The influence of mouse *Ped* gene expression on postnatal development

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The *Ped* (preimplantation embryo development) gene, whose product is Qa-2 protein, is correlated with a faster rate of preimplantation development (*Ped fast* phenotype) in mice that express Qa-2 protein compared with mice with an absence of Qa-2 protein (*Ped slow* phenotype). In the current study, we have used two congenic mouse strains differentially expressing the *Ped* gene, strain B6.K1 (*Ped slow*; Qa-2 negative) and strain B6.K2 (*Ped fast*; Qa-2 positive), to investigate the effects of *Ped* gene expression on postnatal growth profiles, systolic blood pressure and adult organ allometry. At birth, B6.K1 mice were moderately lighter than B6.K2 mice. B6.K1 mice became heavier during postnatal life ($P < 0.05$) and had elevated systolic blood pressure at 21 weeks of age when compared with B6.K2 mice ($P = 0.006$). B6.K1 mice also demonstrated elevated serum angiotensin-converting enzyme (ACE) activity, a known regulator of blood pressure ($P = 0.037$). Altered organ:body weight ratios were also observed, with the B6.K1 females having a higher ratio for lungs than B6. K2 females ($P = 0.014$). These data provide evidence of an association between the rate of preimplantation embryo development, postnatal growth and later cardiovascular function.

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During preimplantation development, the rate of embryo cleavage divisions can be influenced both by genetic determinants and environmental factors (reviewed in Warner & Brenner, 2001; Fleming *et al.* 2004; Warner *et al.* 2004). One gene that has been shown to influence the rate of mouse cleavage is the *Ped* (preimplantation embryo development) gene (Goldbard *et al.* 1982). Subsequent studies have linked *Ped* to the mouse major histocompatibility complex (MHC) (Warner *et al.* 1987*b*), in particular the *Q7* and *Q9* genes (Cai *et al.* 1996; Wu *et al.* 1999). The *Q7* and *Q9* genes are duplicated genes, which differ by a single nucleotide only; this leads to a glutamine in *Q7* and a glutamic acid in *Q9*. Mouse strains express *Q7* or *Q9*, or both, and regardless of how many genes are expressed, they show the *Ped fast* phenotype. Mouse strains that have a deletion of both genes do not express Qa-2 protein and therefore show the *Ped slow* phenotype (Cai *et al.* 1996). The *Q7* and *Q9* genes encode Qa-2 protein, a MHC class Ib molecule that is expressed throughout preimplantation development (Warner *et al.* 1987*a*). In blastocysts, Qa-2 protein is expressed on both inner cell mass and trophectoderm cells (McElhinny *et al.* 1998). Two phenotypes of the *Ped* gene, *fast* and *slow*, have been defined based upon the number of cells contained within preimplantation embryos at specific times (Goldbard *et al.* 1982; Brownell & Warner, 1988). The *Ped fast* phenotype corresponds to the presence of Qa-2 encoding genes, whilst the *Ped slow* phenotype corresponds to the absence of Qa-2 protein. *Ped fast* embryos have an increased rate of embryo cleavage and contain more cells than *Ped slow* embryos (Goldbard *et al.* 1982; Brownell & Warner, 1988). Microinjection of the *Q7* and/or *Q9* genes into Qa-2-negative zygotes, leading to the expression of Qa-2 on the surface of the injected embryos, increases the rate of embryo cleavage (Wu *et al.* 1999). Removal of the Qa-2 protein from the embryonic cell surface, through enzymatic cleavage, results in a slowing of mouse preimplantation embryo development (Tian *et al.* 1992). Addition of exogenous Qa-2 protein into the membrane of *Ped*-negative embryos increases the rate of cleavage divisions (McElhinny *et al.* 2000). Therefore, expression of Qa-2 protein on the surface of preimplantation embryos is required for a fast rate of preimplantation cleavage division, the *Ped fast* phenotype.

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Embryonic and fetal development periods represent times of coordinated cell division, differentiation and growth. Perturbations in embryo environment, including reduced growth factors or other metabolites, have been shown to affect the normal patterns of embryonic cell number and rate of development (Fleming *et al.* 2004). Maternal undernutrition has been shown to reduce the rate of preimplantation development (Kwong *et al.* 2000). Offspring developing from these embryos display impaired fetal growth, altered postnatal physiology and adult hypertension. Embryos developing at a faster rate, and containing more cells in response to specific culture conditions, may have an increased chance of fetal survival (Lane & Gardner, 1997). Manipulation of preimplantation cell numbers using different experimental approaches has been shown to delay progression during later gestation after embryo transfer (reviewed in Fleming *et al.* 2004). Therefore, out of a normal population, those embryos that show a faster rate of preimplantation development may enjoy a selective advantage and enhanced chance of postnatal survival.

Expression of the Qa-2 protein during preimplantation development has also been shown to influence postnatal phenotype. Thus, offspring developing from Ped fast embryos have a higher chance of intrauterine survival, are heavier at birth, come from larger litters and are heavier at weaning (Warner *et al.* 1991, 1993; Exley & Warner, 1999). Warner & Brenner (2001) proposed that the Qa-2 protein might have a role in protecting the developing fetus from maternal natural killer cells or macrophages, which could account for the higher levels of fetal loss in embryos lacking Qa-2 protein.

Several studies have shown that altering embryonic and/or fetal development results in the subsequent onset of hypertension in the adult offspring (Langley-Evans *et al.* 1996, 1999; Kwong *et al.* 2000). Aspects of the renin–angiotensin system (RAS) in particular have been shown to be susceptible to certain challenges administered during these windows of development. The cleavage of angiotensin I to angiotensin II, a potent vasoconstrictor, by angiotensin-converting enzyme (ACE) results in an elevation of blood pressure (Skeggs *et al.* 1956). The angiotensin II type 1 (AT_1) receptor is located in vascular smooth muscle cells, kidney, heart, adrenal glands and the brain, where its activation results in vasoconstriction, the release of aldosterone and vasopressin, renal tubular sodium reabsorption, and decreased renal blood flow (reviewed in Unger, 2002). Along with these, activation of AT_1 also results in the vascular wall remodelling, typically characterized by a reduction in lumen diameter and an increase in media thickness (Unger, 2002). Angiotensin II has also been linked to endothelial dysfunction and elevated levels of superoxide anion levels (Rajagopalan *et al.* 1996), thus further contributing to the development of hypertension. Receptor blockade of the AT_1 receptor using the agonist losartan has been shown to significantly reduce blood pressure in offspring from maternal low-protein-diet-fed rats (Sherman & Langley-Evans, 2000). As well as cleaving angiotensin I, ACE also acts to stimulate the release of aldosterone from the adrenal cortex, thus leading to the re-absorption of sodium, and it inactivates bradykinin, again facilitating in the raising and overall regulation of blood pressure (Yang & Erdos, 1967; Yang *et al.* 1970).

Using two congenic strains of mice, B6.K1 (Ped slow; Qa-2 negative) and B6.K2 (Ped fast; Qa-2 positive), we have investigated the influence of embryonic *Ped* gene expression on postnatal development, growth and physiology. Collectively, our data indicate that *Ped* gene expression alters postnatal growth and susceptibility to high systolic blood pressure.

Methods

Animals

B6.K1 and B6.K2 mouse colonies were established at the University of Southampton Biomedical Research Facility from the original colonies at Northeastern University, Boston Massachusetts (Warner *et al.* 1991); they were maintained on a 07.00–19.00 h light cycle, with controlled temperature and standard chow and water *ad libitum*. Animals were housed and used in accordance with Home Office regulations and approved project licence protocols. Single B6.K1 and B6.K2 females (6–8 weeks old, derived from breeding pairs that were 8–9 weeks old) were individually housed overnight without superovulation with single males of the same strain and age. Each morning, females were checked for a vaginal plug as a sign of mating. Plug-positive females were individually housed throughout gestation whilst plug negative females were replaced into original cages during the day and returned to the same male in the afternoon for up to five nights attempted mating. In total, eight females of each strain, B6.K1 (Ped slow; Qa-2 negative; experimental) and B6.K2 (Ped fast; Qa-2 positive; control), were allowed to develop their pregnancies to term. Offspring were weighed on an electronic balance on day of birth and subsequently on the same day every week for 27 weeks. On day of birth, care was taken by wearing gloves and washing hands not to transmit the smell of other mice onto the newborn pups to prevent rejection of pups by their mother. At 3 weeks of age, offspring were weaned from their mothers, and the sexes caged separately. Mice had access to standard chow and water *ad libitum*.

Systolic blood pressure

Systolic blood pressure was determined at 8 and 21 weeks of age by tail-cuff plethysmography using an ITC model

229 blood pressure monitor (Linton Instruments, Norfolk, UK). Mice were allowed to acclimatize to a room temperature of 27–30°C for at least 90 min before readings were taken, and to the tail-cuff apparatus for several minutes before use. Four systolic blood pressure recordings were taken per mouse at each age studied. If after 20 min all four recordings had not been taken, the mouse was released and allowed to recover for 30 min before obtaining the remaining blood pressure values. Heart rate was monitored as an indicator of stress and if it rose above 500 beats min^{-1}, readings were stopped until the rate returned below this value (Langley-Evans *et al.* 1996).

Measurement of organ weight

At 27 weeks of age, following cervical dislocation, blood samples were removed via heart puncture and allowed to clot on ice. Liver, left and right kidneys, heart and lungs were dissected out, weighed, and snap frozen in liquid nitrogen and stored at −80°C. Blood samples were centrifuged at 10 000 *g*, and 4°C for 10 min, after which serum was aliquoted and stored at −80°C.

Serum ACE activity

The method of Raimbach & Thomas (1990) was used with modifications. A 5 μl volume of serum was mixed with 70 μl buffer (300 mM KH_2PO_4, 150 mM NaCl, pH 8.3) to which 50 μl hippuryl-L-histidine-L-leucine acetate salt (5 mM; Sigma, Poole, UK) in buffer was added. Immediately after mixing, samples were transferred to a shaking water bath for 45 min at 37°C. The reaction was terminated by addition of 875 μl ice-cold chloride-free buffer (100 mM KH_2PO_4, pH 8.3) and transferring samples to ice for 10 min. Samples containing only serum and chloride buffer were used as negative controls. Subsequently, 500 μl 0.16 M cyanuric chloride (2-4-6-trichloro-1-3-5-triazine; Sigma) in 1,4 dioxane (Sigma) was added to each sample, mixed and left at room temperature for 10 min for yellow colouration to develop. After centrifugation at 3000 *g* for 10 min at room temperature, the clear yellow supernatant was removed. From each sample, four 200 μl aliquots were pipetted into a 96-well plate and analysed on a plate reader (Dynatech, MR5000) at 380 nm against a blank containing 1 ml 100 mM buffer (pH 8.3) and 0.5 ml cyanuric chloride in 1,4 dioxane. For each analysis, a standard curve over the range of 1 nM to 50 μM was prepared from a 1 mM hippuric acid (Sigma) solution in 100 mM chloride buffer, pH 8.3, and treated exactly as the incubates. Each sample was analysed in duplicate, and an average activity taken. Serum ACE activity was expressed as nanomoles of hippurate formed per millilitre of serum per minute. The intra- and interassay variations were 5.75 and 8.51%, respectively.

Lung tissue ACE activity

The method of Forhead *et al.* (2000) was used with modifications. Samples of lung (approximately 50 mg) were homogenized in 300 μl ice-cold buffer (0.2 M H_3BO_3, 2 M NaCl, pH 8.3) using a PowerGen 125 homogenizer. Samples were centrifuged at 25 000 *g* for 10 min at 4°C, and the supernatant was removed and stored at −80°C until analysis. The pellets were homogenized in a further 300 μl buffer and the supernatants removed after centrifugation as before. Samples (10 μl) of each lung extract were mixed with 50 μl buffer and 20 μl deionized water before incubation at 37°C for 5 min. A 20 μl volume of hippuryl-L-histidine-L-leucine acetate salt (20 mM; Sigma) in the above buffer was added to each tube. Negative controls included the addition of 100 μl 1 M HCl prior to addition of the hippuryl-L-histidine-L-leucine. Samples and blanks were incubated at 37°C for 30 min. The reaction was terminated by the addition of 100 μl 1 M HCl, 100 μl 1 M NaOH, 400 μl 0.2 M KH_2PO_4 (pH 8.3) and 400 μl 0.16 M cyanuric chloride in 1,4 dioxane. Samples were mixed and left at room temperature for 10 min for yellow colouration to develop, before analysis as for serum ACE.

A standard curve over the range 20 μM to 4 mM comprising hippuric acid in 1 M NaOH was treated exactly as the incubates. Samples were analysed in duplicate, and an average activity was taken. Tissue protein content was measured using the Bio-Rad protein assay kit. Tissue ACE activity was expressed as nanomoles hippurate formed per milligram of protein per minute. The intra- and interassay variations were 7.31 and 8.04%, respectively.

Statistical analyses

Student's *t* test was used to analyse mean litter size of B6.K1 and B6.K2 mice (SigmaStat statistical software, version 2.0). All postnatal data (birth weights, postnatal weights, blood pressures, organ weights and ratios, serum and lung ACE levels and ACE correlations) were analysed using a multilevel random effects regression model (procedure XTREG in STATA 7 statistical package, StataCorp 2001) that takes into account between-litter and within-litter variation and different parameters measured from individual animals (Kwong *et al.* 2004). Thus, differences identified between strains throughout the study are independent of variation within strains comprising either litter size or maternal origin of litter. Results given in the text and figures are means ± S.E.M., with $P < 0.05$ regarded as significant in the random effects regression model.

Table 1. Litter and growth criteria of B6.K1 and B6.K2 matings

		P value
Number of B6.K1 litters analysed	8	
Number of B6.K2 litters analysed	8	
Mean B6.K1 litter size	7.25 ± 0.53	
Mean B6.K2 litter size	6.38 ± 0.71	0.34
B6.K1 male:female ratio	1.53	
B6.K2 male:female ratio	1.8	0.75
Combined B6.K1 birth weight (g)	1.24 ± 0.027 ($n = 58$)	
Combined B6.K2 birth weight (g)	1.33 ± 0.016 ($n = 51$)	0.19
B6.K1 males birth weight (g)	1.23 ± 0.048 ($n = 28$)	
B6.K2 males birth weight (g)	1.34 ± 0.023 ($n = 25$)	0.19
B6.K1 females birth weight (g)	1.26 ± 0.026 ($n = 30$)	
B6.K2 females birth weight (g)	1.30 ± 0.022 ($n = 26$)	0.19

Results

Litter size, birth weights and postnatal growth

Litter size and offspring gender ratio were not significantly different between B6.K1 and B6.K2 strains (Table 1). Although B6.K1 males and females had lower mean birth weights (1.23 ± 0.048 and 1.26 ± 0.026, $n = 28$–30, respectively) than B6.K2 males and females (1.34 ± 0.023 and 1.30 ± 0.022, $n = 25$–26, respectively), these differences were not significant. At birth B6.K1 males were 8.21% lighter, whist B6.K1 females were 2.99% lighter than B6.K2 males and females, respectively.

Mean weekly weights of B6.K1 and B6.K2 mice for up to 27 weeks of age are shown in Fig. 1. During weeks 4 and 5, both male and female mice exhibited accelerated growth, after which mean weight gain decreased. From week 5 onwards, B6.K1 males had an elevated mean weight compared with B6.K2 males. At week 6, both the male and female B6.K1 mice were significantly heavier ($P < 0.05$) than B6.K2 mice. Subsequently, during specific weeks (7, 8 and 10), B6.K1 mice were significantly heavier than B6.K2 mice when male and female data are combined ($P < 0.05$). At weeks 25 and 26, B6.K1 females became significantly lighter than the B6.K2 females ($P < 0.05$).

Systolic blood pressure

The mean systolic blood pressures of B6.K1 and B6.K2 mice at 8 and 21 weeks are shown in Fig. 2. At 8 weeks, no significant difference in blood pressure between the strains is evident. At 21 weeks, the B6.K1 mice had a significantly elevated systolic blood pressure when compared with the B6.K2 mice (110.31 ± 0.58 *versus* 107.38 ± 0.54 mmHg, $P = 0.006$) (Fig. 2*A*). Whilst both male and female B6.K1 mice had a higher systolic blood pressure than the B6.K2 mice when analysed separately (Fig. 2*B* and *C*), this difference was only significant in the females (110.221 ± 0.88 *versus* 106.69 ± 0.75 mmHg, $P = 0.032$) (Fig. 2*C*).

Organ allometry

The mean organ:body weight ratios in B6.K1 and B6.K2 mice at 27 weeks are shown in Fig. 3. There was no significant difference between male B6.K1 and B6.K2 mice for any of the organs analysed. However, B6.K1 males did

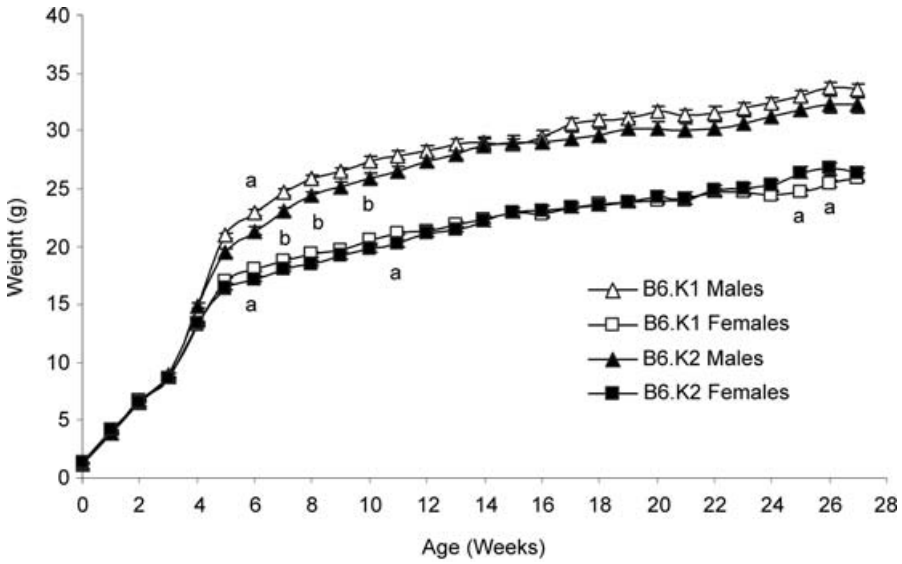

Figure 1. Mean body weights for B6K1 and B6.K2 mice
Values are mean weights (±S.E.M.) for B6K1 and B6.K2 male and female offspring at birth and until 27 weeks of age; a denotes a significant difference between the B6.K1 and B6.K2 strains for either the males or the females ($P < 0.05$); b denotes that there is an overall significant difference between the two strains, but only when the male and female data are combined ($P < 0.05$, $n = 23$–30 per group).

have a lower ratio for all of the organs when compared WITH B6.K2 males. B6.K1 females had a significantly elevated ratio for lungs when compared to B6.K2 females ($P = 0.014$). B6.K1 females also had an elevated ratio for their left and right kidneys and hearts, although these ratios were not significant. When data for male and female lung:body weight ratios were combined, B6.K1 mice had a significantly elevated ratio when compared with the B6.K2 mice ($P = 0.013$). Organ weights analysed independently of body weight showed no significant difference between male B6.K1 and B6.K2 mice for any of the organs studied; however, B6.K1 females had a significantly elevated lung weight when compared with B6.K2 females ($P = 0.038$, data not shown).

ACE activity

Mean ACE activity of B6.K1 and B6.K2 serum and lung tissue for individual animals is shown in Fig. 4. B6.K1 males had a significantly elevated serum ACE activity when compared with B6.K2 mice (302.1 ± 7.0 *versus* 247.42 ± 8.32 nmol hippurate ml^{-1} min^{-1}, respectively, $P = 0.02$, Fig. 4*A*). When data for male and female mice were combined, B6.K1 mice had a significantly elevated serum ACE activity when compared with B6.K2 mice (246.87 ± 7.82 *versus* $209.19 \pm$

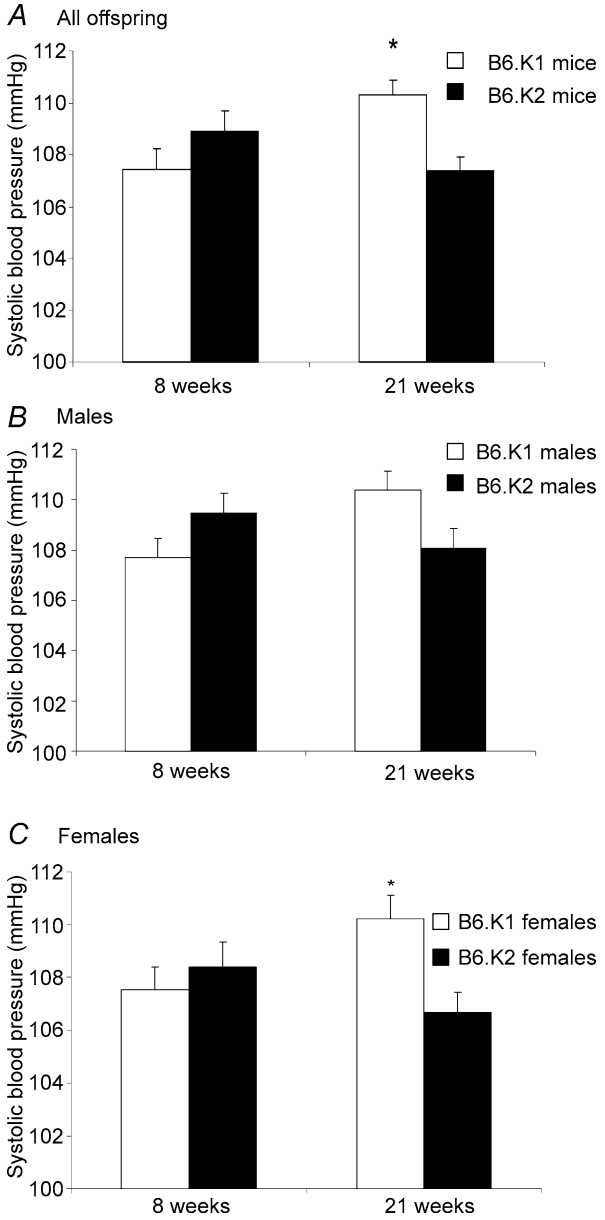

Figure 2. Mean systolic blood pressures for B6.K1 and B6.K2 mice
$n = 46$–54 per group; *A*, $^*P = 0.006$; *C*, $^*P = 0.032$.

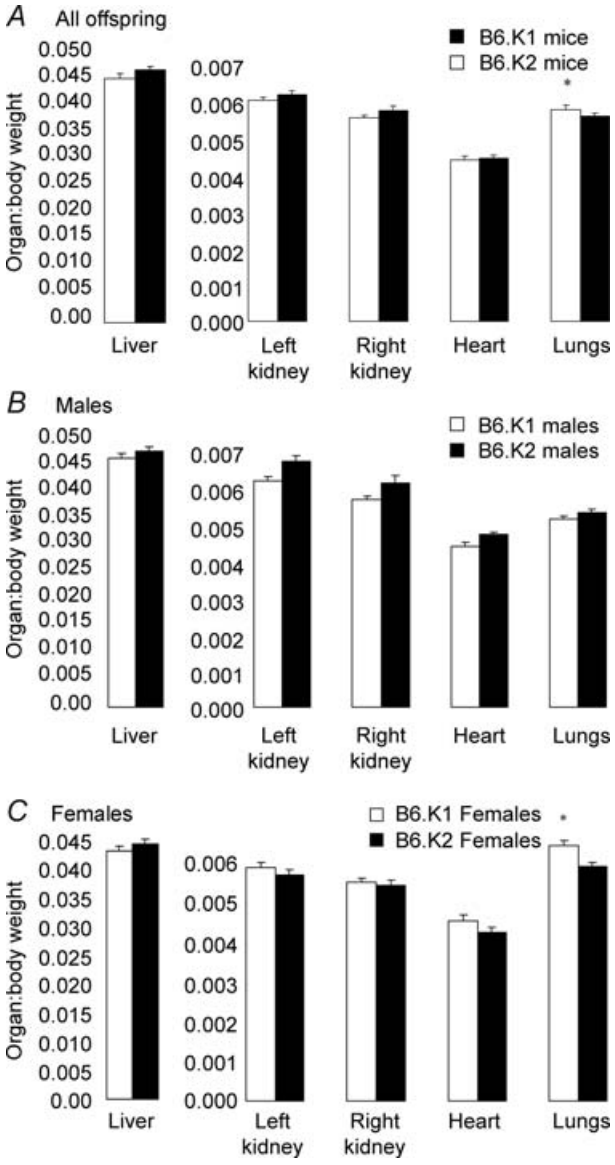

Figure 3. Mean organ:body weight ratios for B6.K1 and B6.K2 mice
$n = 23$–27 per group; *A*, $P = 0.013$; *C*, $^*P = 0.014$.

6.74 nmol hippurate ml^{-1} min^{-1}; $P = 0.037$). Although B6.K1 mice (both males and females) had higher lung ACE activity than B6.K2 mice (10.67 ± 0.62 *versus* 10.07 ± 0.62 nmol hippurate ml^{-1} min^{-1}, males; 11.38 ± 1.32 *versus* 8.05 ± 0.64 nmol hippurate ml^{-1} min^{-1}, females), this was not significant ($P = 0.393$ and 0.236, respectively). B6.K1 mice had an 18.01% increase in their serum ACE activity, whilst B6.K1 males had a 22.1% increase and B6.K1 females had a 12.1% increase when compared with B6.K2 offspring. B6.K1 mice had a 22.8% increase in their lung ACE activity, whilst B6.K1 males had a 6.01% increase and B6.K1 females had a 43.8% increase when compared with B6.K2 offspring.

The relationship between serum and lung ACE activity is shown in Fig. 5. No correlation was evident in B6.K1 mice, whilst B6.K2 mice displayed a significant positive correlation. This correlation was primarily attributed to B6.K2 males (Fig. 5*B*, $P = 0.004$), and not B6.K2 females (Fig. 5*C*, $P = 0.49$). No significant correlation was found between serum ACE activity and body weight for B6.K1 and B6.K2 mice either as males and females analysed independently or together (data not shown). No significant correlation was found between lung ACE activity and body weight for B6.K1 and B6.K2 mice (data not shown). However, when data for males and females were analysed independently, B6.K2 males had a significant negative correlation ($P < 0.001$) not apparent for B6.K2 females or for B6.K1 males or females.

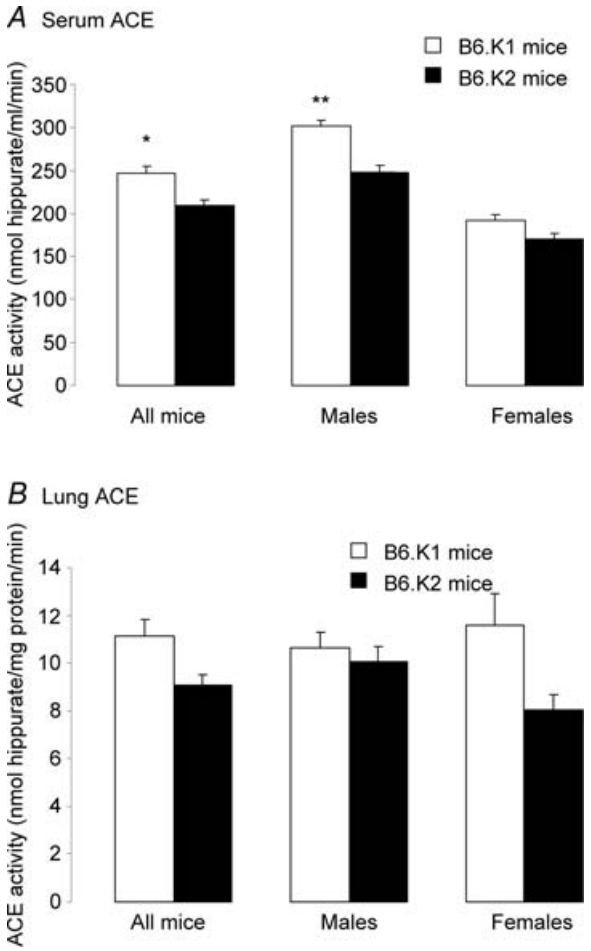

Figure 4. Mean ACE activity of B6.K1 and B6.K2 mice
A, serum, $^*P = 0.037$; *B*, lung tissue, $^{**}P = 0.02$; $n = 40$ per group.

Discussion

Numerous animal studies have demonstrated that alterations to the normal pattern, environment or rate of embryonic or fetal development can have long-term effects on postnatal growth, development and physiology (Thompson *et al.* 1995; Kwong *et al.* 2000; Young *et al.* 2001; Ecker *et al.* 2004). Whilst previous focus has been directed upon the environmental effects of embryo development, we investigated the postnatal development and physiology of two congenic mouse strains, B6.K1 (*Ped slow*; Qa-2 negative) and B6.K2 (*Ped fast*; Qa-2 positive), which differ only in the absence or presence of the major histocompatibility complex *Q7* and *Q9* genes, and thus subsequently the absence or presence of Qa-2 protein expression, respectively.

B6.K1 mice were lighter than B6.K2 mice at birth, as reported in previous studies (Warner *et al.* 1991, 1993), but this was not significant in the current study. Similarly, no significant differences in litter size or weight at weaning were observed, in contrast to previous studies (Warner *et al.* 1987*a*, 1991, 1993; Exley & Warner, 1999). This distinction may reflect differences in sample size or the statistical methods applied. Here, the random effects regression analysis takes into account between-litter and within-litter variation including variation in litter size. Thus, litter size between B6.K1 and B6.K2 strains did not contribute significantly to the (non-significant) birth weight relationship between the strains.

It has been proposed that as *Ped fast* embryos proliferate and develop faster compared with Ped slow embryos, they may reach the uterus earlier and have improved vascularized implantation sites leading to better placentation and fetal development (Brownell & Warner, 1988; McElhinny *et al.* 1998; Warner & Brenner, 2001; Warner *et al.* 2004). Studies in mice involving the asynchronous transfer of embryos have shown that more advanced embryos can implant earlier, and can give rise to offspring of increased birth weight (Aitken *et al.* 1977; Marsk, 1977; Ueda *et al.* 2003). Since the *Ped* gene product, i.e. Qa-2 protein, displays many functional and structural similarities to the human HLA-G protein (Geraghty *et al.* 1987; Jurisicova *et al.* 1996; Comiskey *et al.* 2003, 2005; Clements *et al.* 2005), embryos expressing the Qa-2 protein may be protected from unwanted maternal immune responses (Loke & King, 1991; Chumbley *et al.* 1994; Comiskey *et al.* 2005). This may be an additional

mechanism through which *Ped* gene expression might enhance fetal development. Exley & Warner (1999) observed that there was a loss of Qa-2-negative embryos between day 14.5 and birth, demonstrating that the presence of Qa-2-encoding genes in the fetus confers a selective advantage later in gestation in addition to the preimplantation phenotype. However, the exact mechanism conferring any developmental advantage upon Qa-2-expressing embryos/fetuses is currently unknown.

Reduced rates of rodent embryo development have been linked with impaired fetal growth and over-compensatory 'catch-up' growth postnatally, resulting in adult obesity and increased risk of cardiovascular disease (Bowman & McLaren, 1970; Langley-Evans *et al.* 1999; Kwong *et al.* 2000). Over-compensatory catch-up growth may reflect the disparity between initial constrained rates of embryonic/fetal development (whether due to poor maternal nutrition or due to a genetic polymorphism; as in this study) and subsequent postnatal over abundance of nutrition (Hales & Barker, 2001; Gluckman & Hanson, 2004). The current results show a similar phenomenon between 6 and 11 weeks of age, with both B6.K1 males and females becoming significantly heavier than B6.K2 mice, despite being initially lighter at birth, with the B6.K1 males remaining heavier for the remainder of the study. Coupled with this, at 21 weeks, B6.K1 males and females have a significantly elevated systolic blood pressure when compared with the B6.K2 males and females. It would therefore appear that the reduced birth weight and subsequent postnatal catch-up growth are associated with an elevated systolic blood pressure during adult life in B6.K1 mice.

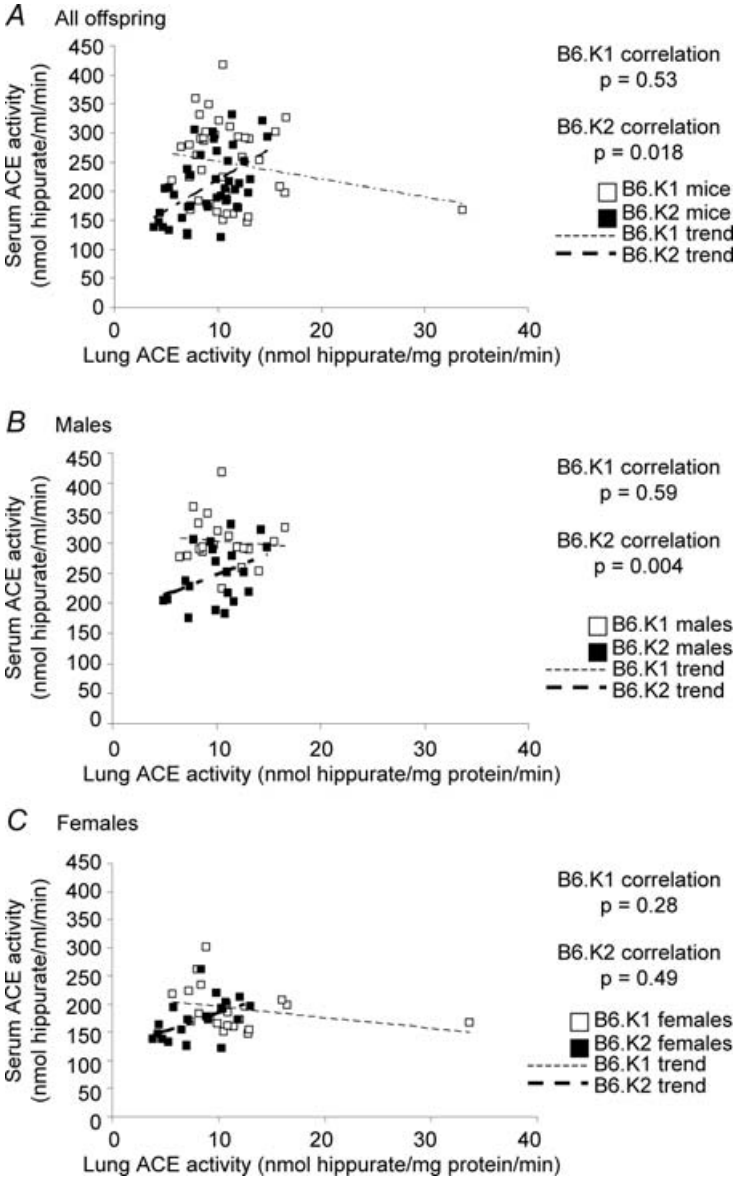

Figure 5. Correlation between serum and lung ACE activity in B6.K1 and B6.K2 mice at 27 weeks of age
$n = 40$ per group.

As hypertension is a multifactorial condition, its origin may lie in more than a single cause, one being altered organ allometry. It was observed that the B6.K1 females had a significantly elevated lung : body weight ratio compared with the B6.K2 females. During periods of impaired fetal development, the fetus makes compensatory adaptations to maintain the development of vital organs such as the brain. These compensations have been shown to have negative impacts on the growth and development of other organ structures (Hales *et al.* 1991; Kwong *et al.* 2000; Whorwood *et al.* 2001). However, overall differences in organ weight between B6.K1 and B6.K2 strains are minimal, do not include the kidney or heart, and therefore are unlikely to be major contributors to the observed difference in blood pressure.

Another factor that influences postnatal blood pressure is ACE activity. ACE cleaves angiotensin I to yield angiotensin II, a potent vasoconstrictor, thus resulting in increased blood pressure. ACE −/− mice are hypotensive, having a systolic blood pressure ∼30 mmHg below wild-type mice (Esther *et al.* 1997; Klein *et al.* 2002). Previous studies have shown that the expression profile of ACE appears to be important in the regulation of blood pressure. In humans, serum ACE levels are significantly higher in hypertensive as compared with normotensive subjects, with serum ACE activity correlating positively with plasma angiotensin II levels (Forrester *et al.* 1997; Nystrom *et al.* 1997). It may therefore be hypothesized that increased expression of ACE enzyme, or an increased activity of existing ACE, may result in an elevated blood pressure. We found that B6.K1 males had a significantly elevated serum ACE activity when compared with the B6.K2 males. When the data for the males and females were analysed independent of gender, the B6.K1 mice had a significantly elevated serum ACE activity when compared with the B6.K2 mice.

Serum ACE activity in humans has also been shown to correlate positively with body mass index (Forrester *et al.* 1997; Nystrom *et al.* 1997). Since lung ACE activity was negatively correlated to body weight in the B6.K2 males, and since serum ACE activity was positively correlated to lung ACE, then, it may be assumed that as body weight increases, overall ACE activity decreases. This reduced activity could be one factor in the lower systolic blood pressure observed for the B6.K2 mice when compared with the B6.K1 mice. *In vivo*, serum ACE is derived from the cleavage by secretases of endothelial-bound somatic ACE, predominantly that of the lung (Beldent *et al.* 1995; Sadhukhan *et al.* 1998; Woodman *et al.* 2000; Eyries *et al.* 2001). It may therefore be hypothesized that the more ACE that is expressed on the surface of the epithelia, then the more ACE will be available for cleavage, so raising serum ACE protein and activity levels. However, for the B6.K1 mice, as lung ACE activity increases, the serum ACE activity falls slightly. There could be several possible reasons for this, such as a decrease in the expression, availability or enzymatic activity of the secretases. However, since the B6.K1 mice had elevated serum ACE activity when compared with the B6.K2 mice, it may be that another source, such as the vasculature or the kidneys, is contributing to the elevated serum ACE activity observed.

In conclusion, it has been shown that *Ped fast* (Qa-2 positive) and *Ped slow* (Qa-2 negative) congenic mouse strains differ in their postnatal development and physiology. One explanation could be that the reduced birth weight and subsequent postnatal catch up growth have resulted in the development of aspects of the 'metabolic syndrome' in that the *Ped slow* mice display significantly elevated blood pressure and body weight. From the data presented within this study, the hypertensive condition observed appears to be attributed to altered patterns of serum and lung ACE activity, or to the differences in the postnatal growth patterns, since there are little differences in organ:body weight ratios between the two strains. The data therefore support the concept that an altered rate of preimplantation embryo development may lead to long-term consequences on postnatal growth and physiology.

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