridizations were performed with RNA from all organs of each five individual mutant mice (in total 10 hybridizations) against the identical pool of the same organ of control RNAs (reference RNA pool; wt). For each individual mouse the chip experiments included a color-flip experiment.

If differential gene expression will be detected between mutant and control mice, additionally control/control poll experiments will be done to confirm the differences between mutant and wild-type control littermates.

3.10.4 Results

Selected Organs and Isolated RNA

Brain was selected as organ for expression profiling analysis. We isolated total RNA of this organ of five *mPtpg*-mutant mice and four control individuals (Table 25).

Table 25: Amount of total RNA [µg] isolated from brain.

Mouse ID	Brain
30025477	216
30025476	367
30025429	266
30025433	309
30025434	351
30025495	214
30025494	264
30025492	316
30025479	263

Analysis of Gene Expression in Brain Tissue

Table 26 summarizes the results of 10 chip hybridizations performed with RNA from brain of *mPtpg*-mutant mice. In total, 3673 probes showed signals in all 10 chip hybridizations.

Table 26: Chip hybridization of brain: labeling and number of detected spots Numbers indicate the ID of mutant mice.		
Chip ID	**Cy5/Cy3**	**Detected Spots**
#1	476 / ref	10259
#2	ref / 476	9649
#3	495 / ref	10207
#4	ref / 495	11838
#5	494 / ref	13158
#6	ref / 494	8579
#7	492 / ref	9016
#8	ref / 492	7776
#9	479 / ref	9975
#10	ref / 479	6505
		3673 overlap

Genes were evaluated for the significance of differential gene expression. Genes were ranked according the lowest absolute ratio of signal intensities (*mPtpg*-mutant versus reference) in 10 microarray experiments (Table 27). This ranking is independent of the reproducibility in terms of up- and down-regulation. The number of genes with non-reproducible up- or down-regulation („non-uniform patterns“) is given for different selections of genes in the ranking („ranked genes“). The number of non-differentially expressed genes („NDE, false positives“) among genes with reproducible patterns was calculated for significance level $p<0.05$.

For example, the selection of the top 20 ranked genes with either up- or down-regulation contains 19 genes with non-reproducible chip data. The remaining gene with reproducible up- or down-regulation is non-differentially expressed with a significance level $p<0.05$. The minimal ratios of expression for this selection ranged from 4.58 to 1.14 fold induction/repression.

Table 27: Chip hybridization of brain RNA: evaluation of data			
Ranked Genes (According Lowest of 10 Ratios)	**Non-uniform Patterns**	**NDE (False Positives) p<0.05**	**Fold Induction (Minimum of 10 Chips)**
1 - 20	19	≥ 1	4.58 – 1.14
1 - 40	37	≥ 1	4.58 – 1.12

According to 95% non-reproducible chip data of the top 40 ranked genes, no gene with differential expression in brain of *mPtpg*-mutant mice was observed in all experiments.

3.10.5 Discussion

Using the selection criteria described above, we could not identify any genes that are differentially expressed in brain of the *mPtpg*-mutant mice. Please, contact us if you have questions concerning the analysis.

3.10.6 References

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Seltmann M., M. Horsch, A. Drobyshev, Y. Chen, M. Hrabé de Angelis and J. Beckers (2005): Assessment of a systematic expression profiling approach in ENU-induced mouse mutant lines. Mammalian Genome 16: 1-10

3.11 Metabolic Screen

3.11.1 Summary

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

In the primary metabolic screen 12 control mice (five males and seven females) and 13 mutants (seven males and six females) were available. They were first fed under *ad libitum* conditions for two weeks, followed by a period of food restriction to 60°% of *ad libitum* for seven days to analyze adaptive responses of metabolism. The primary metabolic screen focuses on investigation of metabolic demands of mice determining daily body weight, energy uptake, metabolizable energy and body temperature and adaptive capacity of metabolic processes. Comparing the sexes within both genotypes indicated males heavier than females with significantly higher food and energy uptake. Genotype specific differences could not be found – hence, no metabolic phenotype was monitored.

3.11.2 Mice

Five adult control males and seven adult mutant males entered the Metabolic Screen at the beginning of calendar week 03 in 2005. The females (seven control and six mutants) 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 seven days of chronic fasting to analyze adaptive responses of metabolism.

3.11.3 Material and Methods

Recorded Data

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

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

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

3.11.4 Parameters

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

3.11.5 Results and Discussion

No information about metabolic properties were available prior the metabolic screening of mPtpg mutant mouse line. Comparing the sexes within both genotypes revealed differences in food and energy uptake, and metabolized energy, respectively. Males were heavier and consumed more food and energy. A genotype specific comparison revealed no statistical differences in any of investigated parameters. The results of the primary metabolic screen do not indicate to a metabolic phenotype of *mPtpg*-mutant mice.

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

3.11.6 References

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Abbreviations

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

Table 28: Metabolic parameters recorded in the primary screen
Data are presented as mean ± standard error of mean.

Parameter	Control (A)					Mutant (B)					A~B	
	ad libitum			food reduction, 7 days to 60%		*ad libitum*			food reduction, 7 days to 60%			
	Male	Female		Male	Female	Male	Female		Male	Female	Male	Female
	(n=5)	(n=7)	*p - value*	(n=5)	(n=7)	(n=7)	(n=6)	*p - value*	(n=7)	(n=6)	*p - value*	*p - value*
Body weight [g]	27.2 ± 0.61	21.3 ± 0.3	< 0.001	23.8 ± 0.6	16.9 ± 0.43	29.2 ± 0.91	20.7 ± 0.36	< 0.001	24.5 ± 0.8	16.4 ± 0.55	n.s.	n.s.
Rectal body temperature [°C]	37.1 ± 0.15	36.8 ± 0.13	n.s.	34.9 ± 0.66	34.7 ± 0.3	37.1 ± 0.12	37.0 ± 0.17	n.s.	34.9 ± 0.21	33.2 ± 0.86	n.s.	n.s.
Food consumption [g day^{-1}]	4.35 ± 0.25	2.5 ± 0.25	< 0.001	60% of *ad libitum*		4.14 ± 0.24	2.3 ± 0.22	< 0.001	60% of *ad libitum*		n.s.	n.s.
Energy uptake [kJ day^{-1}]	80.2 ± 4.64	49.5 ± 5.25	< 0.001	48.1 ± 2.78	29.6 ± 3.64	76.4 ± 4.33	42.4 ± 4.08	< 0.001	45.8 ± 2.6	25.4 ± 2.45	n.s.	n.s.
Energy uptake BW^{-1} [kJ g^{-1} day^{-1}]	3.35 ± 0.11	2.15 ± 0.19	0.001	1.76 ± 0.07	1.64 ± 0.17	3.11 ± 0.16	2.04 ± 0.18	0.001	1.57 ± 0.09	1.54 ± 0.11	n.s.	n.s.
Feces production [g day^{-1}]	0.73 ± 0.05	0.48 ± 0.05	< 0.01	0.56 ± 0.03	0.25 ± 0.03	0.74 ± 0.04	0.44 ± 0.06	< 0.001	0.47 ± 0.04	0.28 ± 0.05	n.s.	n.s.
Energy content feces [kJ g^{-1}]	15.5 ± 0.03	15.4 ± 0.07	n.s.	15.0 ± 0.09	15.1 ± 0.09	15.6 ± 0.09	15.5 ± 0.08	n.s.	15.1 ± 0.09	14.9 ± 0.12	n.s.	< 0.02
Metabolized energy [kJ day^{-1}]	67.3 ± 3.84	37.7 ± 3.8	< 0.001	38.8 ± 2.41	22.7 ± 2.75	63.4 ± 3.81	34.7 ± 3.12	< 0.001	37.8 ± 2.19	20.7 ± 1.77	n.s.	n.s.
Metabolized energy [kJ g^{-1} day^{-1}]	2.81 ± 0.09	1.76 ± 0.17	< 0.001	1.42 ± 0.06	1.34 ± 0.16	2.58 ± 0.15	1.67 ± 0.14	< 0.001	1.3 ± 0.08	1.26 ± 0.09	n.s.	n.s.
Food assimilation coefficient [%]	84.0 ± 0.25	81.7 ± 0.84	< 0.05	80.5 ± 0.71	83.7 ± 0.88	82.8 ± 0.64	83.3 ± 0.98	n.s.	82.5 ± 0.97	81.8 ± 1.66	n.s.	n.s.

3.12 Pathology Screen

3.12.1 Summary

The Pathology screen performed a complete morphological analysis with standard stains. Our analysis in the primary screen did not reveal any mPtpg mutant mouse line specific morphological phenotypes.

3.12.2 Mice

A total of 41 mice, 23 mutants (14 females, 9 males), and 18 control animals (14 females, four males) were analyzed. Due to the workflow in the GMC, mice of different ages were received from different screens (Table 29).

Table 29: *mPtpg*-mutant mice and their control littermates analyzed.

Origin	Mutant		Control		Number of Animals	Age [weeks]
	Female	Male	Female	Male		
Lung Screen	5	-	5	-	10	15
Dysmorphology Screen	3	2	3	-	8	19 - 22
Metabolic Screen	6	7	6	4	23	21 - 22
Total Number of Animals	**14**	**9**	**14**	**4**	**41**	

3.12.3 Materials and Methods

Mice received in the laboratory of pathology were sacrificed with CO_2. The animals were analyzed macroscopically and weighed (www.eulep.org/Necropsy_of_the_Mouse/index_2004.php). The thymus and left lobe of the liver were measured. Blood samples were taken, centrifuged and the serum was saved at –20°C. Tails were preserved at -70°C for further genetic analysis. Following a complete dissection, an x-ray of the complete bone structure was taken, when indicated (Hewlett Packard, Cabinet X-Ray System Faxitron Series). All organs were fixed in 4% buffered formalin and embedded in paraffin for histological examination. Two-µm-thick sections from skin, heart, muscle, lung, brain, cerebellum, thymus, spleen, cervical lymph nodes, thyroid, parathyroid, adrenal gland, stomach, intestine, liver, pancreas, kidney, reproductive organs, and urinary bladder were cut and stained with haematoxylin and eosin (H&E). Prussian's Blue staining was performed when indicated.

3.12.4 Results

Overview

Table 30: Genotype-specific morphological alterations of *mPtpg*-mutant mice:

Organs	Alteration	Organs	Alteration
Skin	No	Pancreas	No
Musculoskeletal system	No	Cervical lymph node	No
Eyes	No	Thymus	No
Cerebrum	No	Spleen	No
Cerebellum	No	Thyroid	No
Heart	No	Parathyroid	No
Trachea	No	Adrenal gland	No
Lung	No	Kidneys	No
Teeth	No	Urinary bladder	No
Salivary gland	No	Testes	No
Esophagus	No	Epididymis	No
Stomach	No	Funiculus spermaticus	No
Small intestine	No	Ovaries	No
Large intestine	No	Uterus	No
Liver	No	Vagina	No

No morphologic phenotypes of mutant mice were detected in our primary screen.

Body Weight
Both, female and male mutant mice had a similar average body weight as their control littermates (see Table 31 for more details).

Table 31: Mean body weight ± standard deviation of *mPtpg*-mutant mice and their control littermates.

Origin	Mutant		Control		Age [weeks]
	Female	Male	Female	Male	
Lung Screen	21.6 ± 2.0	-	22.0 ± 0.6	-	15
Dysmorphology Screen	24.3 ± 0.5	29.0 ± 1.0	20.7 ±0.5	-	19 - 22
Metabolic Screen	21.8 ± 1.0	28.1 ± 1.7	21.7 ± 1.4	26.3 ±1.8	21 - 22

Secondary, non-genotype-specific results

Brain

An absence of the corpus callosum was found in mutant (11/23, 47.8%) and control mice (6/18, 33.3%). There seems to be no significant differences between the groups (two-tailed Fisher's Exact Probability Test: p=0.52).

Uterus

In six of 14 females (four mutants, two controls) a bilateral hydrometra was detected. The diameter of the uteri was markedly enlarged (Fig. 7). Uteri were filled with a clear, watery fluid.

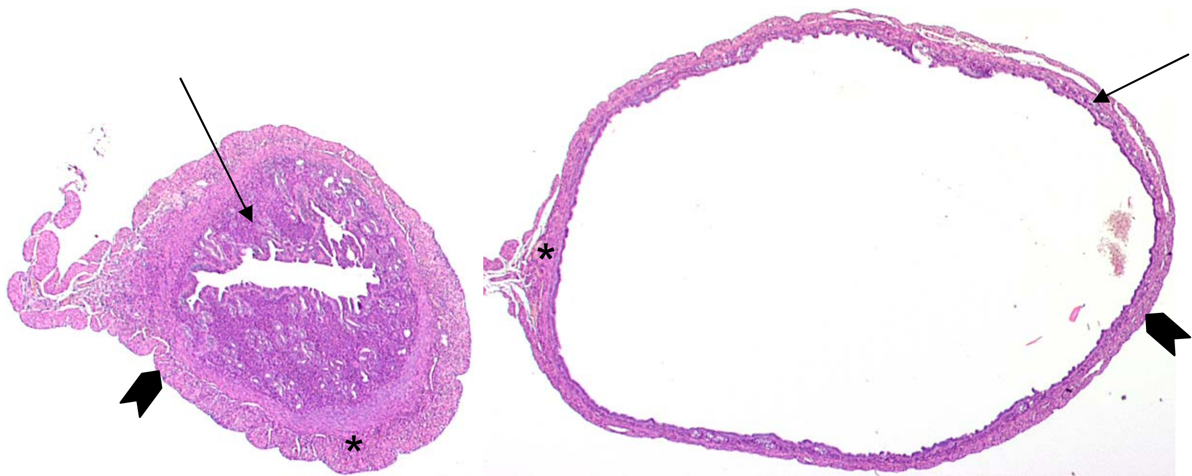

Figure 8: Cross sections through uteri

Left: Cross section through a normal uterus horn (H&E, 25x); endometrium (arrow), myometrium (asterisk), and epimetrium/serosa (arrowhead) are indicated.

Right: Hydrometra, cross section through a uterus horn (H&E, 25x). The diameter is markedly enlarged and all layers of the uterus wall are compressed (arrow: endometrium; asterisk: myometrium; arrowhead: epimetrium).

Testes
One male mutant mouse developed a benign gonadal teratoma, composed of nerve cells, bone, bone marrow, and large cystic structures lined by a monolayer epithelium (Fig. 8).

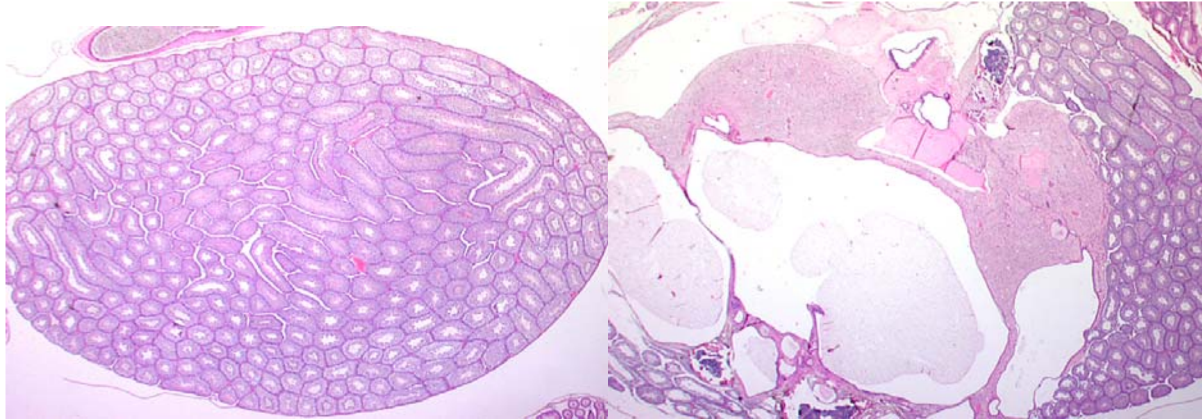

Figure 9: Cross sections through testes
Left: A normal testis is depicted (H&E 12x).
Right: Gonadal teratoma of a male 129 mouse. Normal testicular tissue is still present at the right side and left corner of the picture. The teratoma, a large, pleomorphic, cystic mass, is located in the center of the testis (H&E, 12x).

3.12.5 Discussion of secondary results

In mice the **absence of the corpus callosum** is well described for mice with a 129 heritage. About 60% of 129 substrains do not develop a corpus callosum. The Behavior Screen of the GMC was not able to detect any differences between mice with and without corpus callosum in the primary screen; neither in this mouse line nor in several other tested mouse lines (unpublished observation). Accordingly, absence of the corpus callosum was found in mutant (43.5%) and control mice (33.3%). The corpus callosum serves as the main nerve fibre tract between both hemispheres of the brain. In humans the corpus callosum is thought to be important to coordinate movement and it concerts certain specialties of the right and left hemispheres (language patterns, memory, complex learning).

Female mice of this batch showed a tendency to develop **hydrometras** (42.9%). The causing agent remains unknown. In general "hydrometra" is not a common disease for females of the 129 strain. Nevertheless it must be considered to be a non-specific alteration in this batch, because there were not only mutants (4/14) but also controls (2/14) affected (two tailed Fisher's Exact Probability Test: p=0.65).

In 1% of male 129 mice a **testicular teratoma** can be found on routine pathological examinations. In particular substrains the susceptibility is even higher (up to 30% in strain 129/T1 and 129/T2). Except for the 129 strain, spontaneous teratomas are an extremely rare finding in mice (see http://www.informatics.jax.org).

3.12.6 Reference

http://www.informatics.jax.org/external/festing/mouse/docs/129.shtml

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Appendix:
Tables

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