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Multivariate Genetic Analysis of Chronic Pelvic Pain and Associated Phenotypes

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Chronic pelvic pain (CPP) is a common condition in women that is difficult to diagnose. Although heritability estimates have been published for some conditions potentially underlying pelvic pain, the heritability of CPP itself has never been investigated. Using data from 623 MZ and 377 DZ female twin pairs aged 29–50 from an Australian twin cohort, we found an increased CPP concordance among MZs compared to DZs, with tetrachoric correlations of 0.43 (95% CI: 0.26–0.58) and 0.11 (95% CI: −0.16–0.38), respectively. This corresponded to a heritability of 0.41 (95% CI: 0.25–0.56). Lack of correlations with environmental indicators suggested that violation of the equal environments assumption was not responsible for this effect. Multivariate Cholesky decomposition models incorporating CPP and significantly correlated phenotypes showed that the entire CPP heritability could be explained by genetic variance underlying endometriosis (38%), dysmenorrhoea (23%), fibroids (24%), and somatic distress (15%), the latter a possible indicator of increased nociception. CPP itself is unlikely to be a useful independent phenotype to conduct genetic aetiological studies; contributing conditions such as endometriosis and variation in nociception are likely to provide more useful phenotypes.

KEY WORDS: Endometriosis; Fibroids; heritability; Pelvic Pain; Somatic distress.

INTRODUCTION

Chronic pelvic pain (CPP)—defined as recurrent or constant lower abdominal pain of at least six months' duration—is a very common condition. Estimates of prevalence rates during any three-month period among women of reproductive age have varied from 14.7% in the US (Mathias *et al.*, 1996) to 24.0% in the UK (Zondervan *et al.*, 2001b) and 25.4% in New Zealand (Grace *et al.*, in press) depending on inclusion of mid-cycle pain.

The CPP is difficult to diagnose because it can be related to many different conditions, such as endometriosis (endometrial-like deposits found in ectopic sites in the pelvis diagnosed through surgery), adhesions after pelvic inflammatory disease or surgery, uterine fibroids (benign growths of uterine muscle diagnosed through pelvic examination and ultra-sound), irritable bowel syndrome (abnormal bowel habit diagnosed by a set of standard criteria), and interstitial cystitis (a chronic inflammatory condition of the bladder wall) (Moore and Kennedy, 2000). In addition, for many women who undergo investigations for CPP no diagnosis can be found. Since symptoms of depression and anxiety are often found in women with CPP, reduced psychological well-being has also been regarded as a potential cause (rather than a result) of the persistent pain (Grace, 1998; Reiter, 1998).

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One way to learn more about the entity CPP and its possible underlying causes is to study its genetic characteristics in relation to those of correlated phenotypes. One such phenotype is endometriosis, a condition causing pelvic pain and infertility. It is commonly accepted that the aetiology of endometriosis has a genetic component (Kennedy *et al*., 1996; Simpson and Bischoff, 2003; Zondervan *et al*., 2001a). Family-based studies have shown a 3–9 times increased risk among female first-degree relatives of endometriosis cases *versus* controls (Coxhead and Thomas, 1993; Lamb *et al*., 1986; Moen and Magnus, 1993; Simpson *et al*., 1980). The heritability of endometriosis has been estimated at 51% in an Australian twin sample comprising 2694 women (Treloar *et al*., 1999b). Heritability estimates for uterine fibroids severe enough to require hospitalisation or hysterectomy have also been shown to be non-trivial, with respective estimates of 55% in a Finnish cohort of 13,872 female twins (Luoto *et al*., 2000), 64% in a sample of 5961 Australian twins (Treloar *et al*., 1998a), and 69% in UK cohort of 1256 female twins (Snieder *et al*., 1998).

Although heritability estimates have been published for some conditions that may explain pelvic pain in a subset of women, the heritability of CPP itself has never been investigated. We used data obtained from a large longitudinal study of Australian twin pairs to assess the heritability of CPP and conducted multivariate genetic analyses to examine the extent to which the genetic influences are attributable to other measured phenotypes, including the diagnoses of endometriosis and fibroids.

METHODS

Samples

The sample consisted of 2000 twin women aged 29–50 years—623 monozygotic (MZ) and 377 dizygotic (DZ) twin pairs—from a larger sample of 2862 twin women aged 29 and above who participated in the third wave of a longitudinal twin cohort study. The twin cohort was originally identified in 1980–1982 (first wave) from the Australian Health and Medical Research Council Twin Register and followed up subsequently in 1988–1990 (second wave) and 1993–1994 (third wave). Details about the study and the different follow-up waves have been published elsewhere (Treloar *et al*., 1998b, 1999a). In short, the questionnaire data collected in the first two waves focussed on aspects of twinning, demographics, life-style, health and allergies, personality characteristics, and reproductive factors. The third wave, focussing on gynaecological health, included information on gynaecological problems that might predispose to hysterectomy (e.g., endometriosis, fibroids), other surgical interventions, medical and hormone treatments, childbirth, menstrual problems and pelvic pain. Of the 1570 female twin pairs who were asked to participate in the third wave, a response was obtained from 1431 pairs (pairwise response rate: 91%); the sample for the present study consisted of the 1000 pairs who were aged 50 or younger at the time of the third wave. Women older than 50 were excluded since diagnostic patterns for potential conditions underlying CPP, such as endometriosis, were likely to differ dramatically in older women (laparoscopy only became routine in the 1970s), and because a differential recall of pelvic pain symptoms was likely. Median age at participation of this subset was 39 years.

Zygosity was determined through two items concerning similarity in appearance and being mistaken for the other twin. Pairs who gave inconsistent responses were recontacted for clarification. This method had been shown to give at least 95% agreement with diagnosis based on extensive blood typing (Martin, 1975); recent blood typing provided a sensitivity estimate of 0.98 (95% CI: 0.94–0.99) and a specificity of 1.00 (95% CI: 0.97–1.00) (Duffy, 1994).

Measures

The primary phenotype, CPP, was assessed by the questionnaire item *Have you had persistent or chronic (long-standing) pelvic pain?*, and if so: *did you have investigations or tests by a gynaecologist for this?* In addition to CPP, a number of other items potentially related to CPP were analysed. Women were asked if they had ever been diagnosed with endometriosis by a gynaecologist. Where possible, medical confirmation of the diagnosis was obtained (Treloar *et al*., 1999b). Diagnosis of uterine fibroids by a gynaecologist was also based on self-report by the woman. Medical confirmation of this comparatively less equivocal diagnosis could be obtained for 33.3% of the women, since they had had a hysterectomy with fibroids as the main reason, or uterine pathology at surgery showed fibroid tissue (leiomyoma).

Menstrual problems were assessed in the second and the third waves. Although data on dysmenorrhoea (pain during periods) were also collected in the first wave (Silberg *et al*., 1990; Treloar *et al*., 1998b), we used the more recent data in the present analyses. In the second wave questionnaire, women were asked whether periods were heavy, moderate or light, and whether they were very painful, moderately painful or no trouble. In the third wave, women were asked whether they had ever consulted for period problems, and if so, whether this was for painful, heavy, irregular, or unusual periods. In both waves, information about hysterectomy and menopause was collected. Using these data, the symptom variables ''dysmenorrhoea'' and ''menstrual flow'' were constructed with four categories of severity: (1) *Severe* (having consulted a doctor for the symptom and having reported either that the symptom was severe or a hysterectomy); (2) *''Moderate I''*(having consulted for and reported symptom of moderate severity); (3) *Moderate II* (having consulted but reported mild or no symptom, or not having consulted but reported moderate/severe symptom); and (4) *Mild/none* (never consulted for symptom and reported that symptom was either mild or not present. The third menstrual variable used in the analyses was having consulted a doctor for otherwise ''unusual or irregular periods''.

Personality characteristics were taken from the second wave survey which included 14 items from the Delusion States Inventory (DSSI/sAD) (Bedford *et al*., 1976) and a 19 item subset from the Symptom Check List (SCL) (Derogatis *et al*., 1976) personality scales. From responses to these items, four factors were generated:somatic distress/anxiety; cognitive distress/anxiety; depression, and panic/agoraphobia (Gillespie *et al*., 1999). Additional characteristics assessed were neuroticism and extraversion from the Eysenck short form EPQ-R(S) (Eysenck *et al*., 1985), self-esteem (Rosenberg, 1965), perceived control (Pearlin and Schooler, 1978), and interpersonal independency (Hirschfeld *et al*., 1977). All variables were coded so that higher scores reflected higher levels of the measured characteristic.

Descriptive Statistical Analyses

Unadjusted associations between CPP and categorical variables were assessed by Pearson's chi-square statistic or Fisher's exact test. Associations with continuous variables were assessed by *t*-test (when normally distributed) or by the Wilcoxon sign rank test (when not normally distributed). For the adjusted analysis, a forward stepwise logistic regression model was used in which all variables that were significantly associated in the unadjusted analyses ($p < 0.05$) were entered and kept if removal did not significantly worsen fit ($p < 0.10$).

Proband-wise concordance rates (the probability that the co-twin of an affected twin will also be affected) for CPP were calculated as: $(2*CA)/(2*CA+\mathrm{D})$, where CA = concordant affected pairs and D = discordant pairs (Sham, 1998). Recurrence risk ratios among MZs and DZs were calculated as the proband-wise concordance rate divided by the prevalence of CPP. Polychoric MZ and DZ twin pair correlations for CPP and the variables significantly associated with CPP were calculated, assuming that the categories of each variable measure thresholds of an underlying normal distribution (Neale and Cardon, 1992). To check the equal environments assumption of the twin model (Neale and Cardon, 1992), indicators of environmental similarity were assessed by asking twins to rate how much they currently see and contact each other and how much they had been raised in a common environment (sharing the same room, being dressed alike, sharing the same playmates, and being in the same class). General linear models were used to assess whether the degree of concordance was associated with any of the indicators of environmental similarity, after adjustment for zygosity and age.

Variance Components Modelling

Variance components models (Neale and Cardon, 1992) were used to more formally estimate the heritabilities of the measures and to evaluate their statistical significance. For each trait, the widely-used ACE/ADE modelling framework was used to partition the phenotypic variance (V_p) into additive genetic variance (a^2); environmental influences specific to the individual (e^2); and either non-additive genetic variance (d^2) or variance due to environmental influences common to the twin pair (c^2). In this setting, the most complete models that can be fitted include additive genetic and environmental effects plus either common environment (ACE) or dominance effects (ADE). More parsimonious models were assessed by dropping one or more variance components and comparing the value of -2 * log likelihood of the reduced model with that of the

fuller model. The difference between these likelihoods follows a χ^2 distribution with degrees of freedom equal to the number of parameters dropped.

Univariate twin models were first fitted to the phenotype CPP to determine the model that best explained the data (ADE; ACE; AE; CE; or E). The heritability of CPP was assessed from the most parsimonious (AE). These models were also used to derive heritability estimates for the five variables significantly associated with CPP (endometriosis, fibroids, dysmenorrhoea, consulted for irregular/unusual periods, and somatic distress).

To evaluate common genetic influences between CPP and correlated phenotypes, we fitted multivariate Cholesky decomposition models (Neale and Cardon, 1992) that partition the variances attributable to the different phenotypes. Phenotypes were entered in the following order: endometriosis, fibroids, dysmenorrhoea, unusual/irregular periods, somatic distress, and CPP. The first variable entered (endometriosis) is assumed to be caused by a latent (unobserved) variable which explains part of the variance of the four remaining variables in the model, including CPP. This variance is partitioned to estimate the proportion of the genetic variance of the remaining variables explained by the genetic variance of the first variable, endometriosis. The second variable entered into the model (fibroids) is assumed to be caused by a second latent variable that explains the variance of the three remaining variables, etc. The last variable (CPP) is assumed to be caused by a sixth latent variable that can explain the remaining variance of CPP that was not yet explained by the previous variables. All factor loadings were first estimated in full Cholesky models; tests of their significance were conducted by setting them to zero and re-estimating the other parameters (reduced models). Since the object of analysis was to elucidate the heritability of CPP, we fitted reduced models for the genetic paths in the model, but did not do so for the environmental factor structure.

SAS/STAT® version 8.2 (SAS Institute Inc., 1990) was used for the analyses of associations between CPP and different phenotypes. The structural equation modelling package Mx (Linux version 1.47c) was used for the calculation of twin pair correlations and the Cholesky decomposition models (Neale *et al.*, 1999). For all Mx analyses, raw data were used.

RESULTS

Prevalence of CPP

Out of the 2000 women constituting the MZ and DZ twin pairs, 1734 were still menstruating at the time of the gynaecological survey. No women were excluded from the study because of their current menstrual status, since the question by which women with CPP were identified related to life-time symptoms. Fifteen women had missing values for the CPP question.

The prevalence in the twin study sample of life-time history of CPP was 232/1985 = 11.7% (95% CI:10.3–13.1%). Of the 232 women with CPP, 142 (61.2%) had consulted a gynaecologist for the pain. Age at reported onset of CPP ranged from 9 to 45 years, with a median of 27 years. CPP prevalence varied significantly with age ($p < 0.001$), with rates from 7.6% among 29–35-year olds to 16.2% among 41–45-year olds. Among women in MZ twin pairs, the prevalence of CPP was 12.0%, whereas among DZs the prevalence was 11.0%.

Twin Pair Correlations and Heritability

Among MZs, there were 23 concordant CPP affected twin pairs, 102 discordant affected, and 491 concordant unaffecteds. Among DZs, there were 6 concordant affected, 69 discordant affecteds, and 294 concordant unaffecteds. This resulted in a proband-wise concordance rate of 0.311 for MZs and 0.148 for DZs (recurrence risk ratios: 2.6 and 1.3, respectively).

The concordance rates for CPP resulted in a tetrachoric twin pair correlation of 0.43 (95% CI:0.26–0.58) among MZ twins, compared to 0.11 (95% CI:−0.16-0.38) among DZ twins. Other than from a genetic component operating on (self-reported) CPP, this difference could possibly be explained by an increased environmental similarity between MZs compared to DZs. After controlling for zygosity, we found that twin pairs concordant for CPP were not more likely than discordant pairs to have shared a room as children ($p = 0.9$), shared the same playmates ($p = 0.25$), been in the same class ($p = 0.07$), been dressed alike ($p = 0.25$), or to currently see ($p = 0.7$) or contact ($p = 0.5$) each other more often.

Univariate models containing genetic, non-shared environmental, and shared environmental/non-additive genetic variance components were fitted to determine what model best explained the

Table I. Univariate Genetic Model Fitting of CPP Phenotype, Using Maximum Likelihood Estimates

	Parameter estimates				
Model*	a^2 (95% CI)	c^2 or d^2 (95% CI)	e^2 (95% CI)	−2lnlik†	*p*-value (comparison model)
ADE (full)	0.02 (0–0.55)	0.41 (0–0.58)	0.57 (0.42–0.74)	1408.59‡	
ACE (full)	0.41 (0–0.56)	0 (0–0.38)	0.59 (0.44–0.75)	1409.09	–
AE	0.41 (0.25–0.56)	0	0.59 (0.44–0.75)	1409.09	0.48 (ADE)
CE	–	0.33 (0.19–0.56)	0.67 (0.53–0.81)	1412.55	0.06 (ACE)
E	–	–	1.0	1431.80	<0.0001 (AE)

*A–additive genetic variance; C–common (shared) environmental variance; D–non-additive genetic variance; E = environmental variance.
†−2lnlik = −2*the log likelihood of the model. An increased value for −2lnlik indicates a worse model fit.
‡Equalled the fit of the non-decomposed, saturated, model.

CPP correlation among the twin pairs (Table I). The best fitting model was the model containing both additive and non-additive genetic as well as non-shared environmental variance (ADE). However, dropping the non-additive genetic component from the model (model AE) did not significantly worsen model fit. Despite the fact that the majority of genetic variance appeared to enter into the non-additive genetic variance component (d^2), the confidence interval for this component was very wide (0–0.55). As expected from the pattern of correlations showing *r*(MZ) greater than twice *r*(DZ), common environment did not enter into the model either, with the fit of the ACE model being equal to that of the AE model. The fit of the model containing non-shared environmental variance only (E model) was significantly worse than that of the AE model ($p < 0.0001$). Thus, the best fitting and most parsimonious model was the AE model. From this model, the heritability (a^2) of CPP was estimated at 0.41 (95% CI: 0.25–0.56).

Multivariate Models

To examine whether the heritability of CPP could be explained by associated phenotypes, a range of reproductive, psychological and other variables were first explored for their general association with CPP (Table II). The 17 variables found to be significantly associated with CPP ($p < 0.05$) were entered into a stepwise logistic regression model to investigate their adjusted association with CPP. Only five variables remained significantly associated with CPP: endometriosis; fibroids; dysmenorrhoea; somatic distress; and unusual/irregular periods (Table III). After allowing for the association between somatic distress and CPP, none of the other psychological measures were significantly associated with CPP. In addition, none of the demographic or life-style factors remained significantly associated with CPP after adjustment for the main five variables.

For each of the five variables significantly associated with CPP, MZ and DZ polychoric twin pair correlations were calculated, and AE models (which again proved the best fitting, most parsimoneous models) were fitted to determine their heritability (Table IV). For two of the variables (fibroids and unusual/irregular periods) the DZ correlation was negative. However, the confidence interval for this correlation estimate was very wide, owing to the small number of concordant affected DZ twin pairs. Indeed, a separate analysis in which the correlation was fixed arbitrarily to a value of +0.1 did not significantly alter model fit ($p = 0.15$). The relative uncertainty of the DZ twin pair correlationestimates for these variables is reflected in the wide confidence intervals for the heritability estimates. To assess whether the increased MZ correlations could be due to increased environmental similarity, associations between indicators of such similarity and concordance were investigated. Out of all indicators, recent frequent contact between twins was associated with diagnosis of endometriosis ($p = 0.05$) and with degree of somatic distress ($p = 0.03$) (not shown).

To assess the extent to which the heritability of CPP was explained by the genetic influences on the five variables associated with CPP, multivariate Cholesky decomposition models were fitted containing the six variables. The fit of the AE, CE and E models were compared to the fit of the full ADE and ACE models (Table V). The most parsimonious and best fitting model was again the AE model, with a fit that was not significantly worse than that of the ADE model ($\chi^2 = 5.67$; df = 21:$p = 0.99$).

The E model had a significantly worse fit compared to the AE model ($\chi^2 = 178.3$; df = 21: $p < 0.0001$).

The initial six-variate AE model provided estimates for all the genetic Cholesky factor loadings

Table II. Unadjusted Association Between CPP and Other Variables

	CPP		
	Yes ($N = 232$)	No ($N = 1753$)	p-value †
Other gynaecological factors			
Endometriosis	34.1%	2.8%	**<0.0001**
Uterine fibroids	27.2%	5.0%	**<0.0001**
Dysmenorrhoea*			
Severe	29.7%	2.4%	
Moderate I	26.3%	10.8%	
Moderate II	23.3%	35.6%	
Mild/none	20.7%	51.2%	**<0.0001**
Heavy menstrual flow*			
Severe	28.5%	7.5%	
Moderate I	15.5%	6.0%	
Moderate II	33.6%	58.2%	
Mild/none	22.4%	28.3%	**<0.0001**
Unusual/irregular periods	31.0%	10.8%	**<0.0001**
Consulted for infertility	21.2%	11.9%	**0.0001**
Number of live births	2.8 (1.2)	2.9 (1.3)	0.49
Psychological measures [mean (SD)]			
Cognitive distress/anxiety	3.0 (1.8)	2.4 (1.5)	**<0.0001**
Somatic distress/anxiety	2.1 (1.3)	1.7 (1.1)	**<0.0001**
Depression	2.0 (1.8)	1.8 (1.5)	**0.001**
Neuroticism	6.6 (3.0)	5.8 (3.0)	**<0.0001**
Panic	1.7 (1.2)	1.5 (1.2)	**0.0002**
Independence	5.4 (1.4)	5.8 (1.5)	**0.008**
Perceived control	7.1 (1.6)	7.6 (1.6)	**0.006**
Extraversion	7.8 (3.1)	7.8 (3.4)	0.28
Esteem	7.2 (1.4)	7.5 (1.5)	0.11
Demographics			
Age *[mean (SD)]*	40.0 (4.9)	38.6 (5.6)	**<0.0001**
Marital status			
Separated/divorced/widowed	3.3%	4.1%	
Never married	7.5%	12.9%	
Married/cohabiting	89.2%	83.1%	0.06
Social class (self-indicated)			
Working class	28.6%	22.5%	
Middle class	70.9%	75.7%	
Upper class	0.5%	1.8%	**0.04**
Income category *[mean (SD)]*	3.0 (1.6)	3.2 (1.7)	0.38
Highest education			
School/apprenticeship	77.1%	66.3%	
Technical/teachers'' college	10.6%	16.3%	
University	12.3%	17.9%	**0.005**
Other factors			
Body mass index *[mean (SD)]*	22.1 (3.4)	22.0 (3.2)	0.16
Cigarette use /day *[mean (SD)]*	7.6 (11.0)	6.5 (9.9)	**0.01**

* For coding see methods.

† Variables significantly ($p < 0.05$) associated with CPP appear in bold and were subsequently entered into a logistic regression model (Table III).

Table III. Results of the Stepwise Logistic Regression Model, Investigating the Adjusted Association Between CPP and Other Variables

Variable	*p*-value*
Entered into the model	
Endometriosis	<0.0001
Uterine fibroids	<0.0001
Dysmenorrhoea	<0.0001
Somatic distress/anxiety	0.0007
Unusual/irregular periods	0.02
Not entered into the model	
Heavy menstrual flow	0.25
Consulted for infertility	0.81
Cognitive distress/anxiety	0.72
Depression	0.23
Neuroticism	0.34
Panic	0.19
Independence	0.71
Perceived control	0.93
Age	0.16
Social class	0.25
Education	0.61
Cigarette use	0.39

**p*-values are calculated after adjustment for the five main variables in the model.

between the five variables and CPP. In this full Cholesky model, it is assumed that the genetic variance of CPP is determined by a genetic component underlying CPP itself as well as by those underlying each of the other variables in the model. However, eight out of the 21 genetic loading parameters in the model appeared close to zero (not shown). This implied that the genetic correlation structure in the model could possibly be simplified; i.e., the heritability of CPP could be explained by a more limited set of genetic loadings. In fact, a reduced model in which the eight parameters were fixed to zero did not offer a significantly worse fit ($\chi^2 = 5.31$; df = 8: $p = 0.72$). Four out of the eight genetic Cholesky factors estimated as zero involved the irregular/unusual periods variable. This included the genetic loading of this variable on CPP, and therefore the variable was removed from the model. The genetic Cholesky estimates between the remaining variables in the resulting five-variable model were very similar to those estimated in the six-variable model. We thus used the five-variable model to further explore the genetic background of CPP. After allowing for the genetic loadings from endometriosis, fibroids, dysmenorrhoea, and somatic distress, fixing the additional genetic loading on CPP to zero did not result in a significantly worse model fit ($\chi^2 = 0.05$, $p = 0.82$). In addition, three more genetic loadings between the other phenotypes in the full genetic Cholesky model could be fixed to zero without significant loss of fit ($\chi^2 = 1{,}73$, $p = 0.19$). We did not formally test the environmental structure in the model because we wished to absorb as much non-shared environmental variance as possible for our genetic explorations. However, it is interesting to note that the independent environmental Cholesky factor underlying CPP was substantial at 0.42, in contrast to the corresponding genetic Cholesky factor.

Figure 1 shows the genetic and environmental components of the five-variable Cholesky decomposition model, with the arrows representing the genetic and environmental loadings. It shows that the genetic component underlying endometriosis affects the genetic variances of all other variables including CPP. Having taken the genetic loadings from endometriosis into account, an additional genetic component underlying fibroids also loads onto CPP but not onto dysmenorrhoea or somatic distress. Similarly, a genetic component underlying dysmenorrhoea loads onto CPP (but not somatic distress). Lastly, a genetic component underlying somatic distress also loads onto CPP. After allowing for the genetic loadings from endometriosis, fibroids, dysmenorrhoea and somatic distress, there is no additional genetic factor underlying CPP itself that further explains the heritability of CPP.

Table VI shows the estimates of the heritabilities of each of the five variables in the model and the genetic correlations between the five variables. The heritabilities in the multivariate model were very similar to those estimated in the univariate models (Table IV). By squaring the genetic Cholesky factors that load onto CPP (bold in Figure 1), the proportions of CPP heritability attributable to the genetic components underlying the other variables can be calculated. Thus, $(0.39)^2 = 0.15$ of the 0.40 CPP heritability (38%) is explained by heritability of endometriosis, 0.09 (23%) by heritability of dysmenorrhoea, 0.09 (24%) by heritability of fibroids, and 0.06 (15%) by heritability of somatic distress. Genetic correlations between CPP and the four other variables were moderately high (all >0.6), although 95% confidence intervals were wide.

When somatic distress (rather than endometriosis) was entered as the first variable in the model, the relative contribution of heritability of endometriosis to CPP heritability decreased from 38% to 22%, the contributions of fibroids and dysmenorrhoea heritabilities remained approximately equal,

Table IV. MZ and DZ Correlation and Heritability Estimates for the Five Variables Significantly Associated With CPP

Variable	Number of MZ pairs*			Number of DZ pairs*			Proband-wise concordance rate (Recurrence risk)*		MZ polychoric correlation (95% CI)	DZ polychoric correlation (95% CI)	Heritability (95% CI)
	CA	D	CU	CA	D	CU	MZs	DZs			
Endometriosis	13	60	520	2	37	326	0.302 (2.6)	0.097 (0.8)	0.52 (0.32–0.69)	0.16 (−0.24–0.52)	0.51 (0.31–0.67)
Fibroids	10	74	511	1	54	312	0.212 (1.8)	0.036 (0.3)	0.35 (0.13–0.55)	−0.19 (−0.55–0.20)	0.30 (0.08–0.50)
Dysmenorrhoea	–	–	–	–	–	–	–	–	0.36 (0.27–044)	0.15 (0.02–0.27)	0.34 (0.27–0.43)
Somatic distress	–	–	–	–	–	–	–	–	0.40 (0.30–0.48)	0.16 (0.02–0.30)	0.39 (0.29–0.47)
Unusual/irregular periods	25	112	482	5	88	282	0.309 (2.6)	0.102 (0.9)	0.41 (0.24–0.56)	−0.09 (−0.34–0.17)	0.35 (0.19–0.50)
CPP	23	102	491	6	69	294	0.311 (2.7)	0.148 (1.3)	0.43 (0.26–0.58)	0.11 (−0.16–0.38)	0.41 (0.25–0.56)

*Number of concordant and discordant pairs, and proband-wise concordance rate and recurrence risk calculations only apply to dichotomous variables. CA = concordant affected; D = discordant; CU = concordant unaffected.

and the contribution of somatic distress increased from 15% to 29%. This suggested that part of the genetic variance of CPP was attributable to genetic variance shared between endometriosis and somatic distress.

DISCUSSION

In this study, an increased concordance of CPP among MZ compared to DZ twin pairs translated into a heritability of 0.41 (95% CI:0.25–0.56). Co-twins of affected MZs were at 2.6 times increased risk of CPP compared to the whole twin population prevalence, whereas co-twins of affected DZs were at 1.3 times increased risk. Lack of correlations with environmental indicators suggests that violation of the equal environments assumption is not responsible for these effects. Thus, the increased CPP concordance among MZs was likely to be attributable to heritable factors.

The prevalence of CPP among twins in this study (11.7%) was lower than found in other population-based surveys (Mathias *et al.*, 1996; Zondervan *et al.*, 2001b), most likely due to a difference in case definition. In the present study, cases were identified through self-report of ''long-standing (chronic) pelvic pain'', and no further detailed data were collected concerning the exact location, duration, and temporality of the pain. The definition is likely to have resulted in a case group that was at the more severe end of the symptom spectrum compared to cases identified in the general population following standard clinical definitions. This was supported by the relatively high percentage (61%) that had consulted a gynaecologist for their symptoms, compared to the specialist consultation rates (12–19%) found in other surveys (Mathias *et al.*, 1996; Zondervan *et al.*, 2001b). Thus, the heritability results are likely to relate to CPP that is slightly more severe than occurring in the general population, but which is more likely to reflect CPP severity for which women seek medical advice.

Although the AE model was the best fitting and most parsimonious model to explain CPP concordance, there was a suggestion of non-additive genetic effects judging by the amount of genetic variance that appeared accounted for by the d^2 component in the ADE model. The potential presence of non-additive genetic effects was also indicated by the observation that the MZ correlation was more than double the DZ correlation. However, the power to detect interaction between

Table V. Six-variate Genetic Model Fitting of the Phenotypes Endometriosis, Fibroids, Dysmenorrhoea, Irregular/Unusual Periods, Somatic Distress, and CPP

Model*	−2lnlik†	χ^2	Δdf‡	*p*-value (comparison model)
ADE (full)	12994.610	–	0	–
ACE (full)	12997.958	–	0	–
AE	13000.370	5.67	21	0.99 (ADE)
CE	13027.665	29.7	21	0.10 (ACE)
E	13178.641	178.3	21	< 0.0001 (AE)

*A–additive genetic variance; C–common (shared) environmental variance; D–non-additive genetic variance; E–environmental variance.
†−2lnlik = −2 * the log likelihood of the model. An increased value for −2lnlik indicates a worse model fit.
‡df = difference in degrees of freedom between reduced and comparison model.

genetic effects was very low, as indicated by the wide confidence interval for d^2.

The CPP is a condition that is notoriously difficult to diagnose. Benign pathology such as endometriosis certainly can be a cause for the pain in a proportion of women, but not all women with endometriosis—in particular those with milder stages of disease—experience symptoms (Waller *et al.*, 1993). Similarly, many women with uterine fibroids do not have pelvic pain. In women who undergo medical or surgical treatment for a diagnosed condition thought to underlie the pain, relief may not always be obtained, thus questioning either the effectiveness of the treatment or the appropriateness of the diagnosis. Since diagnostic uncertainty is unlikely to be different between MZ and DZ twin pairs, variance components analysis in twins allows us to investigate what proportion of the genetic variance of CPP, if any, could be attributable to genetic variance underlying such diagnoses.

In order to determine whether related phenotypes may account for the heritability of CPP, we first investigated the crude associations between CPP and hypothesised reproductive, psychological, and life-style covariates. As expected, endometriosis, fibroids, and menstrual characteristics were all associated with CPP, as were many indicators of psychological health such as depression, somatic and cognitive anxiety, and panic. Many such

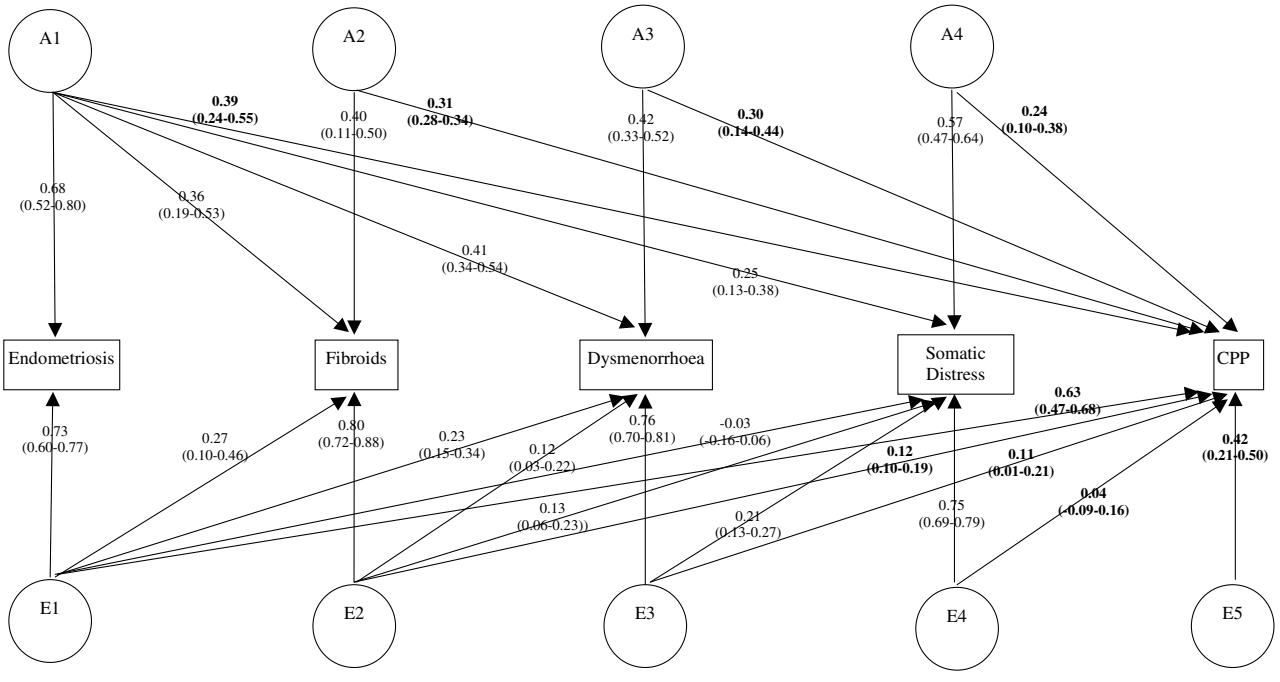

Fig. 1. The five-variate cholesky model, showing genetic and environmental factor loadings (and 95% confidence intervals) on each of the five variables (loadings on CPP shown in bold).

Table VI. Heritabilities* of and Genetic Correlations† Between the Five Variables and Their 95% Confidence Intervals

Variable	Endo.	Fibroids	Dysmen.	Somatic distress	CPP
Endometriosis	**0.47** (0.27–0.63)				
Fibroids	0.67 (0.42–0.98)	**0.29** (**0.22–0.40**)			
Dysmenorrhoea	0.70 (0.56–0.84)	0.47 (0.40–0.53)	**0.36** (**0.27–0.43**)		
Somatic distress	0.40 (0.21–0.54)	0.27 (0.22–0.39)	0.28 (0.17–0.44)	**0.38** (**0.30–0.47**)	
CPP	0.61 (0.57–0.66)	0.78 (0.51–0.85)	0.77 (0.62–0.91)	0.60 (0.54–0.81)	**0.40** (**0.26–0.51**)

*On the diagonal, in bold.
†Off the diagonal.

psychological characteristics have been reported as occurring more frequently in women with CPP compared to those without, although they appear not to be specific to CPP but to chronic pain conditions in general (McGowan *et al*., 1998). However, these psychological measures are known to be highly inter-correlated. Indeed, together with endometriosis, fibroids, dysmenorrhoea, and unusual/irregular periods, only somatic distress remained significantly associated in the multivariate logistic regression model.

Through multivariate twin modelling, we showed that the entire CPP heritability could be explained by genetic variance underlying four related phenotypes, with no genetic influences unique to CPP. The phenotype explaining most of the genetic variance of CPP (38%) was endometriosis, followed by dysmenorrhoea (24%), and fibroids (23%). In interpreting these findings, diagnostic uncertainty should be borne in mind. Although stable genetic influences on menstrual symptoms have been identified previously in data from this twin sample (Treloar *et al*., 1998b), part of the heritability accounted for by dysmenorrhoea or fibroids may in fact be related to undiagnosed endometriosis.

An interesting finding was that the remaining 15% of the CPP heritability appeared to be explained by a genetic component underlying somatic distress, and that this percentage increased to 29% when entered into the model before endometriosis. The reason for this change of model order was hypothesis-driven. Somatic distress is a propensity to report generalised somatic symptoms such as pains in the chest or back, weakness, dizziness and palpitations. It has been identified as a psychological dimension that is distinct from cognitive anxiety and depression (Gillespie *et al*., 2000), but has—to date—not yet been independently associated with CPP. Somatic distress may represent increased nociception (pain perception). Notably, a small experimental study recently found that nociception in pain referral sites was increased in 10 women with endometriosis and pelvic pain, compared to non-referral sites and compared to the same referral site in 10 control women (Bajaj *et al*., 2003). If increased pain sensitivity is associated with the pathological process of endometriosis itself, then the part of CPP heritability explained by somatic distress may represent undiagnosed endometriosis. However, it is also possible that increased pain sensitivity is a characteristic of the pelvic pain syndrome itself, irrespective of the presence of endometriosis. An increased nociception has also been suggested in patients with functional bowel disorders (Bouin *et al*., 2001). Indeed, when we entered somatic distress as the first variable in the multivariate Cholesky model, it reduced the proportion of CPP heritability explained by endometriosis from 38% to 22%, suggesting that genetic variance in increased nociception may explain part of the contribution from this phenotype.

The phenotypes significantly associated with CPP had individual heritability estimates that varied from 0.30 to 0.51. Few other studies independent of the Australian twin panel have reported heritability results for these phenotypes. Our estimate of heritability for fibroids for which a gynaecologist had been consulted was 0.30 (95% CI:0.08–0.50), lower than reported for fibroids requiring hospitalisation (0.55) (Luoto *et al*., 2000) or hysterectomy (0.69) (Snieder *et al*., 1998), but similar to that reported for fibroids diagnosed through ultrasound in a subset of volunteers (0.26) (Luoto *et al*., 2000). Our

estimate of dysmenorrhoea heritability (0.34 (0.27–0.43)), although defined differently, was similar to that reported from an earlier wave in the same twin pairs (Treloar *et al*., 1998b), and was also similar to that reported in 1992 in a sample of 827 US twin pairs (0.41) (Kendler *et al*., 1992). The same US twin study also reported estimates of somatic distress heritability between 0.26 and 0.36 among female twins (Kendler *et al*., 1995), similar to our estimate of 0.35 (95% CI:0.19–0.50).

The association between environmental similarity and concordance for the five phenotypes was examined. The extent of current contact between twins was associated with a life-time diagnosis of endometriosis ($p = 0.05$) and with degree of somatic distress ($p = 0.03$); no effects of childhood environmental similarity were identified. It may be that current contact was stimulated by a pre-existing "shared" condition or symptom similarity, but increased contact in adulthood could equally have preceded symptom onset. Although increased current contact may have inflated the individual heritability results for these two phenotypes, it is unlikely to have confounded the degree in which CPP heritability was explained by the heritability of these phenotypes since degree of environmental sharing was not associated with CPP concordance itself.

Although we investigated several diagnoses associated with CPP that may account for its heritability, we were unable to incorporate data on bowel or urinary symptoms since these were not collected for the study. To date, no estimate has been published on the heritability of urological diagnoses that may show overlapping symptomatology with CPP, such as interstitial cystitis. In an Australian twin sample of 686 men and women, a heritability estimate of functional bowel disorder was found of 57%, but due to the small sample size no separate estimate was provided for women (Morris-Yates *et al*., 1998).

This study is the first to report a heritability estimate for CPP, a very common condition in women of reproductive age. However, it showed that this heritability can be explained by genetic variance underlying endometriosis, fibroids, dysmenorrhoea, and somatic distress. Thus, CPP itself is unlikely to be a useful independent phenotype to conduct studies elucidating heritable aetiological aspects of the condition. Contributing conditions such as endometriosis, genetic aspects of which remain under investigation (Treloar *et al*., 2002; Zondervan *et al*., 2001a), are more likely to provide a fruitful approach. In addition, variation in nociception (which may contribute to a wide range of chronic pain conditions (Clauw *et al*., 1997; Goldenberg 1991), may be a potentially useful phenotype for genetic exploration.

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REFERENCES

Bajaj, P., Bajaj, P., Madsen, H., and Arendt-Nielsen, L. (2003). Endometriosis is associated with central sensitization:a psychophysical controlled study. *J. Pain*. **4**:372–380.

Bedford, A., Foulds, G. A., and Sheffield, B. F. (1976). A new personal disturbance scale (DSSI/sAD). *Br. J. Soc. Clin. Psychol*. **15**:387–394.

Bouin, M., Meunier, P., Riberdy-Poitras, M., and Poitras, P. (2001). Pain hypersensitivity in patients with functional gastrointestinal disorders: a gastrointestinal-specific defect or a general systemic condition? *Dig. Dis. Sci*. **46**:2542–2548.

Clauw, D. J., Schmidt, M., Radulovic, D., Singer, A., Katz, P., and Bresette, J. (1997). The relationship between fibromyalgia and interstitial cystitis. *J. Psychiatr. Res*. **31**:125–131.

Coxhead, D., and Thomas, E. J. (1993). Familial inheritance of endometriosis in a British population. A case control study. *J. Obstet. Gynaecol*. **13**:42–44.

Derogatis, L. R., Rickels, K., and Rock, A. F. (1976). The SCL-90 and the MMPI: a step in the validation of a new self-report scale. *Br. J. Psychiatry*. **128**:280–289.

Duffy, D.L. (1994). *Asthma and allergic diseases in Australian twins and their families*. Brisbane, Australia: University of Queensland.

Eysenck, S. B. J., Eysenck, H. J., and Barret, P. (1985). A revised version of the psychoticism scale. *Personal. Individual Diff*. **6**:21–29.

Gillespie, N., Kirk, K. M., Heath, A. C., Martin, N. G., and Hickie, I. (1999). Somatic distress as a distinct psychological dimension. *Soc. Psychiatry Psychiatr. Epidemiol.* **34**:451–458.

Gillespie, N. A., Zhu, G., Heath, A. C., Hickie, I. B., and Martin, N. G. (2000). The genetic aetiology of somatic distress. *Psychol. Med.* **30**:1051–1061.

Goldenberg, D. L. (1991). Fibromyalgia, chronic fatigue syndrome, and myofascial pain syndrome. *Curr. Opin. Rheumatol.* **3**:247–258.

Grace, V. M. (1998). Mind/body dualism in medicine:the case of chronic pelvic pain without organic pathology. A critical review of the literature. *Int. J. Health. Serv.* **28**:127–151.

Grace, V. M., Zondervan, K. T., and Vares, T. (2004). Chronic pelvic pain in New Zealand: prevalence, pain severity, diagnoses and use of the health services. *Aust. N. Z. J. Med.* **28**:369–375.

Hirschfeld, R. M., Klerman, G. L., Gough, H. G., Barrett, J., Korchin, S. J., and Chodoff, P. (1977). A measure of interpersonal dependency. *J. Pers. Assess.* **41**:610–618.

Kendler, K. S., Silberg, J. L., Neale, M. C., Kessler, R. C., Heath, A. C., and Eaves, L. J. (1992). Genetic and environmental factors in the aetiology of menstrual, premenstrual and neurotic symptoms:a population-based twin study. *Psychol. Med.* **22**:85–100.

Kendler, K. S., Walters, E. E., Truett, K. R., Heath, A. C., Neale, M. C., Martin, N. G., and Eaves, L. J. (1995). A twin-family study of self-report symptoms of panic-phobia and somatization. *Behav. Genet.* **25**:499–515.

Kennedy, S., Hadfield, R., Mardon, H., and Barlow, D. H. (1996). Age of onset of pain symptoms in non-twin sisters concordant for endometriosis. *Hum. Reprod.* **11**:403–405.

Lamb, K., Hoffmann, R. G., and Nichols, T. R. (1986). Family trait analysis: a case-control study of 43 women with endometriosis and their best friends. *Am. J. Obstet. Gynecol.* **154**:601.

Luoto, R., Kaprio, J., Rutanen, E. M., Taipale, P., Perola, M., and Koskenvuo, M. (2000). Heritability and risk factors of uterine fibroids–the Finnish Twin Cohort study. *Maturitas.* **37**:15–26.

Martin, N. G. (1975). The inheritance of scholastic abilities in a sample of twins. II. Genetical analysis of examinations results. *Ann. Hum. Genet.* **39**:219–229.

Mathias, S. D., Kuppermann, M., Liberman, R. F., Lipschutz, R. C., and Steege, J. F. (1996). Chronic pelvic pain: prevalence, health-related quality of life, and economic correlates. *Obstet. Gynecol.* **87**:321–327.

McGowan, L. P. A., Clark-Carter, D. D., and Pitts, M. K. (1998). Chronic pelvic pain: a meta-analytic review. *Psychol. Health* **13**:937–951.

Moen, M. H., and Magnus, P. (1993). The familial risk of endometriosis. *Acta Obstet. Gynecol. Scand.* **72**:560–564.

Moore, J., and Kennedy, S. H. (2000). Causes of chronic pelvic pain. *Baillieres. Clin. Obstet. Gynaecol.* **14**:389–402.

Morris-Yates, A., Talley, N. J., Boyce, P. M., Nandurkar, S., and Andrews, G. (1998). Evidence of a genetic contribution to functional bowel disorder. *Am. J. Gastroenterol.* **93**:1311–1317.

Neale, M. C., Boker, S. M., Xie, G., and Maes, H. H. (1999). *Mx Statistical modelling*. VCU Box 900126, Richmond, VA 23298: Department of Psychiatry.

Neale, M. C., and Cardon, L. R. (1992). *Methodology for genetic studies of twins and families*. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Pearlin, L. I., and Schooler, C. (1978). The structure of coping. *J. Health Soc. Behav.* **19**:2–21.

Reiter, R. C. (1998). Evidence-based management of chronic pelvic pain. *Clin. Obstet. Gynecol.* **41**:422–435.

Rosenberg, M. (1965). *Society and the adolescent self-image*. Princeton, NJ: Princeton University Press.

SAS Institute Inc. (1990). *SAS/STAT user's guide*. Cary, NC, USA: SAS Institute Inc.

Sham, P. (1998). The analysis of continuous and quasi-continuous characters. In: B. Everitt (ed.), *Statistics in human genetics*. London: Arnold Publishers, pp. 187–266.

Silberg, J. L., Heath, A. C., Kessler, R. C., Neale, M. C., Meyer, J. M., Eaves, L. J., and Kendler, K. S. (1990). Genetic and environmental effects on self-reported depressive symptoms in a general population twin sample. *J. Psych. Res.* **24**:197–212.

Simpson, J. L., and Bischoff, F. (2003). Heritability and candidate genes for endometriosis. *Reprod. Biomed. Online.* **7**:162–169.

Simpson, J. L., Elias, S., Malinak, L. R., and Buttram, V. C. J. (1980). Heritable aspects of endometriosis. I. Genetic studies. *Am. J. Obstet. Gynecol.* **137**:327–331.

Snieder, H., MacGregor, A. J., and Spector, T. D. (1998). Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. *J Clin. Endocrinol. Metab.* **83**:1875–1880.

Treloar, S. A., Do, K. A., and Martin, N. G. (1998a). Genetic influences on the age at menopause. *Lancet.* **352**:1084–1085.

Treloar, S. A., Martin, N. G., and Heath, A. C. (1998b). Longitudinal genetic analysis of menstrual flow, pain, and limitation in a sample of Australian twins. *Behav. Genet.* **28**:107–116.

Treloar, S. A., Martin, N. G., Bucholz, K. K., Madden, P. A., and Heath, A. C. (1999a). Genetic influences on post-natal depressive symptoms: findings from an Australian twin sample. *Psychol. Med.* **29**:645–654.

Treloar, S. A., O''Connor, D. T., O'Connor, V. M., and Martin, N. G. (1999b). Genetic influences of endometriosis in an Australian twin sample. *Fertil. Steril.* **71**:701–710.

Treloar, S., Hadfield, R., Montgomery, G., Lambert, A., Wicks, J., Barlow, D., O'Connor, D., and Kennedy, S. (2002). The international endogene study: a collection of families for genetic research in endometriosis. *Fertil. Steril.* **78**:679.

Waller, K. G., Lindsay, P., Curtis, P., and Shaw, R. W. (1993). The prevalence of endometriosis in women with infertile partners. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **48**:135–139.

Zondervan, K. T., Cardon, L. R., and Kennedy, S. H. (2001a). The genetic basis of endometriosis. *Curr. Opin. Obstet. Gynecol.* **13**:309–314.

Zondervan, K. T., Yudkin, P. L., Vessey, M. P., Jenkinson, C. P., Dawes, M. G., Barlow, D. H., and Kennedy, S. H. (2001b). The community prevalence of chronic pelvic pain in women and associated illness behaviour. *Br. J. Gen. Pract.* **51**:541–547.

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