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Research paper

Epistatic interaction between common *AGT* G(−6)A (rs5051) and *AGTR1* A1166C (rs5186) variants contributes to variation in kidney size at birth

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ABSTRACT

Low nephron number has been recognised as an important cardiovascular risk factor and recently a strong correlation between renal mass and nephron number has been demonstrated in newborns. The aim of this study was to investigate individual, as well as combined, effects of common variants of genes which encode for major components of the renin–angiotensin system (*REN* G10601A, *AGT* G(−6)A, *ACE* I/D, *AGTR1* A1166C) on kidney size in healthy, full-term newborns. A significant additive main effect of the *ACE* I/D polymorphism, as well as an additive-by-additive interaction between *AGT* G(−6)A and *AGTR1* A1166C variants, were found. The variance attributed to the epistatic effect was 27.9 ml^2/m^4, which accounted for 73.8% of the interaction variance (37.8 ml^2/m^4), 66.4% of the genetic variance (42.0 ml^2/m^4) and 4.4% to the total phenotypic variance (628 ml^2/m^4). No other statistically significant main or epistatic effects were detected. Our results highlight the importance of considering gene–gene interactions as part of the genetic architecture of congenital nephron number, even when the loci do not show significant single locus effects. Unravelling the genetic determinants of low nephron number, along with early molecular screening, may well help to identify children at risk for cardiovascular disease.

1. Introduction

Much research has been aimed at determining the factors that contribute to congenital nephron number, fuelled by observations which have suggested that low nephron number is an important risk factor for cardiovascular disease later in life (Amann et al., 2004; Brenner et al., 1988; Keller et al., 2003), and early identification of children at risk may prove decisive in the prevention of end-organ damage (Amann et al., 2004).

The number of nephrons is a quantitative trait and as such is influenced by multiple genetic and environmental factors, as well as their interactions (Luyckx et al., 2011). According to the current view, quantitative traits are controlled by large numbers of loci (known as quantitative trait loci, QTL), each of which has a relatively small effect (Flint and Mackay, 2009). Epistasis, or interaction between two or more alleles at different loci, has been recognised as an important component of the genetic architecture of quantitative traits (Carlborg and Haley, 2004; Mackay, 2014; Moore, 2005; Phillips, 2008) but the extent to which it can contribute to those traits may vary (Carlborg and Haley, 2004). In the literature a number of commonly used definitions of epistasis exist (Cordell, 2002). For the purpose of quantitative genetics, epistasis can be defined as a deviation from an additive expectation of allelic action (Phillips, 2008). As a result of this, the effects of alleles will change according to genetic background (i.e. via alleles from other loci) giving context-dependent effects (Flint and Mackay, 2009).

In humans, the number of nephrons is completely set during foetal life (Guron and Friberg, 2000) and a strong correlation between renal mass and nephron number has been demonstrated in newborns (Zhang et al., 2008). As a consequence kidney volume has been used, in several association studies with newborns, as a surrogate marker of nephron endowment (El Kares et al., 2010; Quinlan et al., 2007; Zhang et al., 2008, 2011). Recently, Pruijm et al. (2013) found that renal length and volume had high heritability (approximately 50%), thereby justifying studies aimed at characterising the genetic determinants of kidney size. Not only have several loci with significant major effects on kidney

Abbreviations: *ACE*, angiotensin I converting enzyme gene; *AGT*, angiotensinogen gene; *AGTR1*, angiotensin II receptor type I gene; ANGII, angiotensin II; NOIA, natural and orthogonal interaction model; RAS, renin–angiotensin system; *REN*, renin gene; TKV/BSA, total kidney volume standardised to body surface area.

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size in newborns been identified (*PAX2*, *RET*, *OSR1*, *ALDH1A1*), but also attempts have been made to determine their combined effects (El Kares et al., 2010; Quinlan et al., 2007; Zhang et al., 2008, 2011).

The renin–angiotensin system (RAS) has been classically viewed as merely a regulator of blood pressure and sodium/water homeostasis (Sparks et al., 2014), but an accumulating body of evidence implicates additional roles in organogenesis (Schütz et al., 1996) and kidney development in particular (Song et al., 2011; Tufro-McReddie et al., 1995; Yosypiv, 2004, 2014). All major components of RAS are expressed in kidneys very early in human development (at 30 to 35 days of gestation), and at higher levels than in adults (Schütz et al., 1996; Yosypiv, 2004). In rodents, targeted inactivation of genes that encode for renin (*Ren*), angiotensinogen (*Agt*), angiotensin converting enzyme (*Ace*) and angiotensin II receptor type 1 (*Agtr1*) have resulted in various renal phenotypes: from mild histological changes limited to the juxtaglomerular apparatus, to severe lethal damage to arterioles, tubules and interstitium (Guron and Friberg, 2000). In humans, a mutation-dependent disruption in RAS signalling has been linked to the pathogenesis of non-syndromic congenital anomalies of the kidney and urinary tract (CAKUT) (Yosypiv, 2014). Angiotensin II (ANGII), the major effector peptide of RAS, has recently emerged as an important stromal factor regulating key steps in kidney development such as ureteric bud (UB) branching (Song et al., 2011), mesenchymal-to-epithelial transition (Yosypiv, 2004), tubular development (Wolf, 2002) and angiogenesis (Madsen et al., 2010). Branching of the ureteric bud is a crucial step during kidney organogenesis and even minor developmental flaws during this process may markedly reduce nephron endowment (Sakurai and Nigam, 1998). ANGII has been demonstrated to stimulate ureteric bud branching in in vitro and ex vivo experiments (Yosypiv, 2008). It has been suggested that ANGII can be generated locally in stromal mesenchymal cells, so that it can act in a paracrine fashion on ureteric bud cells expressing angiotensin II receptor type 1 (AT1) (Yosypiv, 2004). Blockage of AT1 receptor in rats resulted in reduced glomerular size and number (Tufro-McReddie et al., 1995).

In line with the role of the RAS system in kidney development, we have recently described an association between a common insertion/deletion polymorphism in the angiotensin-converting enzyme gene (*ACE*) and kidney size in newborns (Kaczmarczyk et al., 2013b). Following this, the purpose of the current study was to investigate individual, as well as combined, effects of common variants of genes which encode for major components of the renin–angiotensin system (*REN* G10601A, *AGT* G(−6)A, *ACE* I/D, *AGTR1* A1166C) on kidney volume in healthy, full-term newborns. Main genetic effects in single-locus models were studied, as well as main and epistatic genetic effects in two-locus models. The genetic effects and corresponding variances were obtained by using the Natural and Orthogonal InterActions (NOIA) model, which was developed for association studies involving quantitative traits and for estimation of genetic effects including gene–gene interactions (Alvarez-Castro and Carlborg, 2007).

2. Materials and methods

2.1. Individuals and genotyping

Full-term, healthy newborns (n = 205) with birth mass greater than 2500 g were recruited for this study from a cohort of 210 newborns which have been described in detail previously (Kaczmarczyk et al., 2013b). Five newborns were excluded from this cohort with failed genotyping. The procedures for measuring kidney size were as described previously (Kaczmarczyk et al., 2013a, 2013b). Total kidney volume (TKV) is the sum of left and right kidney volumes and was normalised for body surface area (TKV/BSA) (Quinlan et al., 2007).

Genomic DNA from cord blood was isolated (using a QIAamp Blood DNA Mini Kit; QIAGEN, Hilden, Germany). The *ACE* insertion/deletion (I/D) polymorphism was identified by polymerase chain reaction (PCR) according to the protocol proposed by Lindpaintner et al. (1996). Three single nucleotide polymorphisms (*REN* G10601A, *AGT* G(−6)A and *AGTR1* A1166C) were typed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Primer sequences, restriction enzymes, length of PCR products and length of allele-specific restriction fragments are presented in Table 1. The study was approved by the ethics committee of the Pomeranian Medical University, Szczecin, Poland, and parents gave informed written consent.

2.2. Statistical analyses

A commercial software package (Statistica, StatSoft, Inc., 2014, version 12) was used for descriptive statistics and for comparing TKV/BSA between male and female newborns (by Student's *t*-test). Quantitative data are expressed as mean ± standard deviation (SD). The statistical platform R (R Development Core Team, 2013) was used with the package *genetics* to test for Hardy–Weinberg equilibrium.

2.2.1. Analysis of gene–gene interactions

To examine gene–gene interactions between the loci, the statistical formulation of the Natural and Orthogonal InterActions (NOIA) model of genetic effects was used which allows for orthogonal (i.e. statistically independent) decomposition of genetic effects and corresponding variances (Alvarez-Castro and Carlborg, 2007). The statistical main or epistatic effects (population-referenced (Alvarez-Castro, 2012)) were measured as deviations from the sample mean (as the reference point). Single-locus (with only main genetic effects) and two-locus (with main and epistatic effects) models were examined.

A thorough description of NOIA framework theory can be found in Alvarez-Castro and Carlborg (2007). Briefly, using matrix notation, a vector G of genotypic values (defined as the average phenotype for each genotype), can be expressed as resulting from the product of a genetic-effect design matrix (reflecting the coefficients of the genetic effects) S and a vector of genetic effects E:

$$G = S \cdot E.$$

The statistical genetic-effect design matrix S_S for one locus is as follows, and would apply to, for example, locus A with two alleles, A1 and A2:

$$S_S = \begin{bmatrix} 1 & -p_{12}-2p_{22} & -\frac{2p_{12}p_{22}}{p_{11}+p_{22}-(p_{11}-p_{22})^2} \\ 1 & 1-p_{12}-2p_{22} & \frac{4p_{11}p_{22}}{p_{11}+p_{22}-(p_{11}-p_{22})^2} \\ 1 & 2-p_{12}-2p_{22} & -\frac{2p_{11}p_{12}}{p_{11}+p_{22}-(p_{11}-p_{22})^2} \end{bmatrix},$$

where p_{11}, p_{12}, p_{22} are the frequencies of A1A1, A1A2 and A2A2 genotypes, respectively. The vector of genotypic values is given by

$$\begin{bmatrix} G_{A1A1} \\ G_{A1A2} \\ G_{A2A2} \end{bmatrix} = S_S \cdot \begin{bmatrix} \mu \\ \alpha \\ \delta \end{bmatrix},$$

where G_{A1A1}, G_{A1A2}, G_{A2A2} are genotypic values for the genotypes A1A1, A1A2 and A2A2, respectively, μ is the reference point, α is the additive effect (the average effect of allele substitution in a population sample), δ is the dominance effect (difference between the heterozygote and the average of the homozygotes). Statistical genetic effects can be inferred by multiplying both sides of the equation above by the inverse matrix of $SS(S_S{}^{-1})$

$$\begin{bmatrix} \mu \\ \alpha \\ \delta \end{bmatrix} = S_S{}^{-1} \cdot \begin{bmatrix} G_{A1A1} \\ G_{A1A2} \\ G_{A2A2} \end{bmatrix}.$$