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Inbred wild type mouse strains have distinct spontaneous morphological phenotypes

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Summary. The mouse is the most commonly used animal for modelling human disease. New approaches for generating genetically manipulated mouse models to represent human disease, as well as target the function of specific genes, has increased the importance of mice in biomedical science. For the correct interpretation of alterations in mouse phenotype the basic morphology of background mouse strains must be known. Despite on-going efforts to create publicly available baseline phenotypic data, the information concerning spontaneous lesions in wild-type mice is incomplete and scattered so far, and further studies are needed. We addressed this problem by screening haematoxylin-eosin stained sections of brain, reproductive organs, urinary bladder, kidney, thyroid, parathyroid, heart, lung, spleen, thymus, lymph nodes, adrenal glands, stomach, intestine, liver, skin and pancreas of six commonly used inbred mouse strains (C57BL6/J, C57BL6/NTac, C3HeB/FeJ, BALB/cByJ, 129P2/OlaHsd and FVB/N) for inherent spontaneous morphological lesions. Interesting spontaneous phenotypes were seen in morphology of the liver, pancreas, adrenal glands, lungs, intestines and heart. In conclusion, care should be taken when choosing the background mouse strain for genetic manipulations, since different mouse strains harbour different inherent lesions that can affect the function of targeted genes, interpretation of results and translation of results to model human disease.

Key words: Mouse model, Inbred strain, Morphology, Phenotype, Pathology

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Introduction

The generation of genetically engineered mice has become an indispensable tool for the identification of the function of specific genes, as well as for understanding how genes affect the development of human disease. Inbred mouse strains are being used as raw material for the generation of genetically engineered mice. Therefore, knowing the inherent morphological phenotypes of the background mouse strains is of utmost importance when designing experiments, as well as for the correct interpretation of results gained from studies of genetically engineered mice (Hancock et al., 2009; Blake et al., 2011; Maddatu et al., 2012).

The mouse is the most commonly used animal for modelling human disease. Over 450 distinct inbred mouse strains have been described so far, providing a multitude of different genotypes and phenotypes to be utilized. The C57BL6 strain is perhaps the most frequently used, and its phenotype is characterized by secure expression of most mutations and a low tumour incidence (for information about inbred strain characteristics see Blake et al., 2011; Maddatu et al., 2012). C57BL6/J is the most widespread substrain for studying genetically engineered mice. The C57BL6/N substrain was separated in the F32 generation of C57BL6/J and has been housed at the National Institute of Health since 1951 (Bailey, 1978; Matsuo et al., 2010). C3HeB/FeJ is a substrain of the C3H strain that was developed by L.C. Strong in the 1920s as a mouse strain with a high incidence of mammary tumours (Medina, 2010). Since this incidence was due to exogenous transfer of the mouse mammary tumour virus (MMTV) through breast milk, it was possible by the use of foster mothers to develop strains free of exogenous MMTV,

C3HeB/FeJ being one of them. C3H mouse is a common research model for the investigation of cancer and is routinely used for ENU (N-ethyl-N-nitrosourea) mutagenesis (Bailey, 1978).

The BALB/cByJ mice belong to a less aggressive and more efficiently reproducing substrain of the bagg albino Strain (BALB/cJ). The well-known use of this strain is in the production of monoclonal antibodies. 129 substrains are known for their use in the production of genetically engineered animals. Their embryonic stem cells are very robust and easy to culture and have been used in the construction of libraries for gene targeting experiments (Ohtsuka et al., 2006). The FVB strain is widely used because of its good reproductive behaviour, with high litter size and large prominent pronuclei in fertilized eggs, which makes it an appealing strain for easy microinjection of DNA (Taketo et al., 1991).

Although the sequencing of the mouse genome has been completed, the function of most genes still remains to be elucidated. This can be done by phenotypic analysis of genetically engineered mice. New methods and approaches for mouse mutagenesis, as well as consortiums to characterize the human genome, all increase the amount of mouse lines to be characterized. Although some phenotypic information is already available, including a recent extensive study of older mice of 30 inbred strains (Sundberg et al., 2011), systematic and comprehensive characterization of morphological phenotypes and distinct features of young animals of inbred mouse strains is still lacking. Big efforts are on-going in order to create publicly available baseline phenotypic data (Bogue and Grubb 2004; Grubb et al., 2004; Bogue et al., 2007), but the information is scattered and incomplete so far. Although a multitude of wildtype mouse strains have been investigated concerning the differences in, for example, their visual parameters (Wong and Brown, 2006), auditory activity (Zhou et al., 2006) or social and learning behaviour (Brown and Wong, 2007; Moy et al., 2007), histological data lack a direct comparison of young animals of different wild type inbred strains. Due to this gap in our basic knowledge, there is a risk that results from mouse studies are misinterpreted and translation of the results to model human diseases is affected.

The German Mouse Clinic was designed to offer standardized and comprehensive phenotype analysis of mutant mouse lines (Gailus-Durner et al., 2005). The aim of the pathology screen of the German Mouse Clinic is to correlate the functional abnormalities of mice with anatomical changes to comprehend the functions of mutated genes. To fulfil this aim, a profound knowledge of the normal histology of different inbred mouse strains is needed. In this study, we screened six different, commonly used inbred mouse strains for the inherent morphological phenotypes in order to find out the basic phenotypes and morphological differences that may affect the choice of mouse strain to be used as a base for genetic manipulations. Our data provides interesting new information about inherent differences and spontaneous lesions in C57BL6/J, C57BL6/NTac, C3HeB/FeJ, BALB/cByJ, 129P2/OlaHsd and FVB/N wild-type mouse strains and offers tools for choosing the most appropriate mouse line, as well as for the correct interpretation of results gained using these mouse lines.

Materials and methods

Animals

A total of 154 animals (77 females, 77 males, age 17-23 weeks) were analyzed (Table 1). All experimental

Table 1. Origin, number and age of animals of different mouse strains analyzed for the study.

Mouse strain	Origin *	Repository	Number of generations kept at Helmholtz	Number of animals (Female/Male)	Age (weeks)
B6J	Little to Fekete to Hall at F22, back to JAX (1948) at F24	Jackson Laboratory (Bar Harbor, Maine)	More than 4 generations	16/16	17
B6N	From NIH in 1951; From Jax at F32; to Jax in 1948 from Hall	Taconic (Germantown, New York)	More than 4 generations	10/10	17
C3Fe	C3H/HeJ ova transferred to C57BL/6 by Fekete at JAX (1948)	Jackson Laboratory (Bar Harbor, Maine)	More than 4 generations	16/16	17-21
CBy	Subline of BALB transferred from Bailey to JAX (1975) at F136	Charles River (Sulzfeld, Germany)	No breeding at Helmholtz. Mice imported for the analysis	10/10	17-18
129P2	129P2 developed by Dunn in 1928 from a cross of coat color stocks and a chinchilla stock from Castle	Harlan Laboratories (Blackthorn, U.K.)	In vitro fertilization and breeding more than 4 generations	13/13	21
FVB/N	Outbred NIH General Purpose Swiss mice established at NIH in 1935. Rowe (NIH) to Amsterdam, 1978	Charles River (Sulzfeld, Germany, 6/6) and Jackson Laboratory (Bar Harbor, Maine 6/6)	No breeding at Helmholtz. Mice imported for the analysis	12/12	18-23
Total				77/77	

* From Handbook on genetically standardized JAX mice. The Staff of the Jackson laboratory. Fourth Edition, Editors Margaret C. Green and Barbara A. Witham.

procedures followed the guidelines of the German Animal Protection Law. We studied the histology of six commonly used inbred mouse strains, namely C57BL/6J (hereafter referred to as B6J, n=16 females, n=16 males, age 17 weeks), C57BL/6NTac (B6N, n=10 females, n=10 males, age 17 weeks), C3HeB/FeJ (C3Fe, n=16 females, n=16 males, age 17-21 weeks), 129P2/OlaHsd (129P2, n=10 females, n=10 males, age 17-18 weeks), BALB/cByJ (CBy, n=13 females, n=13 males, age 21 weeks) and FVB/N (n=12 females, n=12 males, age 18-23 weeks). All animals were healthy and did not suffer from any visible infections at the time of sacrifice. Upon entry into our animal facility, animals were housed in groups of 4-5 animals per cage in type II polycarbonate cages in individually ventilated caging (IVC) systems to guarantee specific pathogen-free (SPF) conditions (Fuchs et al., 2011) and kept in a 12-hr light/12-hr dark cycle. Food (standard chow) and water were available *ad libitum*.

Histology

Formalin-fixed, paraffin-embedded tissues (brain, reproductive organs, urinary bladder, kidney, thyroid, parathyroid, heart, lung, spleen, thymus, lymph nodes, adrenal glands, stomach, intestine, liver, skin and pancreas) were stained with haematoxylin and eosin (H&E). Morphological analysis was performed in H&E stained sections using Zeiss Axioplan 2 imaging microscope and pictures taken using the Olympus virtual microscope system Dotslide 2.0.

Statistical analysis

The prevalence of morphological phenotypes was assessed and compared between different mouse strains and between genders in each mouse strain. A mouse was considered affected with the phenotype if one or more lesions were found. No distinction was made evaluating the number of lesions in an individual affected animal. Fisher's exact test was used to evaluate statistical significance of observed group differences in percentage of morphological findings. Since the total number of interesting comparisons was high, inflation of type I error (increase of number of false significant tests with increasing number of comparisons) had to be addressed. Therefore, to correct p-values for the multiple test issue, the Bonferroni-Holm correction method was applied to all p-values relying on the total number of comparisons being conducted. Accordingly, for confirmatory purpose, only the differences yielding to adjusted p-values (two-sided) less than 0.05 were considered statistically

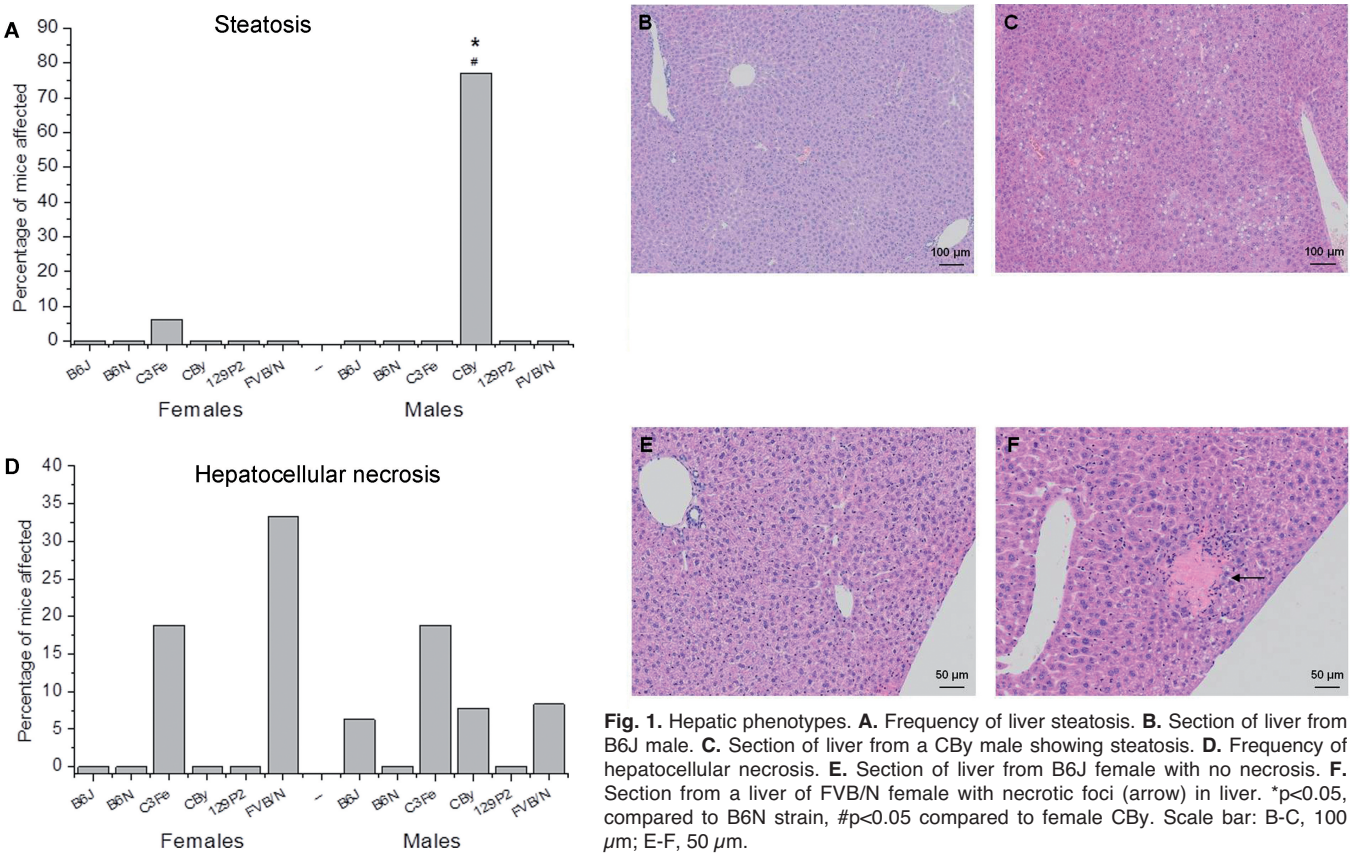


Fig. 1. Hepatic phenotypes. **A.** Frequency of liver steatosis. **B.** Section of liver from B6J male. **C.** Section of liver from a CBy male showing steatosis. **D.** Frequency of hepatocellular necrosis. **E.** Section of liver from B6J female with no necrosis. **F.** Section from a liver of FVB/N female with necrotic foci (arrow) in liver. *p<0.05, compared to B6N strain, #p<0.05 compared to female CBy. Scale bar: B-C, 100 μm; E-F, 50 μm.

significant. Since this adjustment procedure yields rather conservative results, unadjusted p-values were also reported to provide a non-confirmatory effect size, which relies on both strength of the difference and sample size.

Results

Histological differences between mouse strains

The basic histology of the inbred mouse strains is mainly similar. However, some interesting differences between mouse strains were found, the most striking involving the liver, pancreas, adrenal glands, lungs, intestines and heart.

Hepatocellular steatosis and necrosis

In CBy male mice an increase in the occurrence of liver steatosis was observed in 10/13 animals (77%, $p<0.05$ compared to male animals of B6N strain, $p<0.05$ compared to female CBy animals, Fig. 1A-C). Also, a high incidence of multifocal hepatocellular necrosis was observed in female FVB/N mice (4/12 animals, 33%) compared to female animals of other strains (0-19%, Fig. 1D-F). Also C3Fe animals showed an increased number of necrotic foci in the liver (3/16 animals in both genders, 19%) when compared to other mouse strains, which had very few cases of hepatic necrosis.

Pancreatic lipomatosis

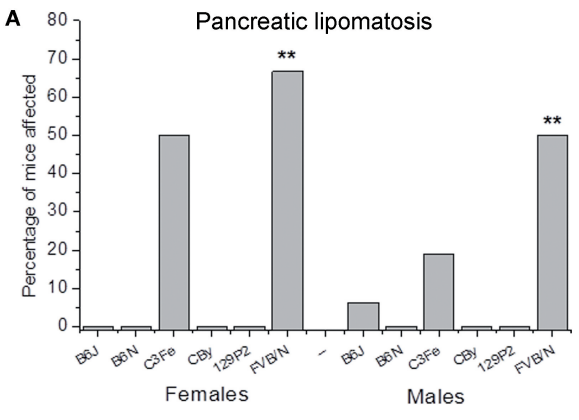
Another inbred lesion was found in the pancreas of FVB/N mice, which displayed an increased amount of interstitial adipose tissue (lipomatosis). In females the occurrence of lipomatosis was 8/12 (66%) and in males 6/12 (50%) (Fig. 2A-C). This increase was also statistically significant when compared to B6N, C3Fe and 129P2 strains ($p<0.01$). Also, C3Fe animals had a non-significant increase in the incidence of pancreatic lipomatosis (8/16 females, 50% and 3/16 males, 19%). An increased deposition of adipose tissue was also found in lower frequency in other organs, such as the salivary glands of C3Fe mice and the heart of FVB/N mice (for frequencies see Tables 4 and 7).

Adrenal subcapsular cell hyperplasia

Subcapsular cell hyperplasia of the adrenal cortex was a common finding in inbred mice in this study. Of

Table 2. Spontaneous morphological lesions in C57BL6/J mice.

B6J	Lesion	Incidence		*p* value	
		Females	Males	Between genders	Between strains
	Adrenal subcapsular cell hyperplasia	13/16	1/16	<0.05	NS
	Peyer's patches	4/16	3/16	NS	NS
	Ectopic thymus	1/16	1/16	NS	NS
	Hydronephrosis	1/16		NS	NS
	Tubular atrophy in kidney	1/16		NS	NS
	Bronchiolar-alveolar adenoma	1/16		NS	NS
	Kidney tubular cell microadenoma		1/16	NS	NS
	Hyperplasia in alveolar epithelium		1/16	NS	NS
	Hepatocellular necrosis		1/16	NS	NS
	Pancreatic lipomatosis		1/16	NS	NS



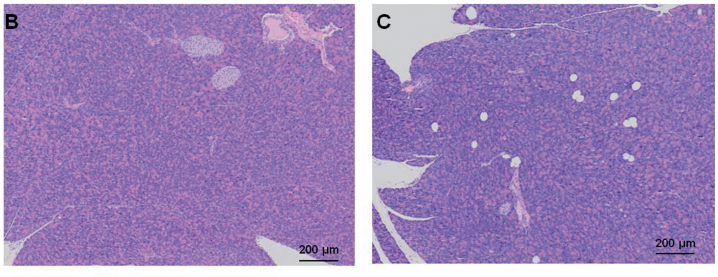


Fig. 2. Pancreatic lipomatosis. **A.** Lipomatosis was mainly found in the pancreas of female C3Fe and FVB/N mice and male FVB/N mice. **B.** Section of a normal pancreas of B6J female mouse. **C.** Section of lipomatotic pancreas of FVB/N female mouse. **$p<0.01$ compared to non-affected strains. Scale bar: 200 μm.

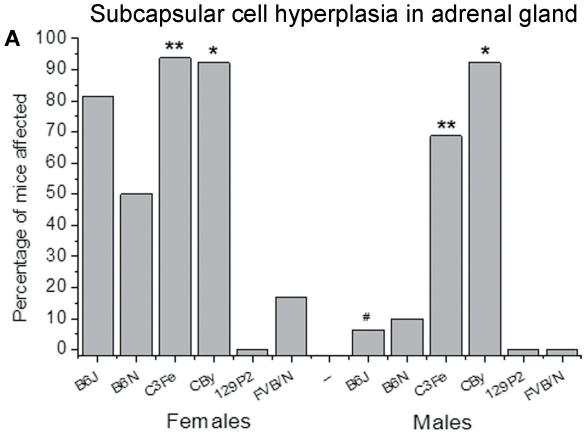
the six mouse strains studied, only 129P2 did not display subcapsular cell hyperplasia. The strains most affected by this phenotype were C3Fe and CBy, both showing a high number of affected animals in both genders (Fig. 3A-C). In detail, 15/16 (94%) C3Fe females and 11/16 (69%) males and 12/13 (92%) CBy of both sexes displayed this phenotype (Fig. 3A-C), with highly significant differences in frequency compared to non-affected strains. Both C57BL6 lines had more subcapsular cell hyperplasia in female animals (13/16 B6J, 81%, and 5/10 B6N, 50%), whereas in C3Fe and CBy strains this phenomenon was common in both genders. There was also a difference in the occurrence

Table 3. Spontaneous morphological lesions in C57BL6/NTac mice.

B6N Lesion	Incidence		*p* value
	Females	Males	
Adrenal subcapsular cell hyperplasia	5/10	1/10	NS
Peyer's patches	4/10	3/10	NS
Ectopic thymus	1/10	1/10	NS
Bronchiolar associated alveolar tissue	1/10	2/10	NS
Accessory adrenal cortical nodule	3/10	1/10	NS
Hydronephrosis	1/10		NS
Pyelonephritis		2/10	NS

Table 4. Spontaneous morphological lesions in C3HeB/FeJ mice.

C3Fe Lesion	Incidence		*p* value
	Females	Males	Between strains
Adrenal Subcapsular cell hyperplasia	15/16	11/16	<0.01
Peyer's patches	12/16	9/16	NS
Bronchiolar associated alveolar tissue	6/16	4/16	NS
Hepatocellular necrosis	3/16	3/16	NS
Pancreatic lipomatosis	8/16	3/16	NS
Lipomatosis in parotid salivary gland	2/16	2/16	NS
Focal infarction in lung		3/16	NS
Follicular cyst in ovary	2/16		NS
Pyelonephritis	2/16		NS
Steatosis in liver	1/16		NS
Accessory adrenal cortical nodule		1/16	NS



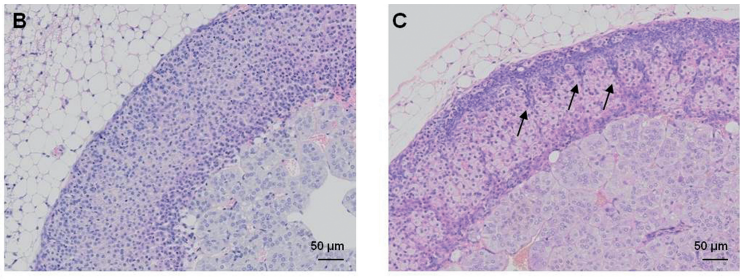
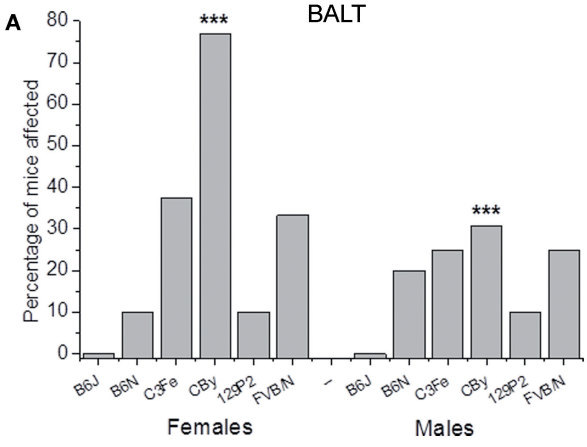


Fig. 3. Subcapsular cell hyperplasia of adrenal gland cortex. **A.** Numerous animals were affected in the B6J, C3Fe and CBy strains. **B.** Section of the adrenal gland from 129P2 female mouse. **C.** Section from C3Fe female mouse with well developed subcapsular cell hyperplasia (arrows). *p<0.05, **p<0.01 compared to 129P2 and FVB/N strains, #p<0.05 compared to female B6J. Scale bar: 50 μm.



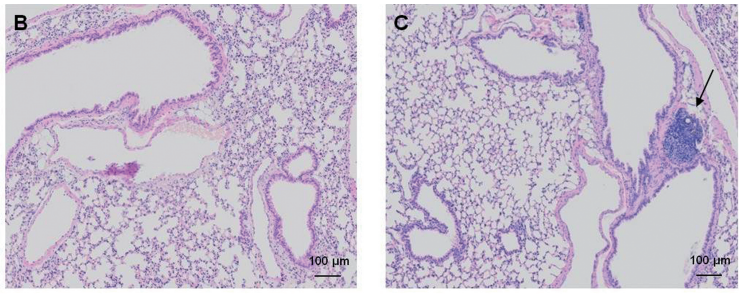


Fig. 4. Bronchiolar associated lymphatic tissue (BALT). **A.** The strain with more prominent BALT was CBy (females). **B.** Section of the lung from a B6J female. **C.** Section of the lung from a CBy female with prominent lymphocytic infiltration in the peri-bronchiolar area (arrow). ***p<0.001 compared to B6N strain. Scale bar: 100 μm.

between B6J and B6N female animals (13/16 B6J females vs. 5/10 B6N females), although this difference was not statistically significant. The difference in a strain between genders was statistically significant only in B6J (81% vs. 6%, p<0.05).

Bronchiolar associated lymphatic tissue

Bronchiolar associated lymphatic tissue (BALT) was seen in low frequency in most of the mouse strains studied. The most strongly affected group of animals were CBy females (10/13, 77%, p<0.001 compared to B6N mouse strain, Fig. 4A-C).

Peyer's patches

The frequency of mucosa-associated lymphatic tissue in the intestines was high, especially in female animals of the two mouse strains, C3Fe (12/16 animals, 75%) and CBy (10/13 animals, 77%, Fig. 5A-C).

Epicardial calcifications

Epicardial calcifications were seen only in hearts of CBy mice (Fig. 6A-C). Both female and male animals were affected (3/13 animals of both genders, 23%), and in both genders the difference in the occurrence of epicardial calcifications compared to other mouse strains was not significant. The calcifications were located mainly on the outer surface of right ventricle.

Other histological findings

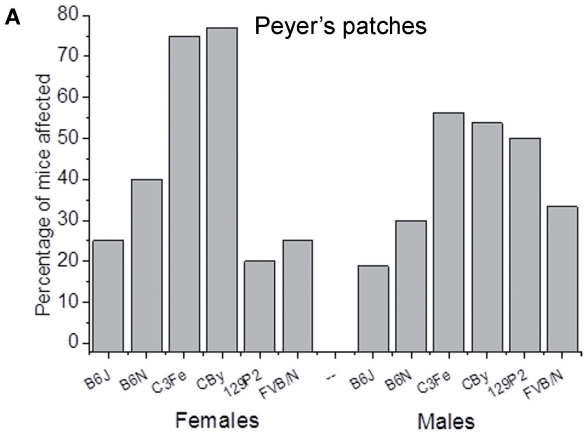
Several other probably coincidental findings were also seen. These findings and the number of mice affected are described in detail in Tables 2-7. A heat-map showing all statistically significant non-adjusted and adjusted p-values is presented in Figure 7.

Discussion

Of the six mouse strains analyzed for this study, both C57BL6 strains, C3HeB/FeJ and BALB/cByJ mice had a high incidence of spontaneous lesions. These four strains were all affected by subcapsular cell hyperplasia of the adrenal gland. Interestingly, C57BL6 strains had this phenomenon predominantly in female animals, whereas in C3HeB/FeJ and BALB/cByJ strains male

Table 5. Spontaneous morphological lesions in BALB/cByJ mice.

CBy	Lesion	Incidence		*p* value	
		Females	Males	Between genders	Between strains
	Adrenal subcapsular cell hyperplasia	12/13	12/13	NS	<0.05
	Peyer's patches	10/13	7/13	NS	NS
	Epicardial calcifications	3/13	3/13	NS	NS
	Bronchiolar associated alveolar tissue	10/13	4/13	NS	<0.001
	Steatosis in liver		10/13	<0.05	<0.05
	Accessory adrenal cortical nodule	1/13		NS	NS
	Atrophic ovary	1/13		NS	NS
	Hepatocellular necrosis		1/13	NS	NS
	Hydrocephalus		1/13	NS	NS
	Thyroid cyst		1/13	NS	NS
	Infarction in kidney		1/13	NS	NS
	Ulceration in stomach		1/13	NS	NS



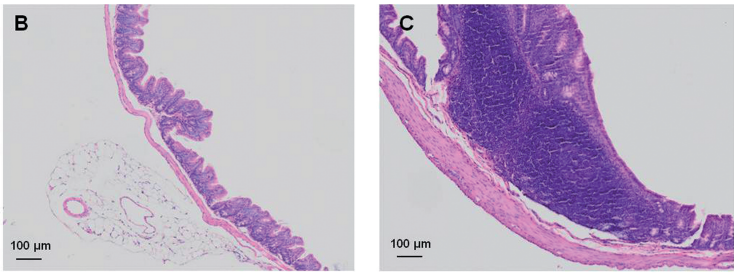


Fig. 5. Peyer's patches in intestines. **A.** C3Fe and CBy animals had high frequency of Peyer's pathes. **B.** Segment of large intestine from a 129P2 female mouse. **C.** Segment of large intestine from a CBy female mouse. Scale bar: 100 μm.

animals were also affected. Prominent Peyer's patches were a common finding in all strains, most severely affecting C3HeB/FeJ and BALB/cByJ female mice. A strikingly specific finding was the epicardial calcifications seen in BALB/cByJ animals. No animals of other mouse strains were affected by this phenotype. Another isolated finding was the presence of hepatocellular necrosis in FVB/N female animals. The mouse strain least affected by spontaneous lesions was the 129P2/OlaHsd strain, which did not display any peculiar phenotype.

Inherent morphological phenotypes can affect the function of targeted genes and proteins of transgenic studies, as well as the interpretation of phenotypes obtained by genetic modelling and/or environmental challenging of laboratory mice. In humans, non-alcoholic fat liver disease (NAFLD) is characterized by fat deposition and necroinflammatory foci in the liver, and it is linked to visceral adiposity, insulin resistance, dyslipidemia and diabetes and may eventually progress to end-stage liver disease. Inasmuch as the incidence of NAFLD is increasing and affects up to 70% of obese persons in Western countries (see Peng et al., 2011 and references therein), animal models have been generated in order to dissect the mechanisms leading to NAFLD and to test possible therapeutic interventions (Hebbard and George, 2011). A high frequency of apparently spontaneous hepatic steatosis was observed in young, not challenged male BALB/cByJ animals in this study. This should be taken into account when designing and interpreting mouse models for NAFLD.

The same risk factors of NAFLD are involved in the occurrence of pancreatic lipomatosis, which can additionally be associated with old age, steroid therapy, pancreatitis, Cushing disease and certain malignancies (Smits and van Geenen, 2011). Pancreatic lipomatosis, manifested as patchy or diffuse fatty infiltration in parts or in the whole pancreas is an uncommon finding in mice. However, in the present study, two mouse strains, C3HeB/FeJ and FVB/N, were affected by this phenotype. Since these animals were young wild-type animals with no challenge, the increased occurrence of pancreatic lipomatosis seems to be due to inherent genetic factors.

Table 6. Spontaneous morphological lesions in 129P2/OlaHsd mice.

129P2 Lesion	Incidence		*p* value
	Females	Males	
Peyer's patches	2/10	5/10	NS
Bronch. associated alveolar tissue	1/10	1/10	NS
Pyelonephritis	2/10	2/10	NS
Brain hemorrhagia	1/10	1/10	NS
Accessory adrenal cortical nodule	1/10	2/10	NS
Pneumonia	4/10		NS
Dilated glands in gland. stomach	2/10		NS
Testicular atrophy		2/10	NS
Lipomatosis in thyroid		1/10	NS
Thrombus in heart left ventricle		1/10	NS
Hydronephrosis		1/10	NS
Abscess in liver		1/10	NS
Fibrosis in salivary gland		1/10	NS

Table 7. Spontaneous morphological lesions in FVB/N mice.

FVB/N Lesion	Incidence		*p* value
	Females	Males	Between strains
Peyer's patches	3/12	4/12	NS
Bronchiolar associated alveolar tissue	4/12	3/12	NS
Pancreatic lipomatosis	8/12	6/12	<0.01
Hepatocellular necrosis	4/12	1/12	NS
Accessory adrenal cortical nodule	2/12	1/12	NS
Lipomatosis in heart	3/12	1/12	NS
Adrenal subcapsular cell hyperplasia	2/12		NS
Pyelonephritis	2/12		NS
Developmental defect in kidney	1/12		NS
Bronchiolar-alveolar adenoma	1/12		NS
Lipomatosis in thymus		1/12	NS

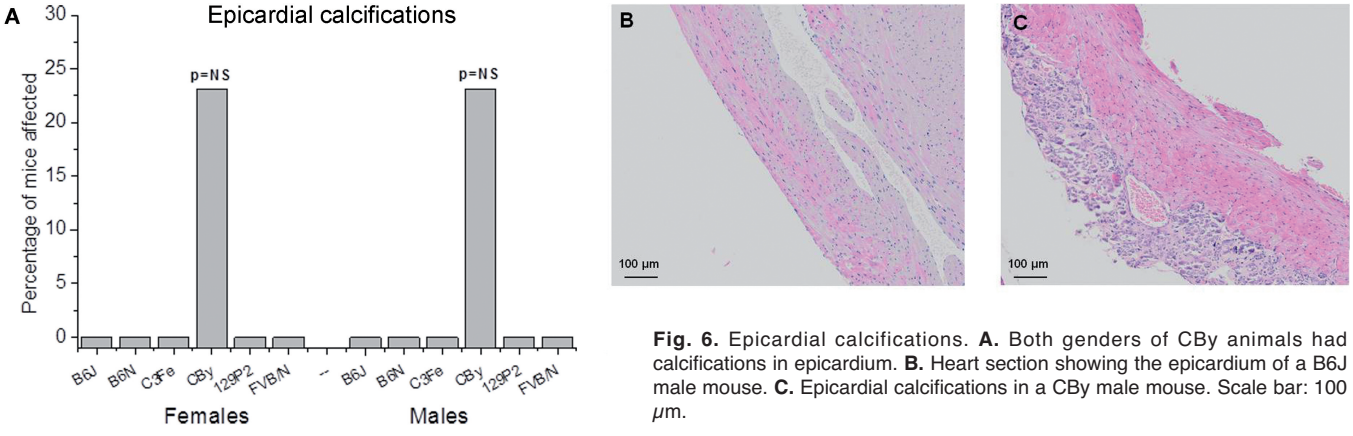


Fig. 6. Epicardial calcifications. **A.** Both genders of CBy animals had calcifications in epicardium. **B.** Heart section showing the epicardium of a B6J male mouse. **C.** Epicardial calcifications in a CBy male mouse. Scale bar: 100 µm.

Subcapsular cell hyperplasia of the adrenal cortex is a frequent phenomenon in old mice and can be induced by gonadectomy, but its etiology in most strains remains elusive (for a review see Bernichtein et al., 2009). In a study by Kim and co-workers (1997), 13-15 month old mice were investigated for the presence of this change. As in our study, a high frequency of subcapsular cell hyperplasia was seen in C3He and BALB/c animals and in female C57BL6 mice, but our study reveals that this phenotype is not always related to aging and can already be seen in 4-5 month-old animals.

Secondary lymphoid organs, characterized here as Peyer's patches in the intestines and bronchiolar associated lymphatic tissue in lungs are occasionally found in mice and are commonly encountered in humans. Their role in immunity is not clear; however there are reports showing that infection or inflammation triggers the organization of lymphoid structure in lungs (Moyron-Quiroz et al., 2004 and references therein) and in the intestines (Kuper, 2006). Histologically, secondary lymphoid organs house subpopulations of T and B lymphocytes and macrophages, and can theoretically give rise to any type of lymphomas (Kuper, 2006). In the present study prominent secondary lymphoid structures were found in C3HeB/FeJ and BALB/cByJ animals. This spontaneous phenotype should be taken into account in studies concerning inflammatory and lymphoid neoplastic processes of lung and intestines. It has to be kept in mind that responses in lymphoid organs can also be due to environmental factors. The health status of the animals used in this study was good and a comprehensive histological analysis of all organs did not reveal any focus of acute or chronic inflammation. However, we cannot totally rule out the possibility that some underlying, clinically and morphologically unapparent infection may have had an impact on these findings.

Dystrophic cardiac calcification (DCC) occurs at

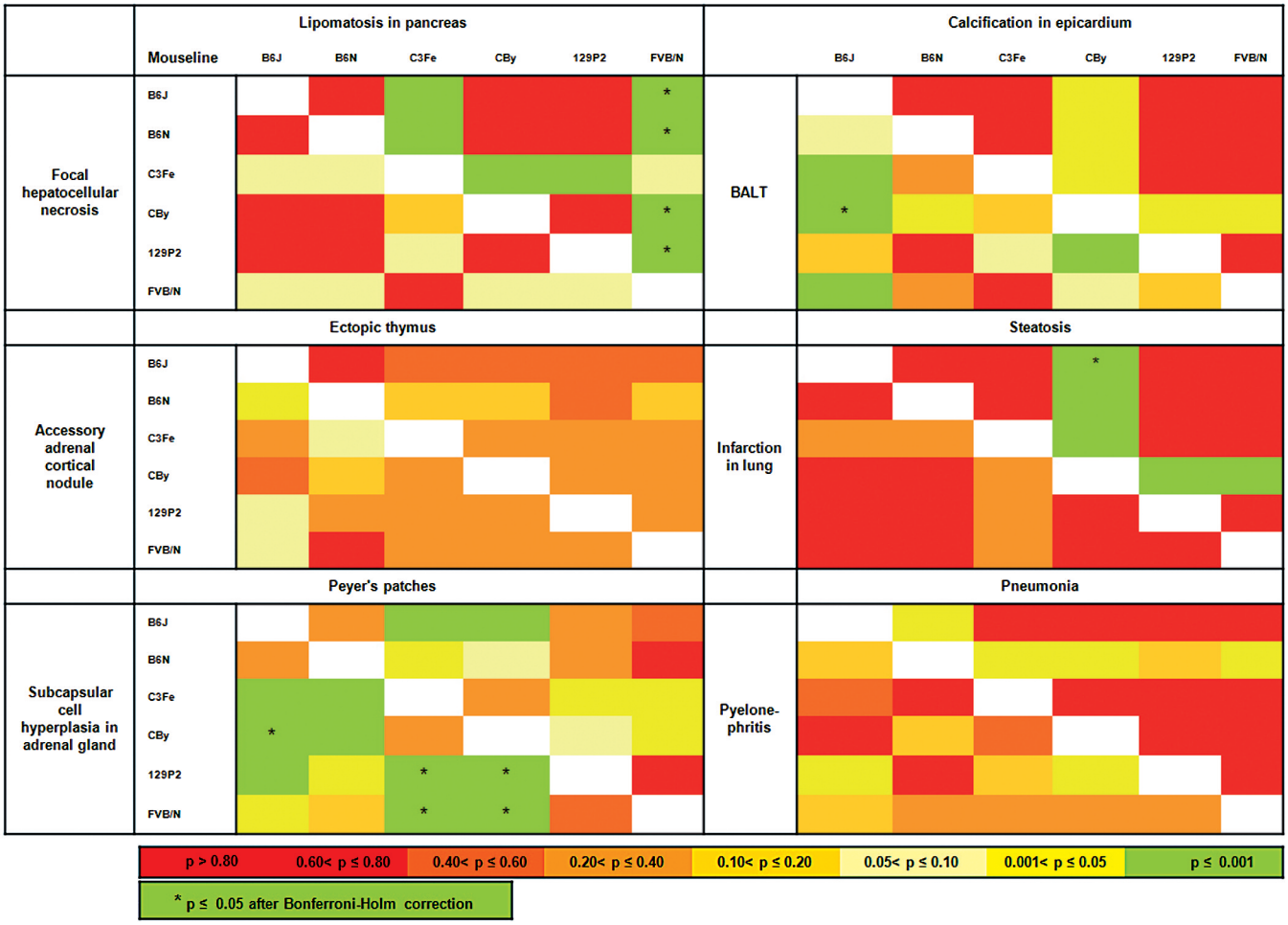


Fig. 7. Heat map of p-values. Statistical evaluation of the comparison of prevalence of most interesting findings between mouse strains is presented. Red colour donates no significance whereas green indicates highly significant differences. Also, p-values significant after Bonferroni-Holm correction are presented (asterisks).

sites of inflammation and necrosis. The occurrence of DCC is independent from plasma calcium levels. In humans, increased cardiac calcification is associated with atherosclerosis, cardiomyopathy and valvular disease, and thus has great impact on cardiac morbidity and mortality (Mohler et al., 2001; London et al., 2005). Cardiac calcifications in BALB/c mice have been reported earlier in both genders (Eaton et al., 1978) or predominantly in male animals (Mossbrugger et al., 2007). We found epicardial calcifications in equal frequency in both male and female BALB/cByJ animals. The susceptibility of BALB/c mice to DCC can offer intriguing possibilities for studying cardiac calcification related diseases.

The need for basic morphological data of wild-type animals used as a basis for creation of genetically modified mouse models is indisputable. Here, we provide new, detailed information to be used as a tool for choosing the right mouse line for transgenic experiments and studies of a certain organ system. Additional information about inbred strain characteristics can be obtained from The Mouse Phenome Database (Maddatu et al., 2012) and from The Mouse Genome Database (Blake et al., 2011). We would also like to draw attention to the importance of proper controls and sample size. As mice already harbour inherent phenotypes at young age, it is of importance to include a proper set of control animals into each experiment to avoid misinterpretation of strain-specific morphological phenotypes (Cardiff et al., 2008). As corollary, professional expertise in mouse pathology should be sought by investigators using mice in biomedical research to avoid misinterpretation of the results.

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References

Bailey D.W. (1978). Sources of sublines divergence and their relative importance for sublines of six major inbred strains of mice. In: Origins of inbred mice. Morse H.-C. III (ed). Academic Press. New York. pp 197-215.

Blake J.A., Bult C.J., Kadin J.A., Richardson J.E., Eppig J.T. and the Mouse Genome Database Group (2011). The Mouse Genome Database (MGD): premier model organism resource for mammalian genomics and genetics. Nucleic Acids Res 39 (Suppl 1), D842-D848.

Bernichtein S., Peltoketo H. and Huhtaniemi I. (2009). Adrenal hyperplasia and tumours in mice in connection with aberrant pituitary-gonadal function. Mol. Cell. Endocrinol. 300, 164-168.

Bogue M.A. and Grubb S.C. (2004). The Mouse Phenome Project. Genetica 122, 71-74.

Bogue M.A., Grubb S.C., Maddatu T.P. and Bult C.J. (2007). Mouse Phenome Database (MPD). Nucleic Acids Res. 35, D643-D649.

Brown R.E. and Wong A.A. (2007). The influence of visual ability on learning and memory performance in 13 strains of mice. Learn Mem.14, 134-44.

Cardiff R.D., Ward J.M. and Barthold S.W. (2008) 'One medicine – one pathology': are veterinary and human pathology prepared? Lab Invest. 88, 18-26.

Eaton G.J., Custer R.P., Johnson F.N. and Stabenow K.T. (1978). Dystrophic cardiac calcinosis in mice. Am. J. Pathol. 90, 173-182.

Fuchs H., Gailus-Durner V., Adler T., Aguilar-Pimentel J.A., Becker L., Calzada-Wack J., Da Silva-Buttkus P., Neff F., Götz A., Hans W., Hölter S.M., Horsch M., Kastenmüller G., Kemter E., Lengger C., Maier H., Matloka M., Möller G., Naton B., Prehn C., Puk O., Rácz I., Rathkolb B., Römisch-Margl W., Rozman J., Wang-Sattler R., Schrewe A., Stöger C., Tost M., Adamski J., Aigner B., Beckers J., Behrendt H., Busch D.H., Esposito I., Graw J., Illig T., Ivandic B., Klingenspor M., Klopstock T., Kremmer E., Mempel M., Neschen S., Ollert M., Schulz H., Suhre K., Wolf E., Wurst W., Zimmer A., Hrabû de Angelis M. (2011). Mouse Phenotyping. Methods 53, 120-135.

Gailus-Durner V., Fuchs H., Becker L., Bolle I., Brielmeier M., Calzada-Wack J., Elvert R., Ehrhardt N., Dalke C., Franz T.J., Grundner-Culemann E., Hammelbacher S., Hölter S.M., Hölzlwimmer G., Horsch M., Javaheri A., Kalaydjiev S.V., Klempt M., Kling E., Kunder S., Lengger C., Lisse T., Mijalski T., Naton B., Pedersen V., Prehn C., Przemeck G., Racz I., Reinhard C., Reitmeir P., Schneider I., Schrewe A., Steinkamp R., Zybill C., Adamski J., Beckers J., Behrendt H., Favor J., Graw J., Heldmaier G., Höfler H., Ivandic B., Katus H., Kirchhof P., Klingenspor M., Klopstock T., Lengeling A., Müller W., Ohl F., Ollert M., Quintanilla-Martinez L., Schmidt J., Schulz H., Wolf E., Wurst W., Zimmer A., Busch D.H. and Hrabé de Angelis M. (2005). Introducing the German Mouse Clinic: open access platform for standardized phenotyping. Nat. Methods 2, 403-404.

Grubb S.C., Churchill G.A. and Bogue M.A. (2004). A collaborative database of inbred mouse strain characteristics. Bioinformatics 20, 2857-2859.

Hancock J.M., Mallon A.-M., Beck T., Gkoutos G.V., Mungall C. and Schofield P.N. (2009). Mouse, man, and meaning: bridging the semantics of mouse phenotype and human disease. Mamm. Genome 20, 457-461.

Hebbard L. and George J. (2011). Animals models of non-alcoholic fatty liver disease. Nat. Rev. Gastroenterol. Hepatol. 8, 35-44.

Kim J.S., Kubota H., Kiuchi Y., Doi K. and Saegusa J. (1997). Subcapsular cell hyperplasia and mast cell infiltration in the adrenal cortex of mice: comparative study in 7 inbred strains. Exp. Anim. 46, 303-6.

Kuper C.F. (2006). Histopathology of mucosa-associated lymphoid tissue. Toxicol. Pathol. 34, 609-615.

London G.M., Marchais S.J., Guérin A.P. and Métivier F. (2005). Arteriosclerosis, vascular calcifications and cardiovascular disease in uremia. Curr. Opin. Nephrol. Hypertens. 14, 525-531.

Maddatu T.P., Grubb S.C, Bult C.J. and Bogue M.A. (2012). Mouse Phenome Database (MPD). Nucleic Acids Res. 40, D887-D894.

Matsuo N., Takao K., Nakanishi K., Yamasaki N., Tanda K. and

Miyakawa T. (2010). Behavioral profiles of three C57BL/6 substrains. Front. Behav. Neurosci. 14, 4-29.

Medina D. (2010). Of mice and women: A short history of mouse mammary cancer research with an emphasis on the paradigms by the transplantation method. Cold Spring Harb Perspect Biol. 2, a004523.

Mohler E.R. 3rd, Gannon F., Reynolds C., Zimmerman R., Keane M.G. and Kaplan F.S. (2001). Bone formation and inflammation in cardiac valves. Circulation 103, 1522-1528.

Mossbrugger I., Hoelzwimmer G., Calzada-Wack J. and Quintanilla-Martinez L. (2007). Standardized morphological phenotyping of mouse models of human diseases within the German Mouse Clinic. Verh. Dtsch. Ges. Path. 91, 98-103.

Moy S.S., Nadler J.J., Young N.B., Perez A., Holloway L.P., Barbaro J.R., Wilson L.M., Threadqill D.W., Lauder J.M., Magnuson T.R. and Crawley J.N. (2007). Mouse behavioural tasks relevant to autism: phenotype of 10 inbred strains. Behav. Brain Res. 176, 4-20.

Moyron-Quiroz J.E., Rangel-Moreno J., Kusser K., Hartson L, Sprague F., Goodrich S., Woodland D.L., Lund F.E. and Randall T.D. (2004). Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. Nat. Med. 10, 927-934.

Ohtsuka M., Ishii K., Kikuti Y.Y., Warita T., Suzuki D., Sato M., Kimura M. and Inoko H. (2006). Construction of mouse 129/Ola BAC library for targeting experiments using E14 embryonic stem cells. Genes Genet. Syst. 81, 143-146.

Peng L., Wang J. and Li F. (2011). Weight reduction for non-alcoholic fatty liver disease. Cochrane Database Syst. Rev. 15, 6:CD003619.

Smits M.M. and van Geenen E.J. (2011). The clinical significance of pancreatic steatosis. Nat. Rev. Gastroenterol. Hepatol. 8, 169-177.

Sundberg J.P., Berndt A., Sundberg B.A., Silva K.A., Kennedy V., Bronson R., Yuan R., Paigen B., Harrison D. and Schofield P.N. (2011). The mouse as a model for understanding chronic diseases of aging: the histopathologic basis of aging in inbred mice. Pathobiol. Aging Age-related Dis. 1, 7179.

Taketo M., Schroeder A. C., Mobraaten L.E., Gunning K.B., Hanten G., Fox R.R., Roderick T.H., Stewart C. L., Lilly F. and Hansen C.T. (1991). FVB/N: an inbred mouse strain preferable for transgenic analyses. Proc. Natl. Acad. Sci. USA 88, 2065-2069.

Wong A.A. and Brown R.E. (2006).Visual detection, pattern discrimination and visual acuity in 14 strains of mice. Genes Brain Behav. 5, 389-403.

Zhou X., Jen P.H., Seburn K.L., Frankel W.N. and Zheng Q.Y. (2006). Auditory brainstem responses in 10 inbred strains of mice. Brain Res. 1091, 16-21.

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