Gender-, strain-, and inheritance-dependent variation in aldosterone secretion in mice

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Abstract

Arterial hypertension represents one of the most common diseases in developed countries and the rennin–angiotensin–aldosterone system is among the major factors in the regulation of blood pressure and sodium balance. With the exception of rare monogenetic diseases, however, inheritance of aldosterone secretion is widely unknown. In this study, we investigated the aldosterone levels in male and female mice of two inbred strains, C3HeB/FeJ and C57BL/6J, as well as their offspring of the F1 and F2 generation. In all cases, female animals displayed lower aldosterone levels than males. Furthermore, C57BL/6J animals had significantly higher aldosterone levels than C3HeB/FeJ mice of the same age and gender. Depending on the paternal origin of the animal, the F1 offspring showed a tendency toward higher aldosterone values when the paternal side was from the C57BL/6J strain. This observation was confirmed in the F2 generation and

over repeated measurements over three consecutive years. Quantification of the aldosterone to renin ratio in the different mouse groups did not show any significant differences, and, similarly, the determination of plasma potassium and kidney parameters did not provide any differences. On the molecular level, investigation of the expression of the enzymes involved in steroidogenesis displayed the same trend as for the aldosterone values, with animals hosting C57BL/6J background in their paternal origin having also the highest expression levels for StAR, cyp11a1, and cyp11b2 enzymes. Taken together, we could demonstrate that the genetic background of the animals plays a significant role modulating their plasma aldosterone levels without clear interference of other parameters in the renin–angiotensin–aldosterone system.

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Introduction

Arterial hypertension represents one of the most common diseases in developed countries. According to recent epidemiological studies, about 20% of the adult population is diagnosed with any form of increased blood pressure (Fardella *et al.* 2000). As one of the major determinates of water and sodium balance, the renin–angiotensin–aldosterone system has profound impact on blood pressure control (Giacche *et al.* 2000). Although low renin hypertension is still considered as a form of essential hypertension, the most frequent cause of secondary arterial hypertension is primary aldosteronism. Primary aldosteronism develops from hypersecretion of the mineralocorticoid aldosterone in which aldosterone production is inappropriately high, relatively autonomous from angiotensin II stimulation, and, thus, non-suppressible by sodium load. The large majority of primary aldosteronism is caused either by an aldosterone-producing adrenal adenoma or by bilateral adrenal hyperplasia, with

two-thirds of patients being diagnosed with the bilateral form of the disease and one-third with unilateral adenomas (Beuschlein & Reincke 2007, Schirpenbach & Reincke 2007). Despite its high prevalence, the genetic causes of primary aldosteronism have been elucidated only in rare familial forms of the disease. In patients with familial hyperaldosteronism type 1 (FH1), symptoms occur already at young age with very high blood pressure levels, which are usually refractory to common antihypertensive medications. Due to the high homology between aldosterone synthase encoded by the *CYP11B2* gene and 11 β -hydroxylase, the product of *CYP11B1* (Kawamoto *et al.* 1990). FH1, also known as glucocorticoid remediable hyperaldosteronism, is caused by unequal crossing-over of *CYP11B1* and *CYP11B2* and the formation of a hybrid gene (Hampf *et al.* 2001). This resulting gene is responsible for ACTH-dependent production of aldosterone instead of being regulated by angiotensin II. Only few families (~50) have been identified worldwide. Only recently, mutations in

KCNJ5 have been determined as the cause of FH3 (Choi *et al.* 2011). Mutations in the selectivity filter of this potassium channel result in an inward flow of sodium in affected glomerulosa cells followed by depolarization and increased steroidogenic activity. Interestingly, somatic mutations in KCNJ5 have been determined in a large proportion of sporadic aldosterone-producing adenomas, further underlining the relevance of this mechanism for autonomous aldosterone secretion (Boulikroun *et al.* 2012). Finally, FH2 is likely to reflect several forms of genetically determined primary aldosteronism that seem to be inherited as an autosomal dominant trait. Likewise, the morphological and functional phenotype is variable from aldosterone-producing adenomas to bilateral hyperplasia. A locus has been mapped on chromosome 7p22 in some but not in all families (Iida *et al.* 1995), but the linkage area has not been resolved to any causative mutation. In addition, the candidate gene approach has been frustrating. Thereby, the genetic basis of FH2 is still unknown.

Mice provide a useful animal model for studying mechanisms involved in blood pressure regulation and specifically for investigation of the renin–angiotensin–aldosterone system. A number of models with targeted genetic alterations including Task1 and Task3 knockout animals (Davies *et al.* 2008, Heitzmann *et al.* 2008) have recently been introduced in the field. Furthermore, we recently used a phenotype-driven mutagenesis screen to generate further mouse models and potentially identify genes with functional impact on aldosterone secretion (Spyroglou *et al.* 2010). Inbred mouse strains, as progenitors of chromosome substitution strains (CSS) and recombinant inbred strains (RIS), can be used for the analysis of traits that are influenced by many genes, such as blood pressure regulation and for the mapping position of a gene effect, as for new candidate genes for primary aldosteronism. Linkage analysis upon crossbreeding of mutant animals from one strain with wild types from another can indicate a relatively small locus on the genome, hosting the responsible gene for the phenotype. However, C57BL/6J and C3H/HeJ inbred strains, which form the genetic background of currently available CSS and one of the RIS panels (BXH set, made by crossing female C57BL/6J (B) with male C3H/HeJ (H) mice), display variations in several physiological and behavioral traits (Singer *et al.* 2004). In this study, we therefore aimed to determine the differences in aldosterone values and the molecular differences in C3HeB/FeJ and C57BL/6J inbred mouse strains as the basis for future exploration of potential genetic mechanisms involved in the modulation of aldosterone secretion.

Materials and Methods

Animals and housing conditions

All animal studies were performed according to protocols examined and approved by the Regierung von Oberbayern

and according to the German Animal Protection Law. Mice were kept in a nonspecific pathogen-free animal facility at an ambient temperature of 22 ± 2 °C (relative humidity 60 ± 5%) and on a 12 h light:12 h darkness circle. The animals were fed standard breeding chow (sodium 0.24% and potassium 0.91%; Ssniff R/M-H, Soest, Germany) *ad libitum* with free access to tap water. The experiments were performed on 12-week-old male and female mice (purchased originally from the Jackson Laboratory, Bar Harbour, ME, USA, and bred at the Helmholtz Zentrum München for more than ten generations), which were maintained in groups of six to eight individuals. Twenty 12-week-old animals were used per gender and genotype for the experiments. The genotypes used were the two C3HeB/FeJ and C57BL/6J inbred strains and F1 and F2 offspring derived from their mating. For details and nomenclature, refer to Table 1.

Blood sampling

The collection of blood samples had to take place under stress-free conditions. To avoid any influence of the examined parameters through hypothalamic–pituitary–adrenal axis activation, handling of the animals until blood sampling was kept to an interval of < 1 min (Spyroglou *et al.* 2009). In all instances, blood sampling took place between 0800 and 1100 h. Blood samples were obtained from anesthetized animals by puncture of the retroorbital sinus with a glass capillary. From each animal, 0.25 ml blood was collected in Li-heparin-coated tubes to avoid coagulation. After centrifugation (10 000 g × 10 min), the plasma was separated and kept at –20 °C until measurement.

Organ sampling

After ether anesthesia, mice were decapitated and the abdominal cavity was opened. Both adrenal glands were identified and removed. Directly after the collection, adrenals were cleaned from surrounding fat tissue using a stereoscope,

Table 1 Nomenclature for C3HeB/FeJ and C57BL/6J mice and their F1 and F2 offspring

Generation	Paternal × maternal origin	Abbreviation
F0	C3HeB/FeJ	CCCC
	C57BL/6J	BBBB
F1	C57BL/6J × C3HeB/FeJ	BBCC
	C3HeB/FeJ × C57BL/6J	CCBB
F2	(C57BL/6J × C57BL/6J) × (C57BL/6J × C3HeB/FeJ)	BBBC
	(C57BL/6J × C57BL/6J) × (C3HeB/FeJ × C57BL/6J)	BBCB
	(C57BL/6J × C3HeB/FeJ) × (C57BL/6J × C57BL/6J)	BCBB
	(C3HeB/FeJ × C57BL/6J) × (C57BL/6J × C57BL/6J)	CBBB

snap frozen, and stored in liquid nitrogen (-80°C) or embedded in paraffin.

Histological procedures

Paraffin sections ($5\text{ }\mu\text{m}$) of the middle part of each adrenal gland were de-waxed by incubation for 3 min in each one of the following solutions: $2\times$ xylol, $2\times$ 100% ethanol, $2\times$ 96% ethanol, and $1\times$ ddH₂O. Then the sections were stained with hematoxylin–eosin for morphological evaluation. After removing the water from the slides, these were dehydrated three times in 100% ethanol, cleared in xylol, and mounted in a resinous medium (Microm, Walldorf, Germany).

Hematoxylin–eosin-stained adrenal sections were examined with a standard light microscope using $\times 50$ magnification. The total adrenal gland surface and zona glomerulosa/total adrenal gland surface were calculated with SPOT 4.0.9 imaging software.

Definition of biochemical profile

Potassium values of the plasma samples were obtained in the Zentrallabor der Medizinischen Klinik Innenstadt (COBAS Integra 800, Roche). Urea and creatinine values were measured in the clinical chemical laboratory of the German Mouse Clinic of the Helmholtz Zentrum München using an AU400 Autoanalyser (Beckman–Coulter, Krefeld, Germany) and adapted reagent kits provided by the manufacturer (Urea: OSR6134; creatinine (Jaffe): OSR6178).

Aldosterone measurement

Aldosterone was determined with an in-house time-resolved fluorescent immunoassay as described in detail elsewhere (Manolopoulou *et al.* 2008).

Renin activity measurement

The plasma renin activity was measured by a commercially available Angiotensin I RIA Kit (DiaSorin, Saluggia, Italy). The method was modified according to Heitzmann *et al.* (2008). Each plasma sample was diluted 1:20 with maleate buffer. The rat renin substrate was diluted 1:3 with maleate buffer. Diluted probe ($50\text{ }\mu\text{l}$), rat renin substrate ($22.2\text{ }\mu\text{l}$), generation buffer ($27.7\text{ }\mu\text{l}$) included in the kit, and PMSF ($2\text{ }\mu\text{l}$) (RENCTK kit) were mixed; $51\text{ }\mu\text{l}$ of this mixture were incubated for 90 min in a water bath at 37°C and the remaining $51\text{ }\mu\text{l}$ were incubated for 90 min on ice (blank sample); $45\text{ }\mu\text{l}$ of both the 37°C sample and the blank sample were dispensed in RIA-coated tubes and incubated for 23 h at room temperature. The same procedure was also performed for the calibrators. After the incubation, the content of the tubes was aspirated and the radioactivity of the 37°C and blank tubes was measured by a Gamma Counter. For the calculation of the results, the corresponding blank value was subtracted from each 37°C sample value. The obtained value

was multiplied by 40.8 (the dilution factor of the sample) and this was divided by the incubation time in hours.

Real-time PCR

Both adrenals from each individual animal were combined and homogenized in extraction buffer while still frozen. RNA extraction was performed using the SV Total RNA Isolation System according to the manufacturer's instructions (Promega). For cDNA synthesis, $1\text{ }\mu\text{g}$ total RNA was reverse transcribed using the reverse transcription system (Promega). Quantification of investigated genes was accomplished using the FastStart DNA MasterPlus SYBR Green I reaction mix in the LightCycler 1.5 (Roche). Real-time PCR conditions were pre-incubated at 95°C for 10 min followed by amplification of 40 cycles at 95°C for 10 s, the annealing temperature (primer dependent as given in Table 2) for 5 s, and extension at 72°C , at which the time is calculated by the product length in base pairs divided by 25 (Roche). The melting curve analysis was performed between 65 and 95°C ($0.1^{\circ}\text{C}/\text{s}$) to determine the melting temperature of the amplified product and to exclude undesired primer dimers. Furthermore, the products were run on a 1% agarose gel to verify specificity of the amplified product. Quantification was adjusted using the house-keeping gene β -actin. To improve comparability of the different groups, the group with the highest expression levels for all examined genes (BBBC) was arbitrary adjusted to 100% and the expression levels for all other groups were compared to this percentage.

Statistical analysis

All results are expressed as mean $\pm$ S.E.M. Statistical significance was determined using the Mann–Whitney *U* test for the comparison of two groups and with the Kruskal–Wallis test for the comparison of multiple groups. Statistical significance was defined as $P < 0.05$.

Table 2 Primer sequences

Gene	Primer sequences	Annealing temperature ($^{\circ}\text{C}$)
β -Actin	F: TCATGAAGTGACGTGGACATCC R: CCTAGAAGCATTTGCGGTGGACGATG	60
<i>Star</i>	F: GACCTTGAAGGCTCAGGAAGAAC R: TAGCTGAAGATGGACAGACTTGC	60
<i>Cyp11a1</i>	F: AGGACTTTCCTGCGCT R: GCATCTCGGTAATGTTGG	53
<i>Cyp11b2</i>	F: CAGGGCCAAGAAAACCTACA R: ACGAGCATTTTGAAGCACCT	63
<i>Agtr1a</i>	F: GATTGGTATAAAATGGCTGG R: TCTGGGTTGAGTTGGTCTCA	60
<i>Agtr2</i>	F: GCTTACTTCAGCTGCATT R: GGACTCATTGTTGCCAGTTG	60

Results

Baseline aldosterone levels vary between inbred strains and among their descendants

Measurement of baseline aldosterone levels in 12-week-old male and female mice of the strain C3HeB/FeJ (CCCC) revealed baseline aldosterone values of 134 ± 11 and 78 ± 5 pg/ml respectively, which resulted in a statistically significant difference between the genders ($P < 0.001$). The same could also be observed for the strain C57BL/6J (BBBB), with male animals having significantly higher aldosterone values than their female littermates (237 ± 12 vs 210 ± 15 pg/ml, $P < 0.01$). In addition, BBBB animals showed significantly higher values than CCCCCs for both genders. To further follow-up on these findings, the aldosterone levels of animals of the F1 generation upon mating of male BBBB to female CCCC or male CCCC to female BBBB animals were defined. In these animals from the F1 generation, gender differences in aldosterone values were found (BBCC males: 120 ± 2 vs females: 81 ± 8 pg/ml, $P < 0.05$; CCBB males: 80 ± 1 vs females: 70 ± 4 pg/ml, $P < 0.05$), which, however, did not reach statistical significance. Furthermore, a tendency toward lower aldosterone values could be observed when the animals' genetic background included C3HeB/FeJ. Further mating of those male and female F1 offspring to inbred BBBBs of both genders gave rise to animals with a mixed genetic background. Depending on the extent of C57BL/6J background on the paternal side, aldosterone values showed a gradual decrease, with male animals having in all cases higher aldosterone than their female littermates (males: BBBC: 225 ± 15 , BBCB: 177 ± 16 , BCBB: 125 ± 18 , and CBBB: 74 ± 14 pg/ml, $P < 0.0001$; females: BBBC: 147 ± 11 , BBCB: 140 ± 16 , BCBB: 80 ± 7 , and CBBB: 75 ± 13 pg/ml, $P < 0.0001$). Within three subsequent years, repeated experiment provided the same pattern of results (Table 3).

Renin activity, serum potassium levels, and kidney parameters are not significantly different between inbred strains

After the last sequential aldosterone measurement of the offspring of this backcrossing strategy (2010), focus was put on the F2 animals and a more detailed phenotypical characterization concerning their renin–angiotensin–aldosterone system was performed. Besides the decreasing aldosterone values, males also showed a tendency toward lower renin values, without, however, reaching statistical significance ($P = 0.27$), whereas the renin values of female animals were comparable among the groups ($P = 0.96$). Likewise, calculation of the aldosterone to renin ratio (ARR), a parameter used in the clinical practice for the diagnosis of primary aldosteronism, showed no differences within the groups for both male ($P = 0.99$) and female ($P = 0.27$) animals (Table 4). As hyperaldosteronism results in hypokalemia, quantification of the plasma potassium levels in those animals was additionally performed. Similar to the ARR, for both genders, the potassium values did not indicate any specific tendency within the examined groups (males, $P = 0.37$; females, $P = 0.10$) and overall remained within the normal range. To further exclude any influence of kidney function for aldosterone regulation, creatinine and urea were also defined in the F2 offspring of both genders. In all instances, however, these parameters remained within the normal range without evident differences among the examined groups (creatinine: males, $P = 0.55$ and females, $P = 0.36$; urea: males, $P = 0.18$ and females, $P = 0.86$, Table 4).

Expression levels of adrenal steroidogenic enzymes are lowest in animals with maternal C3HeB/FeJ background

On the molecular level, the adrenal expression levels of key enzymes involved in the aldosterone biosynthesis were investigated: StAR, responsible for the regulation of the cholesterol transport in the mitochondria, showed the highest expression in BBBC male ($100 \pm 11\%$) and female ($100 \pm 7\%$)

Table 3 Aldosterone values (pg/ml) of male and female C3HeB/FeJ, C57BL/6J mice and their F1 and F2 offspring determined in different cohorts within the years 2007, 2009, and 2010

Strains	2007			2009			2010		
	Males (pg/ml)	Females (pg/ml)	P	Males (pg/ml)	Females (pg/ml)	P	Males (pg/ml)	Females (pg/ml)	P
Aldo									
BBBB	237 ± 13	210 ± 15	<0.01	235 ± 15	188 ± 17	<0.05	250 ± 35	180 ± 21	<0.05
BBBC	225 ± 15	147 ± 11	<0.01	217 ± 18	177 ± 23	<0.05	225 ± 19	172 ± 12	<0.001
BBCB	177 ± 16	140 ± 16	0.12	193 ± 21	164 ± 23	0.25	213 ± 12	169 ± 12	<0.05
BCBB	125 ± 18	80 ± 7	0.07	186 ± 19	146 ± 18	0.09	180 ± 12	140 ± 15	<0.05
BBCC	120 ± 2	81 ± 8	<0.05	181 ± 22	102 ± 13	<0.01	190 ± 11	129 ± 17	<0.01
CCBB	80 ± 1	70 ± 4	<0.05	160 ± 12	106 ± 11	<0.01	186 ± 11	123 ± 10	<0.01
CBBB	74 ± 15	75 ± 13	0.69	154 ± 16	70 ± 8	<0.0001	175 ± 14	115 ± 15	<0.01
CCCC	134 ± 11	78 ± 5	<0.001	160 ± 12	78 ± 6	<0.0001	170 ± 21	71 ± 12	<0.001
P	<0.0001	<0.0001		<0.05	<0.0001		<0.05	<0.0001	

Table 4 Aldosterone (pg/ml), renin (ng/ml per h), ARR (pg/ml per ng per ml per h), potassium (mmol/l), creatinine (mg/ml), urea (mg/ml), and levels of expression of the enzymes StAR, cyp11a1, cyp11b2, Agtr1a, and Agtr2 (expressed as the percentage of expression in comparison to the BBBC group); zona glomerulosa (expressed as percentage of total adrenal surface); and total adrenal area for the groups BBBC, BBBCB, BCBB, and CBBB

	ARR					StAR	cyp11a1	cyp11b2	Agtr1a	Agtr2	Glomerulosa area (% total adrenal area)	Total adrenal area (sq pixels × 10 ⁴)
	Aldo (pg/ml)	Renin (ng/ml per h)	ARR (pg/ml per ng per ml per h)	K ⁺ (mmol/l)	Crea (mg/ml)	Urea (mg/ml)						
Males												
BBBC	225 ± 19	93.7 ± 22	3.8 ± 0.9	5.1 ± 0.13	0.35 ± 0.01	61.4 ± 1	100 ± 11	100 ± 10	100 ± 12	100 ± 10	14.5 ± 0.8	70 ± 3.2
BBBCB	213 ± 12	73.3 ± 9	4.0 ± 1.0	4.7 ± 0.11	0.35 ± 0.01	58.5 ± 2	81.8 ± 3	81.4 ± 5	58.8 ± 7	61.5 ± 4	11.5 ± 0.3	69 ± 4.5
BCBB	180 ± 12	60.7 ± 10	3.8 ± 0.9	4.9 ± 0.11	0.36 ± 0.01	57.3 ± 4	84.7 ± 8	67.6 ± 6	55.4 ± 7	70.8 ± 6	15.4 ± 1.0	55 ± 2.7
CBBB	175 ± 14	42.5 ± 8	3.8 ± 0.6	4.8 ± 0.13	0.34 ± 0.01	56.3 ± 2	61.9 ± 6	52.1 ± 2	89.3 ± 7	112 ± 15	15.7 ± 1.0	60 ± 9.3
P value	<0.05	0.2719	0.9958	0.3737	0.5562	0.1881	<0.05	<0.001	<0.01	<0.01	0.1051	0.3058
Females												
BBBC	172 ± 12	51.5 ± 7	3.8 ± 0.4	4.6 ± 0.16	0.33 ± 0.01	59.5 ± 2	100 ± 7	100 ± 6	100 ± 5	100 ± 9	9.9 ± 0.6	113 ± 4.0
BBBCB	169 ± 12	53 ± 13	3.5 ± 0.6	4.3 ± 0.11	0.35 ± 0.01	50.2 ± 3	78.4 ± 4	88 ± 5	75.6 ± 9	61 ± 5	10.4 ± 0.6	97 ± 7.7
BCBB	140 ± 15	52.2 ± 10	3.2 ± 0.9	4.6 ± 0.12	0.35 ± 0.01	58.2 ± 2	83.3 ± 6	86.3 ± 7	97.7 ± 12	82.1 ± 10	10.2 ± 1.0	111 ± 5.4
CBBB	115 ± 15	53.1 ± 4	2.6 ± 0.2	4.2 ± 0.07	0.34 ± 0.01	49.6 ± 3	73.7 ± 3	58.4 ± 3	131.1 ± 8	73.6 ± 7	9.3 ± 0.1	79 ± 7.6
P value	<0.05	0.9603	0.2660	0.1054	0.3592	0.8599	<0.01	<0.001	<0.01	<0.05	0.5548	0.0928

animals and the lowest in CBBB animals (males: $62 \pm 6\%$, $P < 0.05$; females: $74 \pm 3\%$, $P < 0.01$), indicating a role of the paternal C57BL/6J background in increased aldosterone synthesis. Similar was the trend for cyp11a1, the enzyme converting cholesterol to pregnenolone, which was highest in BBBCs (males: $100 \pm 10\%$; females: $100 \pm 6\%$), with a gradual reduction of its expression levels in BBBCBs (males: $82 \pm 5\%$; females: $88 \pm 5\%$) and BCBBs (males: $68 \pm 6\%$; females: $86 \pm 7\%$) and the lowest levels in CBBBs (males: $52 \pm 2\%$; females: $58 \pm 3\%$, $P < 0.001$). The same observation was obtained for the aldosterone synthase (cyp11b2), which converts 18-OH-corticosterone to aldosterone ($P < 0.05$ for both genders, Table 4).

Additionally, upon investigation of the angiotensin receptor 1a (Agtr1a), a reduction of the expression levels of the receptor could be observed starting from BBBC to BBBCB and toward BCBB, but CBBB animals of both genders displayed again higher Agtr1a levels (males and females $P < 0.01$; Table 4). Similarly, for Agtr2, the expression levels tended to be lower in BBBCB, BCBB, and CBBB animals of both genders, when compared with BBBCs (males, $P < 0.01$; females, $P < 0.05$; Table 4).

Adrenal morphology displays no differences among the animal groups

Upon hematoxylin-eosin staining and quantification, the zona glomerulosa area (expressed as zona glomerulosa/total adrenal gland area in %) proved to be comparable in all four groups of male mice. Similarly, glomerulosa surface did not differ significantly among female BBBC, BBBCB, BCBB, and CBBB animals. Although the total adrenal areas, measured in square pixels, were comparable among mice of all four backgrounds of the same gender (Table 4), the total adrenal surfaces of female animals were significantly larger than those of male mice ($P < 0.0001$).

Discussion

During this study, we observed significant differences in baseline aldosterone values between male and female mice. The consistency of this observation in a large cohort of animals over a long observation period in repeated experiments in two different inbred strains (C57BL/6J to C3HeB/FeJ) and their offspring is indicative of a strong gender-dependent phenomenon. Differences in the regulation of blood pressure between genders have been well described in human, with both pre- and postmenopausal women having lower blood pressure values than men (Burt *et al.* 1995). In fact, there is some evidence that estrogen-dependent effects on the renin-angiotensin-aldosterone system have to be considered: angiotensin II receptor levels are downregulated by estrogen replacement therapy in ovariectomized rats (Roesch *et al.* 2000) and – based on exploration of a group of women with high estrogen levels

– estrogen status appears to influence aldosterone levels (Miller *et al.* 1999). By contrast, hormone-replacement therapy in postmenopausal women does not exhibit any significant effects on blood pressure regulation (Reckelhoff 2001) and renin levels remain higher in men than in women regardless of age (James *et al.* 1986).

From our data, it can also be concluded that independent of gender differences significant variations in baseline aldosterone levels exist between the two investigated inbred strains, with C57BL/6J animals displaying higher levels in comparison with those of C3HeB/FeJ mice. These findings, which again were found to be very consistent over an observation period of 3 years, are further endorsed by results derived from smaller groups of animals reported earlier (Manolopoulou *et al.* 2008, Sun *et al.* 2010). Interestingly, in the current study, in animals from both the F1 and F2 generation, aldosterone levels were higher if the C57BL/6J strain was on the paternal side. Variations in aldosterone levels were mirrored by respective expression patterns of steroidogenic enzymes in the adrenal glands. These findings could be explained by some level of autonomy of aldosterone secretion in those animals with higher levels of aldosterone. However, an alternative explanation would be a higher angiotensin drive on aldosterone secretion, which would also result in higher expression of steroidogenic enzymes. Interestingly, aldosterone–renin ratios remained unchanged among the different groups of animals. While this excludes relevant autonomy of adrenal aldosterone secretion, smaller but potentially relevant differences in angiotensin-dependent stimulation could have been missed by determination of renin activity, which has a number of technical drawbacks (Morganti *et al.* 1987). An overt kidney phenotype as the cause of secondary aldosteronism was, however, not evident in the investigated animals.

In line with this observation, no differences in mean arterial pressure have been reported in the literature between the C57BL/6J and C3HeB/FeJ strains (Mattson 2001). However, as such effects might only be detectable upon physiological stimulation of the renin–angiotensin–aldosterone system (Heitzmann *et al.* 2008), further investigations of blood pressure regulation in the different mouse strains should be supplemented by investigations after a sodium load test or during low-salt diet respectively.

In population-based studies, it has well been recognized that a continuous spectrum of disease activity exists from normal renin–angiotensin–aldosterone function in essential hypertension to low renin hypertension and primary aldosteronism with its normokalemic and hypokalemic variance (Hannemann *et al.* 2012). Thus far, only the rare monogenetic forms, namely FH1 and FH3, have been delineated to a specific genetic mechanism. However, aldosterone secretion is also influenced by genetic factors with a continuous gradient of increasing risk of blood pressure progression across ARR levels in non-hypertensive individuals (Newton-Cheh *et al.* 2007). In fact, the heritability of serum aldosterone is estimated as 10% and the heritability for multivariable-adjusted logARR was found to range around

40% (Newton-Cheh *et al.* 2007, Alvarez-Madrazo *et al.* 2009). ARR is influenced by clinical and genetic factors; although not associated with common variants in the renin gene, a modest linkage to chromosome 11p has been reported (Newton-Cheh *et al.* 2007). Furthermore, in a haplotype analysis of the human *CYP11B2* gene, an association of a polymorphism with plasma aldosterone levels could be documented (Russo *et al.* 2002). Whether different inbred mouse strains carry different haplotypes in these genes as the cause of aldosterone variations remains to be investigated.

Taken together, in the current study, we could show that there exist gender- and strain-dependent differences in the baseline aldosterone levels in age-matched mice. These observations could serve as a helpful tool to delineate genetic mechanisms involved in aldosterone secretion. The findings should also be taken into consideration for example in the organization of phenotype-driven mutagenesis screens that includes an adrenal phenotype as the phenotypic screening parameter.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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