Original Article

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Refined phenotyping identifies links between preeclampsia and related diseases in a Norwegian preeclampsia family cohort

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Objective: Preeclampsia is a complex genetic disease of pregnancy with a heterogenous presentation, unknown cause and potential severe outcomes for both mother and child. Preeclamptic women have increased risk for atherothrombotic cardiovascular disease. We aimed to identify heritabilities and phenotypic correlations of preeclampsia and related conditions in the Norwegian Preeclampsia Family Biobank.

Methods: By applying a variance components model, a total of 493 individuals (from 138 families with increased occurrence of preeclampsia) were classified according to 30 disease-related phenotypes.

Results: Of parous women, 75.7% (263/338) had experienced preeclampsia and 35.7% of women with and 22.4% without preeclampsia delivered children small for gestational age (SGA). We identified 11 phenotypes as heritable. The increased occurrence of preeclampsia was reflected by the presence [heritability (H2r) = 0.60)] and severity (H2r = 0.15) of preeclampsia and being born in a preeclamptic pregnancy (H2r = 0.25). Other heritable phenotypes identified included SGA (H2r = 0.40), chronic hypertension (H2r = 0.57), severity of atherothrombotic cardiovascular disease (H2r = 0.31), BMI (H2r = 0.60) and pulmonary disease (H2r = 0.91). The heritable phenotype preeclampsia overlapped with SGA ($P = 0.03$), whereas pulmonary disease was phenotypically correlated with atherothrombotic cardiovascular disease ($P < 0.01$), SGA ($P = 0.02$) and BMI ($P = 0.02$).

Conclusion: This is the first study identifying the H2r of a range of health-related conditions in preeclamptic families. Our study demonstrates how refinement of phenotypes leads to better H2r estimation and the identification of a biological relationship between preeclampsia and related traits.

Keywords: biobank, family cohort, heritability estimate, phenotypes, phenotypic correlation, phenotyping, preeclampsia

Abbreviations: aCVD, atherothrombotic cardiovascular disease; DM2, diabetes mellitus type 2; H2r, heritability; HELLP, gestational syndrome incorporating haemolysis, elevated liver enzymes and low platelets; IUGR, intrauterine growth retardation; MBRN, the Medical Birth Registry of Norway; SGA, small for gestational age

INTRODUCTION

During the last decade, new clinical and pathophysiological insights have revealed that a diagnosis defined as one clinical disease entity in reality may encompass a spectrum of related conditions with deviating pathogenic mechanisms. Accordingly, a need for higher phenotypic resolution within the field of translational and genetic research has emerged to improve the screening, diagnosis and prediction of complex disorders such as preeclampsia and cardiovascular diseases [1,2]. Classification of phenotypes of common diseases with overlapping biologic mechanisms could further help identify shared genetic and pathophysiologic causes and phenotype-specific therapeutic targets.

Preeclampsia is a major cause of morbidity and mortality of pregnant women and fetuses with 2–8% of all pregnancies affected [3]. There are neither reliable predictive tests nor effective therapies other than delivery. Preeclampsia manifests as elevated maternal blood pressure with proteinuria in the latter half of pregnancy and presents with a range of clinical symptoms and signs, including

Journal of Hypertension 2015, 33:2294–2302

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Received 23 December 2014 **Revised** 10 June 2015 **Accepted** 10 June 2015

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DOI:10.1097/HJH.0000000000000696

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hypertension and proteinuria diagnosed a few days before spontaneous delivery without maternal discomfort or detectable influence on the fetus' well-being, as well as maternal multiorgan failure with fetal demise [4]. In a disease so diverse, it is likely that different pathophysiologic changes underlie the various phenotypic presentations. To identify functional changes behind specific disease presentations, it is necessary to carefully select the preeclampsia phenotype to examine. Any pregnancy will cause haemodynamic changes with physiologic increases in the cardiovascular load. Women who develop preeclampsia in response may be predisposed to react abnormally throughout life to increased burdens on their cardiovascular system. These individuals might have higher risk of developing atherosclerosis, consequently resulting in conditions encompassed by the term atherothrombotic cardiovascular diseases (aCVDs). Systematic research has revealed comorbidity of preeclampsia and aCVD, identifying women experiencing preeclampsia with a subsequent two to eight-fold increased risk of developing established risk factors or confirmed aCVD [5]. The research on preeclampsia and aCVD has progressed considerably in recent years [5], but the genetic contribution to the pathophysiology remains largely unexplained. Stricter phenotypic differentiation of complex diseases with several, possibly diverging, etiologic causes is required to gain knowledge on their biologic background and address possible underlying and interacting disease mechanisms. One way to further refine phenotypes is by utilization of available information aggregated in biobanks.

Biobanks are increasingly used in biomedical research as repositories of biologic specimens associated with collected information regarding health, environment and lifestyle of included individuals [6]. Family-based biobanks include families with increased prevalence of the condition of interest compared with the general population. Unaffected family members can be used as controls for the affected relatives as all share a common genetic background and at least partially comparable exposures to environmental factors [7].

Here we describe the Norwegian Preeclampsia Family Biobank and how we have classified individuals in the cohort according to 30 disease phenotypes. This study aims to identify the prevalence and heritability (H2r) of detailed preeclampsia phenotypes and related health conditions. Detailed phenotyping increases our knowledge on inheritance of specific traits and thereby improve the likelihood of identifying true genetic associations and biologic mechanisms defining a phenotype. To improve classification of diseases and identify possible overlapping disease-related phenotypes within the collected families, we evaluate the phenotypic correlation both between different phenotypes of preeclampsia and between preeclampsia and associated conditions including aCVD and fetal intrauterine growth retardation (IUGR).

METHODS

The Norwegian Preeclampsia Family Biobank

The Preeclampsia Family Biobank is a nested cohort of families with an increased susceptibility for preeclampsia, defined as pairs of either two sisters or a mother and her daughter wherein both individuals have had preeclampsia. The Preeclampsia Family Biobank was established during 2002–2012 to address the genetic basis of preeclampsia [8], and is based on collaboration between the Norwegian University of Science and Technology, Trondheim, Norway, and five hospitals in Mid and West Norway (Supplementary Figure 1, http://links.lww.com/HJH/A513). The population in these regions is ethnically uniform, as only 6.9% is of non-white origin, and migration numbers are low (Statistics Norway, 2012–2013).

For an illustration of the selection of participants, see Supplementary Figure 2 (http://links.lww.com/HJH/A513). The Medical Birth Registry of Norway contains information on all pregnancies and deliveries in Norway since 1967. Women who through their unique identification numbers were found to have a first-degree female relative where both gave birth at one of the participating hospitals between 1967 and 2005 and had the diagnosis 'preeclampsia' in the Medical Birth Registry of Norway were defined as having a familial predisposition. Medical doctors examined hospital records to verify the preeclampsia diagnosis of the 1161 identified preeclamptic pregnancies of 1003 women against the currently used national criteria, reproducible hypertension with proteinuria [9,10]. Where only one woman in a pair fulfilled the criteria, or where one woman of a pair had died or moved to an unknown address, both women of the pair were excluded from the study.

The 426 women where both women of a pair had valid preeclampsia diagnoses and where both were alive and registered with a postal address in Norway at the time of the study did fulfil the study criteria and were invited to participate in the study and extend the invitation to all their relatives above 17 years of age.

During the period 2009–2011, a standardized interview of all 496 included participants was performed and blood samples were collected. For each family, a summary pedigree was constructed in Cyrillic 2.1.3 (CyrillicSoftware, Oxfordshire, UK). The assembled information (Supplementary Table 1, http://links.lww.com/HJH/A513) was deidentified and quality controlled at Haukeland University Hospital and stored at the Western Norway Regional Health Authority database. Blood samples were collected in serum separator, EDTA and Tempus blood RNA stabilizing tubes (Applied Biosystems, Foster City, California, USA) from 98% of the participants. All blood subsets were stabilized and stored at facilities of the HUNT Research Centre and Biobank, Norwegian University of Science and Technology.

The Preeclampsia Family Biobank establishment and estimation of phenotypic heritabilities have obtained the required approvals from the Regional Committees for Medical Research Ethics, the National Data Inspectorate and the Preeclampsia Family Biobank. All participants gave their informed consent when enrolled.

Definitions of phenotypes

Preeclampsia has been defined according to international criteria as hypertension (blood pressure $\geq$ 140/90) with proteinuria ($\geq$+1 of protein on a urine dip-stick or $\geq$0.3 g protein in a 24-h urine collection) measured on at least two separate occasions [4,8,11]. As illustrated in Table 1,

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TABLE 1. Definitions of phenotypes in the Norwegian Preeclampsia Family Biobank

Disease phenotype	Subgroup	Included diagnostic traits
Preeclampsia	Moderate	HT with proteinuria
		Diagnosed ≥gestation 34 weeks + 0 days
	Severe	BP ≥160/110 with proteinuria
		HT with proteinuria ≥3 g/24 h
		Diagnosed <gestation 34 weeks + 0 days
		Symptoms, HELLP, eclampsia
Gestational HT		BP ≥ 140/90
SGA offspring		<5 percentile
		Anamnestic: slow fetal growth/very small child
aCVD[a]	Risk factors	Chronic HT
		Hypercholesterolemia
	Established disease	Coronary heart disease: angina pectoris, acute myocardial infarction
		Cerebrovascular disease: ischaemic/haemorrhagic stroke
		Other peripheral arterial disease: claudicatio intermittens, thrombosis of extremities
Diabetes[a]	Type 1	Insulin demanding
		Childhood or teenage onset
	Type 2	Diet only, oral antidiabetic medication alone or in combination with insulin. Onset >age 20
	Gestational	Diabetes developed and diagnosed during pregnancy
Autoimmune/Inflammatory disease		Hypothyroidism
		Systemic lupus erythematosus, other specified rheumatic disease, psoriasis
		Inflammatory bowel disease
Kidney disease	Kidney disease	Consistent microhaematuria/macrohaematuria, consistent albuminuria
		Glomerulonephritis, nephrosis
		Kidney transplant, need of dialysis
Pulmonary disease	Asthma	
	COPD	

aCVD, atherothrombotic cardiovascular disease; BP, blood pressure; COPD, chronic obstructive pulmonary disease; HELLP, gestational syndrome incorporating haemolysis, elevated liver enzymes and low platelets; HT, hypertension; SGA, small for gestational age.
[a]Intervention (diet or medication) required.

preeclampsia was examined as a binary trait, presence or absence of the condition (phenotype 'preeclampsia'). Preeclampsia was also classified into moderate or severe preeclampsia according to international guidelines, and we further created a probable high-severity phenotype including eclampsia (seizures); gestational syndrome incorporating haemolysis, elevated liver enzymes and low platelets (HELLP; multiorgan failure); and predefined symptoms. Preeclampsia diagnosed before gestational week 34 was defined as early preeclampsia and grouped as severe, whereas preeclampsia diagnosed at later gestations was categorized as late preeclampsia and classified as moderate.

IUGR is a condition of inappropriately low fetal growth estimated by transabdominal ultrasound. In lack of serial ultrasound measurements in this study, small for gestational age (SGA) was used as proxy for IUGR. The birth weight centile for each neonate was computed using common Norwegian unisex reference ranges established for fetal growth according to the week of pregnancy [12]. For the estimated due date, we used the date set either according to an ultrasound scan done before 20 weeks of gestation or, when lacking, based on last menstrual period. Gestational week at birth was calculated based on estimated due date. Neonates were classified as SGA if their birth weight was under the 5th centile estimated for the week of gestation when the birth took place.

To be classified with a disease phenotype, the participant must have stated that he or she had received relevant treatment by a medical doctor for the disorder. We separated each disease modality into several main phenotypes and phenotypic subgroups as defined in Table 1. The conditions included in the term aCVD were divided into treated risk factors for aCVD and established (meaning current or previously treated) aCVD. Established aCVD included coronary heart disease, cerebrovascular disease and other peripheral arterial disease. Diabetes was phenotyped into diabetes mellitus type 1, type 2 (DM2) and gestational diabetes. The registered inflammatory diseases included hypothyroidism, systemic lupus erythematosus, rheumatic disease, psoriasis, inflammatory bowel disease and the category 'other specified inflammatory diseases'. Kidney diseases included glomerulonephritis, nephrosis, consistent microhaematuria or macrohaematuria, need of dialysis, consistent albuminuria and kidney transplantation. Pulmonary diseases encompassed asthma and chronic obstructive pulmonary disease.

The severity scores for preeclampsia and aCVD are constructed scores (Supplementary Table 2, http://links.lww.com/HJH/A513), consisting of grouped variables with scores between 0 and 5 and therefore analyzed as quantitative phenotypes. To estimate severity of preeclampsia for each parous woman, all preeclampsia phenotypes were assigned a value of zero (not present), one (gestational hypertension), two (moderate disease), four (severe disease) or five (very severe disease; symptoms of preeclampsia, multiorgan failure incorporated by the HELLP syndrome or eclampsia) [11,13]. All women were designated a score of disease severity correlating to their sum of the given values, multiplied with her affected births and then divided by her nonaffected births. To score the severity of aCVD for each individual, we designated each aCVD phenotype with a value of zero (absence of phenotype), one (aCVD risk factors) or two (established aCVD). For each participant, an aCVD

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severity score was calculated by adding the values for each aCVD phenotype.

Heritability

Differences in phenotypic presentation between individuals could be caused by variation in genotype or environment, or combinations of these. The general term 'heritability' describes to which amount genes influence the expression of a phenotype, and H2r estimates the relative contribution of genetic factors to the expression of a phenotype in a specific population, not if a trait is genetic. H2r is an estimate of genotypic and phenotypic variance within a population, and not an estimate calculated for individuals.

The correlation between two phenotypes (ρP) is presumed to reflect an association between the two traits and indicate functional relationship. Both genetic and environmental effects are included in phenotypic correlation estimates [14].

Statistical analysis

The computer program SOLAR version 7.2.0 was employed to estimate and test the significance of heritabilities for quantitative phenotypes and disease endpoints using the relevant polygenic commands [15]. Covariates included were age and sex of the participant, and the age–sex interactions along with a weighting factor calculated according to preeclampsia status and assigned each individual to correct for the ascertainment bias created by the inclusion criteria of the cohort. Owing to the potential effects of kurtosis in variance components models, the quantitative phenotypes with high residual kurtosis were normalized using an inverse Gaussian transformation. Analysis of disease endpoints as discrete binary traits was performed using a liability threshold model in SOLAR. This model employs probit-regression for the mean effect component and a standard random effects variance component model for the residual additive genetic component of variance [14,15].

The phenotypes exhibiting significant H2r were cross-examined in pairs for phenotypic correlations, using a likelihood-ratio test that explicitly allows for nonindependence among related individuals [14,15]. The covariates established as significant in the H2r estimation of a specific trait were included in all further calculations involving that phenotype.

RESULTS

Descriptive characteristics

The Preeclampsia Family Biobank includes 138 pedigrees with a total of 496 individuals (71.4% women). The cohort contains 102 pairs of women (30 mother–daughter and 72 sister–sister pairs) with verified diagnoses, and in addition 35 women who fulfil the criteria of inclusion but where the affected first-degree relative was unwilling or unable to participate [8,10]. The families of these 35 women were incorporated in the H2r analyses if any other close family members participated, such as partners or female relatives with healthy pregnancies or without verified preeclampsia diagnosis. Descriptive characteristics of the cohort (Supplementary Table 3, http://links.lww.com/HJH/A513) include information on women who have given birth at least once (parous) or never (nulliparous), and according to preeclampsia status where relevant. As the participants in the cohort are related, subgroups have not been statistically compared according to sex, parity or preeclampsia status. The mean age at inclusion was 46.8 (standard deviation 13.4) years for the whole cohort, whereas for nulliparous women ($n = 14$), the mean age was 25.7 (standard deviation 9.5) years. Mean BMI was 27.1 for all participants. For men, parous women and nulliparous women, mean waist circumference was 98.4, 90.7 and 84.5 cm, respectively. Among parous women, the median number of births was 3.0, ranging from one to eight births, and the median number of preeclamptic pregnancies were 1.5 (span 0–5).

The 263 women recorded with preeclampsia include the women primarily invited to the study who had had their preeclampsia diagnosis verified through medical records, as well as female relatives reporting in interviews to have experienced the disease. Of the 263 preeclamptic women, 81% had the disease in their first pregnancy (Supplementary Table 4, http://links.lww.com/HJH/A513). In 50% of women with an affected first pregnancy, preeclampsia did not recur in another pregnancy, whereas 27% experienced preeclampsia in all their pregnancies (data not shown). The preeclamptic women in the cohort with severe disease phenotype experienced severe preeclampsia in the form of early preeclampsia (26.2%), preeclampsia with severe hypertension (36.1%) and preeclampsia with severe proteinuria (3.8%). Approximately 36% of preeclamptic women and 22.4% of women with pregnancies defined as nonpreeclamptic gave birth to a SGA neonate (Supplementary Table 4, http://links.lww.com/HJH/A513).

Of the relevant disease phenotypes examined, the percentages for chronic hypertension and for aCVD were fairly consistent across all groups except for the nulliparous women, wherein only few individuals were affected (Supplementary Table 5, http://links.lww.com/HJH/A513). The overall prevalence of DM2, the aCVD phenotypes coronary heart disease (myocardial infarction or unspecified angina pectoris) and cerebrovascular disease (ischaemic or haemorrhagic stroke) were 5.8, 4.5, and 2.3%, respectively, with a higher proportion of males affected. Pulmonary diseases were relatively evenly presented in all subgroups, ranging from 12.8 (men) to 15.5% (parous women without preeclampsia). Autoimmune diseases were present in 12.1% of the total cohort and in 13.3% of the participating women.

Heritability

Table 2 presents the selected phenotypes of preeclampsia and relevant diseases with significant H2r estimates. For the complete list of traits examined, see Supplementary Table 6 (http://links.lww.com/HJH/A513). Preeclampsia showed an overall H2r of 0.60 ($P = 0.03$). The estimated genetic effects on phenotypic variance ranged from 15.1% (disease severity of preeclampsia, $P = 0.03$) to 91.0% (pulmonary disease, $P = 0.0002$). The increased susceptibility of preeclampsia in the cohort was supported by significant H2r

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TABLE 2. Phenotypic traits with significant heritability

Phenotype	H2r	*P* value	SE
Preeclampsia	0.600	0.033*	0.309
Total number of births[a]	0.195	0.029*	0.112
Given birth to SGA neonate	0.404	0.012*	0.290
Diabetes mellitus type 2	0.568	0.030*	0.309
Severity of preeclampsia[a]	0.151	0.034*	0.088
Chronic hypertension	0.569	0.0061**	0.235
Severity of aCVD	0.314	0.0046**	0.126
Born in a preeclamptic pregnancy	0.252	0.011*	0.117
Pulmonary disease	0.909	0.0002***	0.232
BMI*	0.603	5.96×10^{-10}***	0.096
Waist circumference[a]	0.575	1.66×10^{-8}***	0.098

aCVD, atherothrombotic cardiovascular disease; SE, standard error; SGA, small for gestational age.
[a]The quantitative phenotype has been inverse normalized.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

estimates of preeclampsia, disease severity of preeclampsia and having been born in a preeclamptic pregnancy (H2r 0.25, $P = 0.01$). Several other assessed phenotypes were shown to be heritable in a variance component model including the predefined covariates (Table 2): chronic hypertension (H2r 0.57, $P = 0.006$), giving birth to neonates SGA (H2r 0.40, $P = 0.01$), total number of births (H2r 0.20, $P = 0.03$) and DM2 (H2r 0.57, $P = 0.03$). Some phenotypes had high residual kurtosis reflecting that a larger amount of the variability in the cohort was caused by few extreme differences from the mean. After normalization of the trait distribution in the cohort, the following phenotypes also had significant H2r estimates and normal residuals: disease severity of preeclampsia, BMI (H2r 0.60, $P = 6.0 \times 10^{-10}$), severity of aCVD (H2r 0.31, $P = 0.005$) and waist circumference (H2r 0.58, $P = 1.7 \times 10^{-8}$). For the other phenotypes examined (Supplementary Table 6, http://links.lww.com/HJH/A513), there were either no significant H2r identified or the residual kurtosis was too high to be corrected in the analyses.

Phenotypic correlations

Paired phenotypes with significant phenotypic correlations (ρP) are presented in Table 3. Possible pleiotropy was demonstrated by the positive phenotypic correlations between preeclampsia status and how many times a woman gave birth (19.5%, $P < 0.01$), as well as between preeclampsia and giving birth to a SGA child (26.2%, $P = 0.03$). BMI exhibited positive correlations to chronic hypertension (26.9%, $P < 0.01$), severity of aCVD (20.5%, $P < 0.01$), DM2 (27.3%, $P < 0.01$), waist circumference (57.5%, $P < 0.01$) and pulmonary disease (17.2%, $P = 0.02$). Development of pulmonary disease was positively correlated to the severity of aCVD (18.8%, $P < 0.01$) and negatively correlated with giving birth to children SGA (25.4%, $P = 0.02$). No significant phenotypic correlations were identified for the other phenotypes examined (data not given).

DISCUSSION

This is a phenotypic description of the Preeclampsia Family Biobank, outlining the phenotypic criteria utilized and how

TABLE 3. Phenotypic correlations (ρP) between heritable traits, *P* values given in parentheses

		Phenotype B								
		Preeclampsia	SGA	Diabetes mellitus type 2	Chronic hypertension	aCVD	Pulmonary disease	Waist circumference	BMI	Total no. of births
Phenotype A	Preeclampsia									
	SGA	0.262 (0.03)*								
	Diabetes mellitus type 2	0.250 (0.23)	−0.179 (0.23)							
	Chronic hypertension	0.199 (0.14)	0.00079 (0.99)	0.345 (4.7e-3)*						
	aCVD	0.051 (0.56)	−0.019 (0.78)	0.375 (1.3e-5)*						
	Pulmonary disease	−0.074 (0.60)	−0.254 (0.02)*	0.249 (0.08)	0.130 (0.21)	0.188 (9.1e-3)*				
	Waist circumference	0.172 (0.12)	0.038 (0.60)	0.093 (0.36)	0.251 (1.2e-4)*	0.207 (1.3e-5)*	0.110 (0.14)			
	BMI	0.157 (0.06)	0.015 (0.82)	0.273 (9.7e-3)*	0.269 (1.6e-5)*	0.205 (1.1e-5)*	0.172 (0.02)*	0.575 (2.6e-37)*		
	Total no. of births	0.195 (8.4e-3)*	0.295 (0.09)	−0.025 (0.79)	−0.034 (0.60)	−0.033 (0.49)	0.007 (0.92)	0.055 (0.24)	−0.068 (0.1)	

aCVD, atherothrombotic cardiovascular disease; ρP, phenotypic correlation; SGA, small for gestational age.
*Significantly correlated phenotypes.

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several examined traits are heritable and phenotypically correlated. To our knowledge, this is the first family study to examine the presence and H2r of defined phenotypes of preeclampsia and combine these with conditions such as aCVD, diabetes and pulmonary disease. The study demonstrates that preeclampsia, chronic hypertension, aCVD, DM2, SGA and pulmonary disease are heritable in the cohort and identifies phenotypic correlations between these heritable traits.

The purpose of the Preeclampsia Family Biobank was to establish a resource for investigation of the genetic background of preeclampsia [8]. Careful definition and selection of phenotypes for genetic studies can improve identification of noneligible subsets, include less heterogeneous phenotypes and select suitable controls. The family study design was chosen to minimize the effects of population heterogeneity, promote discovery of parent-of-origin effects and identification of quantitative trait loci, and estimate the H2r of disease-specific traits [7]. Of other identified family-based cohorts based on preeclampsia, five studies are corresponding most closely to ours [16–20]. These five studies all focus on the genetics of preeclampsia but neither examined other disease phenotypes nor investigated the preeclampsia phenotype in detail except for inclusion of HELLP in the Dutch study [19]. The results of our study support presence of a genetic cause of preeclampsia and the theories of how different complex diseases are influenced by partly shared inherited genetic pathways.

A relation between preeclampsia and aCVD was described as early as in 1927 [21] and since then an increasing number of studies have identified women with preeclamptic pregnancies to have an enhanced risk of subsequently developing aCVD, hypertension and DM2 [5,22,23]. Our results confirm the association between preeclampsia and aCVD. Familial clustering of aCVD and aCVD risk factors, including DM2, and H2r of delivering SGA neonates and pulmonary disease in families have been indicated repeatedly [24–27]. The novel discovery in our study is that hypertension, aCVD, DM2, giving birth to children SGA and pulmonary disease are all heritable in a family cohort based on a different disease, preeclampsia. To our knowledge, this is further the first study including both female and male relatives in the H2r estimates for preeclampsia and delivery of SGA neonates. Obtaining a more comprehensive paternal family information enable us to include the male contribution to development of the conditions in the investigation of trait H2r.

A normal pregnancy augments cardiovascular demands and an increased activation of the immune system. If a woman has a preeclamptic pregnancy, the stress-test theory hypothesizes that she is inclined to react abnormally to increased metabolic and cardiovascular stress. Consequently, preeclampsia indicates that she will be susceptible to developing aCVD in response to metabolic and cardiovascular changes later in life [28]. The identification of preeclampsia, chronic hypertension, aCVD and DM2 as heritable and with a partly overlapping H2r in the cohort supports the stress-test hypothesis. The lack of significant results concerning other aCVD traits examined may in part be due to participant numbers and interfamily variations. The low mean age of the cohort could be another explanation why several aCVD phenotypes did not seem heritable. The prevalence of chronic hypertension (26.8%), aCVD (35.3%) and DM2 (5.8%) in the cohort is consistent with general prevalence estimates in the adult population in developed countries [29–31]. Despite compatible numbers, this might not represent the true prevalence of aCVD in the families. The younger participants from the second and third generations may not have developed established aCVD, conditions diagnosed more prevalently with increasing age [31,32], but rather display the risk factors for the disorder.

An association between preeclampsia and giving birth to neonates SGA has been identified through pathophysiological and epidemiological research [33–35]. This is the first report presenting H2r estimates of SGA and a positive phenotypic correlation between preeclampsia and SGA in a family-based study. In the cohort, the SGA phenotype is not restricted to the preeclamptic women as women without preeclampsia also present with a much higher proportion of small neonates (22.4%) than the 2.0–11.8% expected from population studies [33,36]. We believe the phenotypic clustering of SGA in all the parous women to indicate SGA as an important trait to consider in future studies. Although we do observe a trend indicating an inverse phenotypic correlation between height and giving birth to SGA children (data not shown), the mean height of women, both with and without preeclampsia, was in accordance with the mean age-related height for Norwegian women (Statistics Norway 2012–2013) [37]. The 26.2% positive correlation identified between presence of preeclampsia and SGA is comparable with estimates from two large birth cohorts [33,35]. Earlier studies have identified 27–57% of pregnancies with early onset of preeclampsia (<gestational week 34) and 18–18.3% of late onset (>gestational week 34) to be associated with birth of a neonate SGA. Unlike these reports, we could not identify any phenotypic correlation between SGA and different subgroups of the preeclampsia phenotype. Too few individuals with any preeclampsia subtype combined with large interfamilial variation in phenotype presentation in our cohort may partly explain the differences.

For the very first time, pulmonary diseases are identified as heritable in a cohort based on preeclampsia. The genetic background of conditions included in the phenotype pulmonary disease is illustrated by their accumulation in families [27,38]. Although the H2r estimates in this study are noticeably high (90.9%), they are comparable to heritabilities of 53–92% reported for studies on asthma [38]. The overlap in phenotypic H2r estimates found for pulmonary diseases and aCVD, DM2, BMI and waist circumference are in accordance with previously demonstrated phenotypic associations [39,40]. Several previous studies have identified an increased risk in asthmatic patients both for the development of preeclampsia and for having SGA children, with higher risk estimates for women with severe asthma [41,42]. Contrary to this, our results show a negative correlation between pulmonary diseases and giving birth to neonates SGA. It is possible that the genes that influence expression of the corresponding traits participate in the same genetic pathway and tend to be inherited together.

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We hypothesize that the causative pathophysiologic mechanisms and expression of each phenotype may be influenced by different genes of the same pathway.

There are several strengths to this study. The multigenerational family cohort is selected from a general population covering all potential candidates from four out of 20 Norwegian counties, and based on a nationwide compulsory birth registry. Linking of unique identification numbers to identify the female family relations ensure the biologic relationship of the invited participants. As the preeclamptic women all have validated preeclampsia diagnosis and we have information from medical records as well as from interviews of participants, the likelihood of including true-positive cases in the cohort is increased. In a preeclampsia research setting, we have established a relatively large biobank that contains more affected families and which we believe is better characterized than other family cohorts of this type. Through the combination of genetic homogeneity, strictly defined phenotypes and examination of preeclampsia, risk factors and related conditions during pregnancy and later in life, the Preeclampsia Family Biobank constitutes a unique setting for examination of genetic H2r of diseases and for correlation between the defined phenotypes.

One of the main weaknesses of this study is that despite being the second largest study of its type, the cohort size is still relatively small. The size of the study, with further reduction of sample numbers in the phenotypic subsets, could be a reason why this study did not identify a genetically heritable component of several preeclampsia phenotypes. Furthermore, the study size could cause a disproportionally increased effect on the estimates of H2r as each family and participant may have a relatively high impact on the result. Although populations may differ in which genetic changes underlie the disease, the identified H2r of preeclampsia in our study is comparable to results from twin studies [43], which supports our estimates. As H2r is largely a component of the population being studied, presumably a larger sample size could lead to reduced standard errors for the examined phenotypes and thereby more robust results. Part of the collected information is based on interviews and self-reported data, a research setting associated with decreased diagnostic validity and recall bias. We have required information on relevant medical treatment instituted by a physician for validation and use of the given diagnoses in our analysis. One potential bias in the study with regard to the potential H2r of preeclampsia was introduced as the families included in the study were assumed to be enriched for preeclampsia. This bias has been corrected for by including a calculated weighting for preeclampsia in all analyses.

Novel in this study is the identification of overlapping H2r for several phenotypes in a family cohort. The conditions examined, preeclampsia, SGA, aCVD and pulmonary disease, share risk factors, and studies on the separate conditions have demonstrated that presence of the disorder of interest increases the risk of developing another of the diseases [23,24,40,44,45]. Furthermore, pathologic mechanisms like endothelial dysfunction and inflammation influence disease development of all the disorders [34,46,47]. The overlap in phenotypic H2r discovered in this study indicates shared biological processes for all of these traits.

This study demonstrates a thorough phenotyping of a family-based preeclampsia cohort with estimation of heritable phenotypes. The study supports a genetic cause of preeclampsia. For the first time, phenotypic H2r of preeclampsia-related traits has been estimated in preeclamptic families, identifying several heritable traits including SGA, aCVD, chronic hypertension and pulmonary disease. The phenotypic correlations identified indicate that pathophysiologic mechanisms shared by the examined phenotypes could be in part caused by identical or corresponding genetic pathways. The results constitute a solid basis for further research on causative genetic mechanisms of development of preeclampsia and shared pathophysiologic changes between preeclampsia and the related disease phenotypes.

ACKNOWLEDGEMENTS

Previous presentation of the work: Part of the content has been presented in abstracts at international and Norwegian congresses.

Sources of funding: This research was funded by grants from the Norwegian Women's Public Health Association and the Norwegian Extra Foundation for Health and Rehabilitation through EXTRA funds (2010/2/0252), the Norwegian Association of Heart and Lung Patients, the Nordic Federation of Societies of Obstetrics and Gynecology, Sigurd K. Thoresen's Foundation, the Norwegian Research Council (FUGE II and 205400/V50) and the Regional Health Authorities. This work was partly supported by the Research Council of Norway through its Centres of Excellence funding scheme, project number 223255/F50. Statistical genetic analyses were performed on the advanced computing resources provided by the Western Australian Advanced Computing Consortia (iVEC).

Conflicts of interest

There are no conflicts of interest.

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Reviewer's Summary Evaluation

Reviewer 2

This report originates from the Norwegian Preeclampsia Family Biobank, and describes some early phenotypic data in respect of a cohort of 496 individuals identified as having a familial pattern of preeclampsia (PE) from the Norwegian Birth registry between 1967 and 2005. The main conclusions are that in this cohort, PE, chronic hypertension, atherosclerotic cardiovascular diseases, type 2 diabetes, the delivery of small for dates infants, and pulmonary disease are heritable and that there are significant correlations between these traits. The strengths of this study lie principally in the careful and detailed phenotyping of PE. This is not an easily defined

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entity, but a complex condition with protean manifestations and a dynamic mode of presentation. Here the authors attempt to characterise the condition in its various manifestations and to explore associations between these phenotypes, the extent to which these are heritable, and links with a number of other phenotypes of birth circumstances and subsequent adult diseases. The main new findings in their study are the heritability of pulmonary disease (asthma and COPD) in this cohort based on selection by PE, and the overlapping heritability of several other of the phenotypes identified. The main weakness, so common in many studies in obstetrics, is the relatively small number of patients included. The observations may also be relevant only to a relatively homogeneous Norwegian cohort.

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