The angiotensin-converting enzyme gene insertion–deletion polymorphism in a white British patient cohort with obstetric cholestasis

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Summary: The DD genotype of the angiotensin-converting enzyme (ACE) gene is over-represented in Finnish patients with obstetric cholestasis (OC). The purpose of this study was to establish whether this genotype is associated with cholestasis in UK cases. In a retrospective case-control study, we determined the ACE insertion/deletion frequencies in 166 British cases and 100 control women by polymerase chain reaction analysis. No significant difference in allele frequencies was found between these groups, but allele frequencies differed significantly between Finnish and UK OC cases ($P = 0.0005$). The prevalence of the DD genotype is lower in UK cases than in controls (χ^2 [1 d.f.] = 4.32, $P = 0.05$) and the odds ratio for OC associated with the DD genotype is 0.54, 95% confidence interval 0.30–0.97. In contrast to Finnish OC cases, the DD genotype of the ACE is not increased in UK cases.

Keywords: angiotensin-converting enzyme, gene polymorphism, obstetric cholestasis

INTRODUCTION

The gene for the angiotensin-converting enzyme (ACE) contains an *Alu*-element insertion–deletion (ACE I/D) polymorphism in intron 16 which is tightly associated with the enzyme level in plasma.¹ High concentration and activity of ACE is associated with hypertension, and carriers of the homozygous deletion (DD) genotype are at increased risk of various associated problems such as ventricular hypertrophy, diabetic nephropathy and vascular disorders.^{2–5}

Studies looking at the effect of ACE I/D genotypes on pregnancy-induced hypertension, preeclampsia and changes of blood pressure during pregnancy found no relationship between the DD genotype and these complications.^{6,7} Owing to the effect of the I/D polymorphism on the function of ACE, it can still be considered as a potential contributing factor in obstetric cholestasis (OC), also known as intrahepatic cholestasis of pregnancy. OC is a complex disorder in which genetically predisposed women^{8–10} have an enhanced susceptibility to the cholestatic effect of pregnancy. Raised levels of oestrogen and progesterone in pregnancy are believed to relate to disease aetiology.^{11–13} ACE influences the indirect

regulation of smooth muscle actin expression through transforming growth factor- β_1 , and oestrogen-induced changes of actin microfilaments have been postulated to cause OC.^{14,15} A study of 57 Finnish patients with OC revealed an increased prevalence of the DD genotype when compared with a control group of 115 healthy pregnancies.¹⁶

To examine whether the DD genotype was present more frequently within a population of 166 white British OC patients, we determined the ACE I/D genotypes of these patients and an ethnically matched group of 100 healthy pregnant control individuals.

MATERIALS AND METHODS

Patients

White British women with OC were identified either prospectively by the clinicians responsible for their care or retrospectively and retrospectively by the UK Obstetric Cholestasis Patient Organisation. The diagnostic criteria for OC were met if women had persistent pruritus and biochemical confirmation of the diagnosis in the absence of other known liver disease, including the haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, acute fatty liver of pregnancy, primary biliary cirrhosis and ultrasound abnormality that could result in biliary obstruction, as in previous studies.¹⁷ Women were excluded from the analysis if the hepatic

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impairment did not resolve postnatally, with the exception of cyclical and exogenous oestrogen-induced cholestasis. Women with dermatoses of pregnancy that can result in pruritus were also excluded. Abnormal liver function was defined as one or more of raised serum bile acids and/or raised serum transaminases (alanine amino-transferase, aspartate amino-transferase or gamma-glutamyl transpeptidase) in at least one pregnancy. As hospitals have different normal ranges for liver transaminase levels, the upper end of the normal range in pregnancy was assumed to be 80% of the level quoted outside pregnancy for each hospital, consistent with published studies and any values above this were considered to be abnormal.¹⁸ The normal level for bile acids was $<14 \mu\text{mol/L}$. Serum bile acid results were available in 53% of cases. Women known to have hepatitis C infection were excluded from the study. One hundred and sixty-six women with OC were identified. The control group consisted of DNA extracted from blood specimens taken from 100 parous women who had no history of cholestasis or any other cause of hepatic impairment in pregnancy. These controls were recruited from the postnatal wards at Queen Charlotte's Hospital, and their ethnicity was classified as white British based on self-assessment of themselves and their parents. This study has ethical approval from the Hammersmith and Queen Charlotte's Hospital Trust Ethics Committee (REC 97/5197), and informed consent was obtained from all subjects.

Methods

DNA was extracted from white blood cells using the PureGene kit (Gentra Systems, Minneapolis, MN, USA) and subjected to polymerase chain reaction amplification using standard methodology. To minimize the possibility of an increased presence of the 'D' allele due to genotyping errors, each sample was screened in duplicate.¹⁹ The first method used a two oligonucleotide-based system that spans the insertion.²⁰ The second method used a three-oligonucleotide system that includes a common downstream primer and two forward primers, one upstream of the insertion/deletion polymorphism, and one within the insertion.⁶ In both cases, extension times were increased beyond the necessary period to allow the polymerase ample time to complete the amplification across the insertion. The allele frequencies of the parous controls used in this study were compared with those reported in two large UK studies. The London/Cardiff/Belfast control population frequencies were based on a combination of 386 subjects from the respective areas tested in another study.²¹ To exclude a local effect, these frequencies were compared with the result of a combined meta-analysis of 15 studies of ACE I/D frequencies in the UK and the risk of myocardial infarction

incorporating 5479 controls (UK meta-analysis controls).²² In addition, the allele frequencies were compared with those in parous Finnish women and those of the general population in Finland.¹⁶ They were also compared in women with and without OC from the UK and Finland.

Statistical analysis

Statistical analysis was performed using the chi-squared (χ^2) test for independence, and the results were defined as being statistically significant if $P < 0.05$. Statistical analysis was performed using SPSS 10.1 software.

RESULTS

Genotypes and allele frequencies of the ACE I/D polymorphism in the UK and Finnish populations are shown in Table 1. No significant differences in allele frequency were detected between the UK non-pregnant control groups, nor between the Finnish and UK non-pregnant control groups. Similarly, no differences were found between non-pregnant and pregnant controls in both the UK and Finnish populations, nor between the Finnish and UK pregnant populations. We also detected no significant differences in allele frequency between the UK OC cases and the UK pregnant controls (χ^2 [1 d.f.] = 3.12, $P = 0.08$). A significant difference in the ACE allele frequency was observed between the OC patients in the UK compared with those in Finland ($\chi^2 = 15.3$, $P < 0.001$).

In the UK population, the overall genotype distribution does not differ between cases and controls (χ^2 [2 d.f.] = 4.47, $P = 0.11$). The same is true for the Finnish population if subjected to an identical analysis (χ^2 [2 d.f.] = 4.96, $P = 0.084$).

However, the prevalence of the DD genotype in the Finnish OC cases is higher than the combined (ID + II) genotype frequency when compared with controls (χ^2 [1 d.f.] = 4.96, $P = 0.04$). In contrast, if the same comparison is applied to the UK population, there is a significant under-representation of the DD genotype (χ^2 [1 d.f.] = 4.32, $P = 0.05$ with Yates' continuity correction).

The odds ratio (OR) for OC associated with the DD genotype in the UK study is 0.54, 95% CI 0.30–0.97, compared with an OR in the Finnish population of 2.12, CI of 1.094–21.¹⁶

DISCUSSION

This study has shown that there is no significant difference in ACE allele frequencies between the UK OC patient group and any of the control groups used. Our results show no difference in the distribution of ACE alleles between the Finnish and the UK general and pregnant populations. The only significant

Table 1 Genotype and allele frequencies of the ACE insertion–deletion polymorphism in OC cases and controls from the UK and Finland

	UK meta-analysis controls (n = 5479)	UK LCB controls (n = 386)	Finnish controls (n = 515)	UK pregnant controls (n = 100)	Finnish pregnant controls (n = 115)	UK OC cases (n = 166)	Finnish OC cases (n = 57)
DD	28.3%	117 (30%)	28%	29 (29%)	31 (27%)	30 (18%)	25 (43.9%)
ID	49.0%	180 (47%)	51%	53 (53%)	58 (50.4%)	98 (59%)	22 (38.6%)
II	22.7%	89 (23%)	21%	18 (18%)	26 (22.6%)	38 (23%)	10 (17.5%)
D	52.8%	54%	54%	55%	52.2%	47.6%	63.2%
I	47.2%	46%	46%	45%	47.8%	52.4%	36.8%

OC= obstetric cholestasis

difference is found when comparing the genotypes of UK women with OC with those of Finnish OC cases. While UK OC cases display a tendency towards under-representation of the D allele, the Finnish OC cases show an increased prevalence of the D allele.

The prevalence of OC varies in different populations, with reported rates varying from 0.2% in France to 0.5–2% in Finland and 12% in Chile.^{23–26} It affects 0.7% of pregnancies in UK white caucasians and double this proportion of Indian and Pakistani Asians in the UK.²⁷

Populations of limited genetic variability may harbour founder mutations at the same or a nearby locus which predispose to the condition but are comparatively rare in other, more genetically heterogeneous populations. A founder mutation with epistatic effects may explain the difference in ACE I/D allele frequencies observed in the Finnish OC cases compared with the UK OC cases.

A study of 24 kb surrounding the deletion–insertion polymorphism found 78 variants that resolved into 13 distinct haplotypes, and 17 of the 78 variants were in linkage disequilibrium with the I/D polymorphism.²⁸ Thus, it is possible that one or more of these 17 variants, or an additional polymorphism could either cause or contribute epistatically to the effects that have been described as associated with the I/D genotype. A specific haplotype, which is in linkage disequilibrium with the D allele and has a cholestatic effect on pregnancies, could be overrepresented in the Finnish population due to a founder effect and thus explains the overrepresentation of the D-allele in Finnish OC cases.

Studies of the frequency of the ACE I/D insertion–deletion polymorphism in preeclampsia patients from different populations have reported an association in Chinese cases, but not in white preeclamptic women from the UK, Germany²⁹ or in those from Finland.^{6,16,30} As with OC, the different results in women from China, Finland, Germany and the UK may reflect ethnic variation in allele frequencies. Again, this phenomenon could also be due to the increased presence of an unfavourable haplotype associated with one of the I/D alleles.

A possible explanation for the conflicting results in the UK and Finnish OC populations is study power: the numbers in the Finnish study were adequate to detect a four-fold increase in risk associated with the D allele with 80% power; the UK study was powered to detect a 2- to 2.5-fold increase in risk. The relatively small sample size in each study is a likely reason for the conflicting results. It is important that future studies of the genetic aetiology of OC utilize larger DNA collections. If necessary this should be in the context of national and international consortia.

Another possible reason for the contradictory results is the use of the two-primer assay in the Finnish study: the excess of DD homozygotes is mainly at the expense of ID heterozygotes. This is the pattern that would be observed if there were preferential amplification of the D allele.

In summary, we do not find the increased prevalence of the DD genotype described in the Finnish population and our study of UK cases has shown a decreased prevalence of the deletion genotype.

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CONFLICTS OF INTEREST

No conflicts of interest are known.

CONTRIBUTORSHIP

R Mullenbach: Experimental execution and interpretation of data, manuscript authorship

N Tetlow: Interpretation of results, experimental expertise;

A Bennett: Experimental design, experimental expertise;

F Broughton Pipkin: Study design, manuscript revision;

L Morgan: Study design, statistical evaluation, manuscript revision;

C Williamson: Study design, patient recruitment, manuscript authorship, final approval of manuscript.

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