Preeclampsia and cardiovascular disease share genetic risk factors on chromosome 2q22

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ABSTRACT

Objective: Four putative single nucleotide polymorphism (SNP) risk variants at the preeclampsia susceptibility locus on chromosome 2q22; rs2322659 (*LCT*), rs35821928 (*LRP1B*), rs115015150 (*RND3*) and rs17783344 (*GCA*), were recently shown to associate with known cardiovascular risk factors in a Mexican American cohort. This study aimed to further evaluate the pleiotropic effects of these preeclampsia risk variants in an independent Australian population-based cohort.
Methods: The four SNPs were genotyped in the Western Australian Pregnancy Cohort (Raine) Study that included DNA, clinical and biochemical data from 1,246 mothers and 1,404 of their now adolescent offspring. Genotype association analyses were undertaken using the SOLAR software.
Results: Nominal associations ($P < 0.05$) with cardiovascular risk factors were detected for all four SNPs. The *LCT* SNP was associated with decreased maternal height ($P = 0.005$) and decreased blood glucose levels in adolescents ($P = 0.022$). The *LRP1B* SNP was associated with increased maternal height ($P = 0.026$) and decreased maternal weight ($P = 0.044$). The *RND3* SNP was associated with decreased triglycerides in adolescents ($P = 0.001$). The *GCA* SNP was associated with lower risk in adolescents to be born of a preeclamptic pregnancy ($P = 0.003$) and having a mother with prior preeclamptic pregnancy ($P = 0.033$).
Conclusions: Our collective findings support the hypothesis that genetic mechanisms for preeclampsia and CVD are, at least in part, shared, but need to be interpreted with some caution as a Bonferroni correction for multiple testing adjusted the statistical significance threshold (adjusted $P < 0.001$).

Key words

2q22; cardiovascular disease (CVD); genetic association; preeclampsia; Raine Study

Introduction

Women with a history of preeclampsia and offspring exposed to preeclampsia in utero are at increased risk of cardiovascular disease (CVD) later in life [1-3]. A large review and meta-analysis found that women with a history of preeclampsia have approximately four-fold increased risk of chronic hypertension, and two-fold increased risk of coronary artery disease and stroke 10-15 years after pregnancy [1]. The offspring of women with preeclampsia have higher mean systolic and diastolic blood pressure in childhood and early adult life in both genders, including those with normal birth weight [4-6]. Furthermore, they have almost a two-fold greater risk of stroke in adulthood [3]. Preeclampsia is now widely viewed as an early screening criterion for CVD in women. Pregnancy is a unique opportunity to identify both women and offspring at increased risk of premature CVD [7], and clinical risk assessments and preventive programmes are under development [8].

Preeclampsia and CVD share several constitutional risk factors (e.g. hypertension and obesity) [9], pathological features (e.g. endothelial dysfunction and inflammation) [10, 11], and tend to occur in the same families [12]. These common antecedents have drawn attention to the likelihood of shared genetic susceptibility [13, 14]. Supporting this notion are several cardiovascular risk factors present years before a preeclamptic pregnancy, including increased blood pressure, higher levels of serum cholesterol, higher levels of low density lipoprotein (LDL)-cholesterol and higher levels of triglycerides [15]. Moreover, the positive association between preeclampsia and CVD is more dependent on these shared pre-pregnancy risk factors than the influence of the hypertensive disorder in the pregnancy itself [16]. This has encouraged the search for genetic determinants common to both disorders. However, to date only a few shared genetic risk factors have been identified [17-20].

Recently, our genetic dissection of the 2q22 preeclampsia susceptibility locus identified four independent single nucleotide polymorphism (SNP) risk variants, residing within four genes, to associate with preeclampsia in an Australian family cohort [19]: lactase (*LCT*, rs2322659), low density lipoprotein receptor-related protein 1B (*LRP1B*, rs35821928), rho family GTPase 3 (*RND3*, rs115015150) and grancalcin (*GCA*, rs17783344). Furthermore, these same four SNPs were associated with cardiovascular risk factors in an independent cohort of Mexican American families, suggesting pleiotropic effects for these SNPs [19]. The aim of this study was to determine whether these four SNPs exhibited pleiotropic characteristics with preeclampsia

susceptibility and cardiovascular risk factors in an independent Australian population-based cohort consisting of mothers and their adolescents. Identifying common genetic factors influencing preeclampsia and CVD may provide insight into pathophysiological mechanisms relevant to both disorders.

Materials and methods

Study population

The Western Australian Pregnancy Cohort (Raine) Study is a pregnancy cohort where women were recruited prior to 18 weeks' gestation from the public antenatal clinic at King Edward Memorial Hospital or surrounding private clinics in Perth, Western Australia. The study has been described in detail elsewhere [21]. Pregnant women (n = 2,900) were enrolled between August 1989 and April 1992, and they gave birth to 2,868 live babies. From the original cohort of women, their children have been followed up over the last two decades with detailed assessments performed every 2-3 years. In the current study, data from the pregnant women, the neonates, and the 8-, 14- and 17-year cohort follow-ups were assessed, as shown in Fig. 1. Only subjects that had two Caucasian parents, were biologically unrelated, and who had no congenital deformities, were included in the current study.

Informed written consent was obtained at recruitment and at each follow-up from the mother or legal guardian as well as from the adolescent during the 14- and 17-year cohort follow-ups. Ethical approval was obtained for all protocols from the Human Ethics Committees of King Edward Memorial Hospital, Princess Margaret Hospital Ethics Committee, Perth, Western Australia and The University of Western Australia.

Antenatal information

At recruitment the mothers completed self-administrated questionnaires concerning their pregnancies and demographic information. The presence of preeclampsia and history of preeclampsia were collected from the mother at antenatal visits at the delivery units and later assessed from the medical records. The medical records were reviewed by obstetricians and research midwives to confirm a standardised diagnosis of preeclampsia as a pregnancy-induced increase in systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg in

women who were normotensive before the 24th week of pregnancy, combined with significant new onset proteinuria (≥ 0.3 g/l in a 24-hour specimen) [22].

Blood pressure, anthropometry and blood samples

Detailed information on measures of blood pressure, anthropometry and biochemistry, is given in detail elsewhere [23, 24]. Briefly, blood pressure was measured with an automatic device (Dinamap Vital Signs Monitor 8100, Dinamap XL Vital Signs Monitor or Dinamap ProCare 100; GE Healthcare) after 5 minutes rest and using the appropriate cuff size. Six readings were recorded, and the average value was calculated after excluding the first reading. Height and weight were measured with light clothing and without shoes. Height was measured with Holtain Infantometer and Stadiometer (to the nearest 0.1 cm), and weight was measured on Wedderburn Scales (to the nearest 100 g). Fasting venous blood samples were drawn for DNA and biochemical analyses. Serum insulin, glucose, total cholesterol, high density lipoprotein (HDL)-cholesterol, LDL-cholesterol and triglycerides were measured in the PathWest Laboratory at Royal Perth Hospital as described previously [23, 24].

Cardiovascular risk factors

Cardiovascular risk factors assessed included resting systolic and diastolic blood pressure, height, weight, waist-hip ratio, abdominal skinfold, and fasting insulin, glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides. Maternal data was obtained from an examination and blood sample taken when their children attended the 8-year cohort follow-up and included 1,685 mothers. Adolescent data was obtained during the 14- and 17-year cohort follow-ups and included 1,293 [23] and 1,053 [24] participants, respectively.

DNA extraction and SNP genotyping

DNA was extracted from blood samples taken from mothers and adolescents at the 14- or 17-year cohort follow-ups as described elsewhere [25]. Briefly, DNA was extracted from 4 mL ethylenediaminetetraacetic acid (EDTA) anticoagulated blood using Qiagen PureGene chemistry (Qiagen, Hilden, Germany). Four independent SNPs in four genes, rs2322659 (*LCT*), rs35821928 (*LRP1B*), rs115015150 (*RND3*) and rs17783344 (*GCA*), were genotyped for mothers and adolescents. For the mothers *de novo* genotyping of the four SNPs was performed. For the

adolescents *de novo* genotyping was performed for rs35821928 and rs115015150. The rs2322659 and rs17783344 SNPs had already been genotyped in a previously performed genome wide association study (GWAS) [26]. *De novo* genotyping was commercially performed by KBioscience (KBioscience, Hertfordshire, UK), with the use of their proprietary fluorescence-based competitive allele-specific PCR genotyping assay, KASP™. Genotyping and quality control of GWAS data have been described in detail elsewhere [26]. Briefly, the Raine adolescent samples were genotyped on the Illumina Human 660W-Quad SNP Chip (Illumina Inc., San Diego, CA, USA) at the Centre for Applied Genomics (Toronto, Ontario, Canada). Individual samples were checked (and excluded accordingly) for gender inconsistencies, levels of heterozygosity and inter-sample relatedness.

Statistical analysis

The software package R (www.r-project.org) was used to compute descriptive statistics, means and 95% confidence intervals (CI). Phenotypes of interest included cardiovascular risk factor measurements and maternal pregnancy characteristics.

SNP association analysis

Measured genotype association analyses were undertaken for all phenotypes applying variance-component methods as implemented in SOLAR [27]. Because variance-component methods are sensitive to kurtosis, all quantitative phenotypes were transformed using SOLAR's inverse normalization procedure. Genetic association was tested for each SNP under an additive genetic model allowing mean phenotype value to vary by minor allele. This model was compared with the null model of no difference in mean phenotype value by SNP genotype using a likelihood ratio test. Twice the difference in log-likelihoods of these models was distributed as a χ^2 random variable with 1 degree of freedom. Concordance with Hardy-Weinberg proportions was tested using χ^2 goodness-of-fit statistic. A threshold of $\alpha = 0.05$ was set for statistical significance of all computed analyses. Adjustment for multiple hypothesis testing was performed using Bonferroni corrections (α/(total number of SNPs x total number of phenotypic traits)).

Cardiovascular risk factors including height, weight, blood pressure, and cholesterol, have consistently been demonstrated to correlate between relatives. This could reflect genetic- and/or shared life style effects [28]. Therefore we performed genetic association analysis to examine the

association between total maternal genotype and total adolescent phenotype, and vice versa. We did not look specifically at mother-offspring pairs. In addition, we performed separate association analyses for girls and boys for all cardiovascular risk factors aiming to detect differences in genetic risk profiles between the adolescent's genders.

Results

Clinical characterisation

At the 14-year follow-up 629 (48.6%) girls and 664 (51.3%) boys participated, whereas at the 17-year follow-up 509 (48.3%) girls and 544 (51.7%) boys participated. Of the enrolled women (mothers of the adolescents) with accessible DNA for genotyping, 40 (3.2%) were diagnosed with preeclampsia in the index pregnancy, and 31 (2.5%) had previously experienced a preeclamptic pregnancy. The mean age for the index pregnancy was 28.2 years. Clinical and biochemical characteristics of mothers and adolescents are presented in Table 1.

SNP genotyping and association analysis

De novo DNA data was available for 1,246 of the mothers. *De novo* DNA data was available for 1,461 of the adolescents and GWAS DNA data was available for 1,494 adolescents. After exclusion of children with congenital deformities, siblings and non-Caucasians, DNA from 1,246 mothers and 1,404 adolescents were included in the final analysis. We observed a high genotyping success rate for all four SNPs (>97%). Allele frequencies for mothers and adolescents are presented in Table 2, and are consistent with frequencies observed by Johnson et al. [19]. Except for the *LCT* (rs2322659) SNP for mothers, all SNPs confirmed to Hardy-Weinberg proportions ($P > 0.05$).

Measured genotype association results were undertaken for all phenotypes and adjusted for sex and maternal age (raw $P < 0.05$). The results are presented in Table 3 and 4 for mothers and adolescents respectively. Carrying the A allele of *LCT* rs2322659 was associated with decreased levels of the adolescent's blood glucose in both mothers and adolescents ($P = 0.003$ and $P = 0.022$, respectively) and decreased maternal height ($P = 0.005$) in mothers. Carrying the T allele of *LRP1B* rs35821928 was associated with increased maternal height in both mothers and adolescents ($P = 0.026$ and $P = 0.013$, respectively) and decreased maternal weight ($P = 0.044$) in

mothers. An association between the A allele of *RND3* rs115015150 and decreased adolescent's waist-hip ratio ($P = 0.030$) was observed in mothers, whereas this SNP was associated with decreased level of adolescent's triglycerides ($P = 0.001$) in adolescents. In mothers, carrying the C allele of *GCA* rs17783344 was associated with increased adolescent's height ($P = 0.045$), whereas in adolescents carrying the C allele was associated with lower risk to be born of a preeclamptic pregnancy ($P = 0.003$) and lower risk to have a mother who previously had experienced a preeclamptic pregnancy ($P = 0.033$). The two latter associations were related to male gender ($P = 0.009$ and $P = 0.0372$, respectively). However, after accounting for the four SNPs tested across the 14 phenotypes, none of the association results satisfy our Bonferroni-adjusted statistical significance threshold (adjusted $P < 0.001$).

Discussion

The basis for this study was the recently reported shared genetic mechanisms putatively influencing preeclampsia and cardiovascular risk factors [19]. We have now assessed these independent putative pleiotropic variants representing four genes; rs2322659 (*LCT*), rs35821928 (*LRP1B*), rs115015150 (*RND3*) and rs17783344 (*GCA*), in an independent Australian population-based pregnancy cohort. We observed shared genetic associations between specific SNPs and known cardiovascular risk factors, for mothers and their offspring. However, we were unable to replicate many of the genetic associations previously reported by Johnson et al. [19]. To our knowledge, this is the first published study that has assessed possible shared genetic risk factors for preeclampsia and CVD in both mothers and their offspring.

Johnson et al. found the A allele of the *LCT* SNP protective for preeclampsia, and nominally associated with oxidative stress indicators, inflammatory- and diabetic biomarkers [19]. Supportive of protective pleiotropic effects on preeclampsia and cardiovascular risk factors, we found the A allele of the *LCT* SNP to be nominally associated with decreased glucose levels in the adolescents. To date, there is limited evidence of the association between exposure to preeclampsia in utero and the offspring's fasting glucose metabolism later in life [5, 6]. The *LCT* SNP was out of Hardy-Weinberg equilibrium for mothers, the same observation made by Johnson et al. in their Australian preeclampsia case-control cohort [19]. This could possibly be explained by locus-specific population stratification, and has been thoroughly discussed elsewhere [19, 29].

LRP1B, a member of the LDL receptor gene superfamily, has recently been shown to be involved in cell migration and invasion *in vitro* [30], central elements in the development of preeclampsia. Further, SNPs in the *LRP1B* gene were associated with body mass index (BMI) in a large GWAS [31], and insulin resistance in a follow-up study [32], suggesting that this gene may be involved in body weight regulation. Johnson et al. found the T allele of the *LRP1B* SNP protective for preeclampsia [19]. We observed the T allele of the *LRP1B* SNP to be associated with decreased weight, and increased height. The possibility of *LRP1B* harbouring genetic variants influencing preeclampsia and CVD is possible, as obesity and short stature are risk factors for both preeclampsia and coronary heart disease [9, 33, 34]. A review and meta-analysis including >3 million individuals showed that short stature is associated with increased risk of CVD, and the findings apply to both genders [33]. No clear understanding of the relationship between height and CVD exists, but shared genetic factors have been proposed [35]. Short stature is also a risk factor for preeclampsia, especially in the cases of severe phenotypes [34].

RND3 (RhoE) plays a role in human cytotrophoblast fusion, suggesting an important role in the regulation of trophoblast fusion in pregnancy [36]. *RND3* inhibits the biological activity of a downstream effector protein, Rho-associated protein kinase (ROCK) [37]. ROCK proteins have important roles in abnormal vascular tone, endothelial dysfunction, inflammation, oxidative stress and vascular re-modelling, all of which are influential factors in preeclampsia and CVD pathogenesis. Johnson et al. found the A allele of the *RND3* SNP associated with higher preeclampsia risk and nominally associated with increased adiponectin levels [19], a protein which is inversely correlated with body fat percentage in adults. In accordance with the latter, we found reduced levels of triglycerides and reduced waist-hip ratio for the A allele of the *RND3* SNP. Hence, these data add to the possibility of *RND3* harbouring genetic variants that may have a role in obesity-related pathology.

Grancalcin (*GCA*), a calcium binding protein, is specifically expressed in neutrophils and monocytes/macrophages, and displays calcium-dependent translocation to the granules and plasma membrane upon activation of these innate immune responders [38]. Neutrophil activation leads to the release of toxic factors (e.g. myeloperoxidase) promoting an inflammatory response, oxidative stress and vascular dysfunction [39]. While grancalcin deficiency does not adversely affect neutrophil function, it does however, impact their adhesive properties to fibronectin [40]. Plasma cellular fibronectin, a marker for endothelial and vascular injury, has been reported in

several studies to be elevated in preeclampsia [41, 42]. Furthermore, neutrophil adhesion to fibronectin promotes cytokines such as IL-8 to exert their chemotactic effects, which may explain the pronounced abundance of neutrophils in the maternal systemic vasculature of both preeclamptic [39] and obese [43] women. We observed that the C allele of the *GCA* SNP was associated with lower risk to be born of a preeclamptic pregnancy and lower risk to have a mother who previously had experienced preeclampsia. These results showed association to male gender, and were not associated with preeclampsia in the mothers. This could possibly indicate a paternally inherited role for this SNP. However, Johnson et al. [19] assessed the maternal genotype, and we cannot exclude our association results to preeclampsia as false positives.

There was only a partial replication between the results of Johnson et al. [19] and our study. This could be explained by differences between the studies including constitution of study populations (e.g. ethnicity, sex and age), sampling procedures and the undertaken biochemical measurements. A limitation to our study is that the number of women with preeclampsia is limited, which reduces the power to detect significant associations and making subgroups analysis assessing severe preeclampsia (e.g. early versus late onset) impossible. Severe preeclampsia may be associated with an even greater risk of CVD later in life [1]. However, this relationship was not confirmed in a recently published large review and meta-analysis [2]. Another limitation of our study is that we did not access paternal data due to insufficient information on paternal cardiovascular risk factors. On the other hand, there is no clear evidence of association between preeclampsia and paternal cardiovascular risk factors [44, 45], suggesting that influence of paternal genes for increasing preeclampsia risk differs to the influence of genes increasing cardiovascular risk [45]. Further, the investigated SNPs could be in linkage disequilibrium (LD) with other as yet unidentified causal variants, and this will be a focus of future studies using efficient next-generation sequencing strategies. Strengths of our study include a large homogeneous study population, assessment of both maternal and adolescent data, a relatively high attendance at cohort follow-ups, inclusion of fasting blood samples, standardized endpoint measurements and an accurate diagnosis of preeclampsia [21].

In conclusion, our study has demonstrated in an independent population that all four genetic variants tested (rs2322659 (*LCT*), rs35821928 (*LRP1B*), rs115015150 (*RND3*) and rs17783344 (*GCA*)) were nominally associated with known cardiovascular risk factors including height, weight, waist-hip ratio, blood glucose and triglycerides. The *GCA* SNP was associated

with lower risk to be born of a preeclamptic pregnancy and lower risk to have a mother who previously had experienced a preeclamptic pregnancy, increasing the putative role for this gene locus in preeclampsia susceptibility. Our findings support the hypothesis that underlying genetic mechanisms for preeclampsia and CVD are, at least in part, shared. These results warrant further investigation to determine the potential roles of these variants in preeclampsia and CVD. The complex etiology of these disorders are striking, and targeted analyses and more comprehensive investigation strategies made possible by new technologies will be important in further revealing the genetic susceptibility to preeclampsia and CVD.

Contributors

M.L. contributed to conception and design, data analysis, interpretation of results and was responsible for manuscript preparation. E.K.M. conceived the idea for the project, contributed substantially to conception and design, revision and final approval of the manuscript. M.P.J. and P.E.M. contributed substantially to data analysis, interpretation of results, revision and final approval of the manuscript. W.A., R.C.H, T.A.M., L.J.B. and C.P. were involved in the planning of the project, contributed substantially to acquisition of data, revision and final approval of the manuscript. L.T.R. A.C.I., R.A., C.E.E., J.B. and S.P.B. contributed to interpretation of data, revision and final approval of the manuscript.

Conflict of interest statement

The authors declare that they do not have any conflict of interest.

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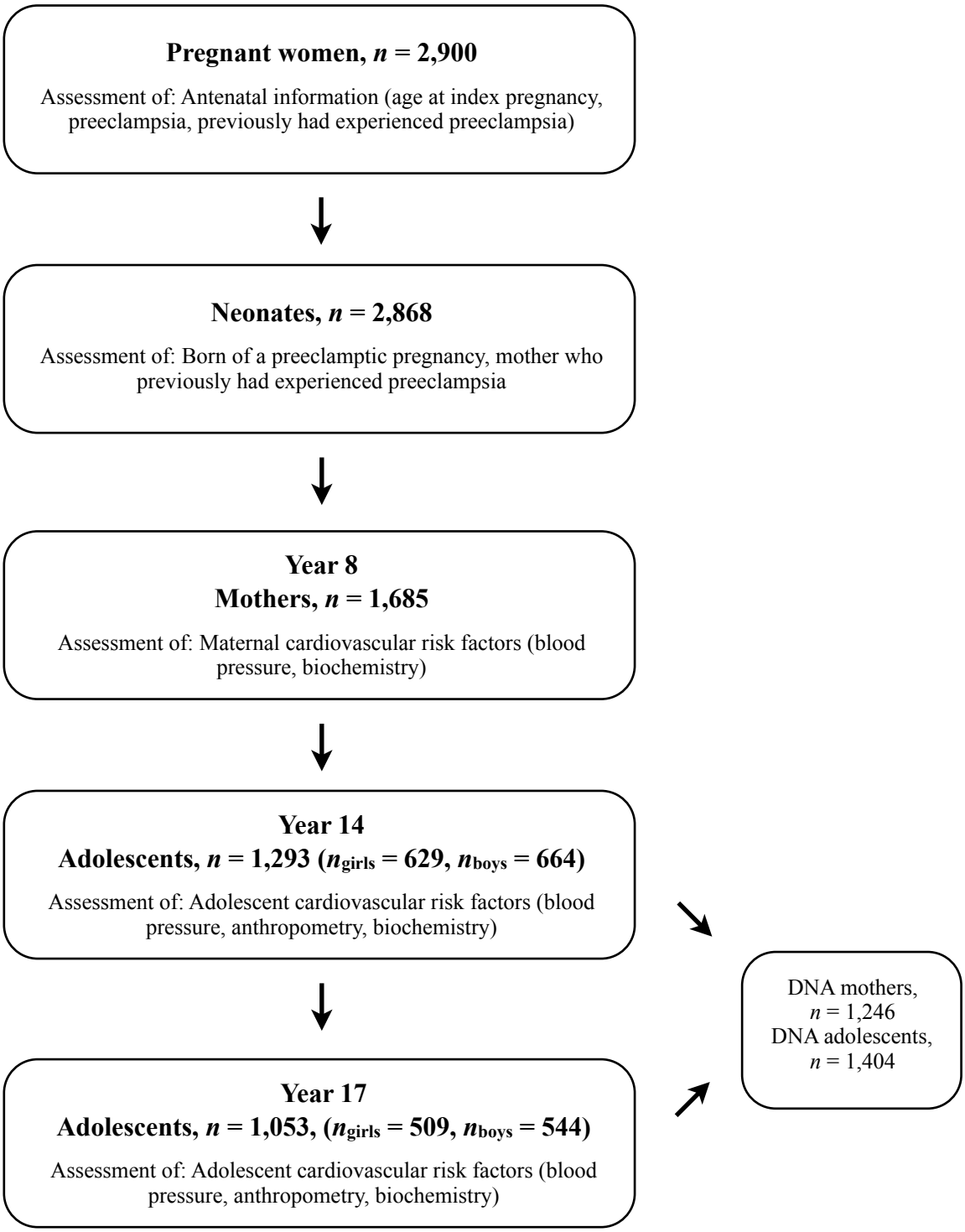
Pregnant women, n = 2,900
Assessment of: Antenatal information (age at index pregnancy, preeclampsia, previously had experienced preeclampsia)
Neonates, n = 2,868
Assessment of: Born of a preeclamptic pregnancy, mother who previously had experienced preeclampsia
Year 8
Mothers, n = 1,685
Assessment of: Maternal cardiovascular risk factors (blood pressure, biochemistry)
Year 14
Adolescents, n = 1,293 (n girls = 629, n boys = 664)
Assessment of: Adolescent cardiovascular risk factors (blood pressure, anthropometry, biochemistry)
Year 17
Adolescents, n = 1,053, (n girls = 509, n boys = 544)
Assessment of: Adolescent cardiovascular risk factors (blood pressure, anthropometry, biochemistry)
DNA mothers, n = 1,246
DNA adolescents, n = 1,404

Fig. 1. Diagram showing numbers of mothers and offspring at the cohort follow-ups which were included in the analysis for the current study.

Table 1
Clinical and biochemical characteristics of mothers at their children's 8-year follow-up, and adolescents at the 14- and 17-year follow-ups.

Trait description	Mothers[ab]	Adolescents 14 yr[a]	Adolescents 17 yr[a]
Systolic blood pressure (mmHg)	118.8 (118.0, 119.6)	111.5 (110.9, 112.1)	118.1 (114.3, 121.9)
Diastolic blood pressure (mmHg)	69.5 (68.9, 70.0)	58.7 (58.4, 59.1)	63.7 (59.7, 67.8)
Height (cm)	163.9 (163.5, 164.3)	165.1 (164.6, 165.5)	174.3 (172.1, 176.4)
Weight (kg)	70.6 (69.6, 71.5)	58.7 (57.9, 59.5)	71.5 (68.3, 74.6)
Waist-hip ratio	-	0.83 (0.83, 0.84)	0.81 (0.80, 0.81)
Abdominal skinfold (cm)	-	-	26.8 (25.3, 28.3)
Insulin (mU/liter)	3.56 (3.45, 3.67)	12.58 (11.85, 13.31)	9.49 (8.83, 10.15)
Glucose (mmol/liter)	4.81 (4.72, 4.90)	4.81 (4.78, 4.84)	4.77 (4.73, 4.80)
Total cholesterol (mmol/liter)	5.07 (4.99, 5.16)	4.17 (4.13, 4.22)	4.12 (4.07, 4.17)
HDL-cholesterol (mmol/liter)	1.51 (1.48, 1.55)	1.39 (1.37, 1.41)	1.30 (1.28, 1.32)
LDL-cholesterol (mmol/liter)	3.10 (3.02, 3.18)	2.32 (2.28, 2.35)	2.34 (2.30, 2.38)
Triglycerides (mmol/liter)	1.02 (0.96, 1.08)	1.02 (0.98, 1.05)	1.06 (1.02, 1.09)

[a]Data are expressed as mean (95% CI).
[b]Clinical and biochemical characteristics were obtained when their children attended the 8-year follow-up.

Table 2
Distribution of alleles for mothers (*n* = 1,246) and adolescents (*n* = 1,404) in the Raine Study.

Gene	SNP	Mothers		Adolescents	
		Major allele *n* (proportion of total)	Minor allele *n* (proportion of total)	Major allele *n* (proportion of total)	Minor allele *n* (proportion of total)
LCT	rs2322659	G 1684 (0.76)	A 522 (0.24)	G 2138 (0.77)	A 656 (0.23)
LRP1B	rs35821928	C 2072 (0.94)	T 142 (0.06)	C 2272 (0.94)	T 156 (0.06)
RND3	rs115015150	G 2171 (0.98)	A 37 (0.02)	G 2392 (0.98)	A 42 (0.02)
GCA	rs17783344	A 1914 (0.86)	C 300 (0.14)	A 2398 (0.85)	C 410 (0.15)

Table 3
SNPs nominally associated ($P < 0.05$) with cardiovascular risk factors for the mothers.

Gene	SNP	Function	Trait description	*n*	*P* value[a]	Direction of association[b]
LCT	rs2322659	Missense	Blood glucose*	875	0.003	↓
			Height	900	0.005	↓
LRP1B	rs35821928	Synonymous	Height	902	0.026	↑
			Weight	863	0.044	↓
RND3	rs115015150	UTR-3	Waist-hip ratio*	739	0.030	↓
GCA	rs17783344	Missense	Height*	830	0.045	↑

*Associated with the adolescent phenotype.
[a]Observed measured genotype *P* value.
[b]Direction of association, for the minor allele.

Table 4
SNPs nominally associated ($P < 0.05$) with cardiovascular risk factors for the adolescents.

Gene	SNP	Function	Trait description	*n*	*P* value[a]	Direction o association[l]
LCT	rs2322659	Missense	Blood glucose	969	0.022	↓
LRP1B	rs35821928	Synonymous	Height*	960	0.013	↑
RND3	rs115015150	UTR-3	Triglycerides	935	0.001	↓
GCA	rs17783344	Missense	Born of a preeclamptic pregnancy	1404	0.003	↓
			Mother with prior preeclampsia	1403	0.033	↓

*Associated with the maternal phenotype.
[a]Observed measured genotype *P* value.
[b]Direction of association, for the minor allele.