An Australian twin study of the genetic basis of preeclampsia and eclampsia

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OBJECTIVE: We investigated maternal versus fetal genetic causes of preeclampsia and eclampsia by assessing concordance between monozygotic and dizygotic female co-twins, between female partners of male monozygotic and dizygotic twin pairs, and between female twins and partners of their male co-twins in dizygotic opposite-sex pairs.

STUDY DESIGN: Two large birth cohorts of volunteer Australian female twin pairs (N = 1504 pairs and N = 858 pairs) were screened and interviewed, and available medical and hospital records were obtained and reviewed where indicated, with diagnoses assigned according to predetermined criteria.

RESULTS: With strict diagnostic criteria used for preeclampsia and eclampsia, no concordant female twin pairs were found. Collapsing diagnoses of definite, probable, or possible preeclampsia or eclampsia resulted in very low genetic recurrence risk estimates.

CONCLUSION: Results from these two cohorts of female twin pairs do not support clear, solely maternal genetic influences on preeclampsia and eclampsia. Numbers of parous female partners of male twins were too low for conclusions to be drawn regarding paternal transmission. (Am J Obstet Gynecol 2001;184:374-81.)

Key words: Genetic, preeclampsia, twin study

Current understanding of the cause of preeclampsia and eclampsia of pregnancy is limited, although specific aspects of the pathogenesis are understood[1] and preconceptional and pregnancy-associated risk factors have been identified.[2] Genetic influences have long been regarded as etiologically important in preeclampsia and eclampsia,[3] and although several candidates have been identified for preeclampsia[4] and pregnancy-induced hypertension,[5] predisposing genes have yet to be indisputably found. A family history of preeclampsia or eclampsia is associated with a 4-fold increase in the relative risk of severe preeclampsia in primigravid women.[6] After a prolonged period of questioning the (maternal) genetic basis of preeclampsia and eclampsia because no concordant monozygotic twin pairs had been described, 3 monozygotic female twin pairs concordant for preeclampsia have now been reported.[7] No twin pair concordance and discordance rates were available from that study, however, so no conclusions were possible concerning maternal genetic influences. A recent British study reported negligible heritability of confirmed proteinuric preeclampsia, on the basis of data from a cohort of adult female twin pairs.[8] Whether the genetic origin of preeclampsia and eclampsia is fetal rather than stemming from the maternal genotype alone or is the result of an interaction between the two is still unresolved.[9] Implantation failure is a key feature of preeclampsia and eclampsia.[10]

Pairs of monozygotic twins share the same genotype and, if raised in the same family, share the same family background. Dizygotic twins share, on average, 50% of their genes. Differences between monozygotic twins in a pair must be caused by either environmental influences or measurement error, whereas differences between dizygotic twins may be caused by effects of differing genetic or individual-specific environmental effects. We describe an exploratory epidemiologic study that sought to identify concordance and discordance rates for a history of preeclampsia or eclampsia in pregnancies of adult twin pairs. To investigate a fetal versus a maternal cause of preeclampsia and eclampsia, we explored concordance between parous female partners of monozygotic and dizygotic male co-twins and between female partners and female co-twins in dizygotic opposite-sex pairs. If paternal

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genetic and therefore fetal effects were important in preeclampsia and eclampsia, we would have expected greater concordance of preeclampsia and eclampsia between partners of monozygotic male co-twins than between partners of dizygotic male co-twins or between female twins and partners of their male co-twins in dizygotic opposite-sex pairs.

Methods

Sample. Twins from two cohorts already recruited for general health–related research were involved in this study. In addition, we aimed to identify affected mothers with preeclampsia or eclampsia who were twins from the hospital records of the Royal Women's Hospital in Melbourne, one of Australia's largest maternity hospitals, to assess co-twin concordance for preeclampsia and eclampsia. Approval to conduct the research was obtained from the Macquarie University Ethics Committee, the Bancroft Centre Research Ethics Committee (Queensland Institute of Medical Research), the Royal Women's Hospital Research and Ethics Committees, and the Australian National Health and Medical Research Council Twin Registry.

Cohort 1. A cohort of 1979 female twin pairs, born from 1913 to 1964 and ascertained originally in 1980-1982 from the Australian (National Health and Medical Research Council) Twin Register, was followed up in 1988-1990, when the minimum age of respondents was 25 years.[11] Two items concerning similarity in appearance and being mistaken by others were included to determine zygosity.[12] Pairs giving inconsistent responses were recontacted for clarification. Such questionnaires have been shown to give ~95% agreement with the diagnosis on the basis of extensive blood typing.[13] More recently, a subsample of 198 same-sex pairs from this group was typed for 11 independent, highly polymorphic markers in the course of an asthma study; no errors in our previous zygosity diagnosis were detected.[14] The 1988-1990 questionnaire replicated most of the 1980-1982 questions and added new health items for women, including obstetric factors relating to each pregnancy. Responses to the 1988 questionnaire were obtained from both members of 1504 female twin pairs (952 monozygotic pairs and 552 dizygotic pairs). Additional twins participated in an abbreviated telephone interview form of the questionnaire (excluding the screening item), giving a total pairwise response rate of 82% (1620 of the original 1979 pairs).

Cohort 2. The second cohort of twins, born from 1964 to 1971, was recruited through the Australian Twin Registry in 1989. More than 3000 pairs (N = 3269) were mailed a health questionnaire in 1990-1992,[15] and response was obtained from 1437 pairs. This cohort was followed up from 1996 for a study of alcoholism and psychiatric morbidity. A question concerning preeclampsia was included in this interview as a screening tool for the present study.

Screening. Screening was performed to maximize the efficiency of case finding, given resource constraints, and to minimize demands on twin research participants in whom preeclampsia or eclampsia was unlikely.

Cohort 1. The older cohort of twins was screened on the basis of the 1988-1990 responses to a questionnaire item concerning high blood pressure or toxemia of pregnancy (a commonly used lay term) relating to each birth. Twins were selected for interview if they reported high blood pressure or toxemia during the first pregnancy but not during any subsequent pregnancy. Initially 641 individual twins were identified as having reported high blood pressure or toxemia during the first pregnancy. However, this number was reduced to 367 when criteria were tightened to exclude twins who also reported blood pressure problems during subsequent pregnancies. Twenty-three twins had died or were lost between the questionnaire screen (1988-1990) and our follow-up (1996-1997). Finally, 344 of these female twins in 338 twin pairs were selected for interview on the basis that they had been "active" twin study participants at last contact (1992-1993). Of the 338 pairs, 158 were monozygotic female, 112 were dizygotic female, and 68 were dizygotic opposite-sex pairs. There were 15 female pairs (13 monozygotic female and 2 dizygotic female) who were concordant on the screening criteria, and the remaining 255 female pairs were discordant.

Cohort 2. Twins from the younger birth cohort (born 1964-1971) were screened on the basis of responses to the interview question, "Have you or your current partner (if male twin) had preeclampsia (high blood pressure or proteinuria) in your first pregnancy?" Female partners of twins in male pairs were included in cohort 2 but not in cohort 1 because no useful screening information was available concerning pregnancies in the spouses of cohort 1 male twins. By the cutoff date for follow-up (July 31, 1997) a total of 2532 cohort 2 twins had been screened; 205 individuals (10%) had responded affirmatively for themselves or their partners to the interview question. This number comprised 151 individual female respondents (55 in monozygotic female, 60 in dizygotic female, and 36 in dizygotic opposite-sex pairs) and 57 male respondents (23 in monozygotic male, 23 in dizygotic male, and 11 in dizygotic opposite-sex pairs).

Of the 109 female pairs (51 monozygotic female pairs and 58 dizygotic female pairs) in which one or both twins had positive screening results, 5 female pairs were concordant (all monozygotic female) and 94 were discordant on the screening question. One dizygotic opposite-sex pair and 1 male pair were concordant on the screening question (male respondents reporting on the female partner).

Histories. A structured interview schedule obtained detailed pregnancy and obstetric care histories including symptoms and treatments indicative of preeclampsia and information concerning preexisting health problems that might influence hypertension or be confused with a diagnosis of preeclampsia or eclampsia (eg, diabetes, renal disease, adrenal gland disease, epilepsy, brain tumor, systemic lupus erythematosus, or other hypertensive condition). Questions included those on blood pressure, proteinuria, edema, epigastric pain, nausea and vomiting, visual disturbances, dizziness, and seizures in the last 4 months of all pregnancies. Known family history was also investigated at interview.

Midwives specially trained for the study interviewed participants during 1996 and 1997. For example, interviewers were alerted to pick up and explore clues about possible preeclampsia from older women's descriptions of management features (eg, use of darkened rooms, boiling of urine, placement of wooden spoon under bed as tongue protector in case of seizure). This process was important because of the relatively advanced age of twins in cohort 1 and because some of the symptoms of preeclampsia may have been associated with impaired recall of events surrounding the delivery.

Interviewers were not informed of the zygosity of participants, although they were informed of their responses to the screening items. After interviews were completed, twins were asked to provide written consent for the research team to access medical and hospital records relating to their pregnancies. Three types of records were requested—hospital records of the confinement, obstetrician records of the pregnancy and follow-up, and general practitioner records for a prepregnancy or early pregnancy blood pressure recording.

Diagnosis. Criteria consistent with published definitions were used for classification.[16] These definitions indicate that the women had had preeclampsia (1) if they were nulliparous, (2) if they were normotensive (blood pressure <140/90 mm Hg), with no proteinuria up to and including 20 weeks' amenorrhea, (3) if they were hypertensive (blood pressure ≥140/90 mm Hg) after 20 weeks' amenorrhea, (4) if they had proteinuria (urinary protein concentration >0.3 g/24 hours) after 20 weeks' amenorrhea, and (5) if proteinuria and hypertension had disappeared by 3 months post partum. Eclampsia was diagnosed in women who met these criteria and who had convulsions or unconsciousness in the perinatal period. Absence of proteinuria in the presence of the pattern of hypertension outlined in criteria 2, 3, and 5 indicated mild preeclampsia. A diagnosis of pregnancy-associated hypertension was made in cases of preexisting hypertension or known renal disease. Women who had been pregnant at least twice and who had high blood pressure only in the first pregnancy were considered to have had preeclampsia irrespective of the other symptoms.

Hospital and medical records were sought for all interviewees, with the exception of a small number of subjects with false-positive findings. In some cases medical records were not available because the hospital had closed or the physician had died. In more cases records had been destroyed after the legally required period had elapsed. When all possible records had been retrieved, all interviews and records were reviewed. The clinical members of the team (Shaun P. Brennecke and Madonna M. Grehan) assigned diagnoses. A subsample of participants was reviewed by each independently to check consistency of classification. Agreement was 100%. We used the following 6 categories of diagnoses: (1) definite preeclampsia or eclampsia (positive interview history with or without complete records; good history plus confirming records), (2) probable preeclampsia or eclampsia (convincing history but only partial records), (3) possible preeclampsia or eclampsia (likely history with or without records), (4) mild preeclampsia or eclampsia, (5) pregnancy-associated hypertension, and (6) none of the above. When classification resulted in definite or probable preeclampsia, we sought information from the co-twin (or the co-twin's partner) if the co-twin or partner had not already been selected for interview on the basis of screening criteria. At final diagnostic classification, reviewers were masked to zygosity, to the co-twin's participation status, and to the co-twin's diagnosis.

Data analysis. The SAS statistical package, version 6.11 (SAS Institute Inc, Cary NC),[17] was used for preliminary data analyses. The liability to preeclampsia or eclampsia is assumed to have an underlying normal distribution, although diagnosis in this case requires dichotomous categorization. The appropriate statistic that estimates a correlation in liability between twins is the polychoric correlation, of which the tetrachoric correlation is the special case for dichotomous variables. For monozygotic and dizygotic pairwise concordance comparisons, if the dizygotic twin pair correlation is less than half the monozygotic twin pair correlation, genetic nonadditivity (dominance or epistasis) is indicated, whereas shared environmental influence increases the dizygotic correlation to more than half the monozygotic correlation.[18] Genetic risk ratios with the use of the λ statistic were computed as probandwise concordance divided by prevalence.[19] Because population prevalence was not known, we used sample prevalences for each cohort. We computed probandwise concordances for each cohort separately and for the combined cohorts of twins, using the following formula: $2 \times \text{Concordant pairs}/[2(\text{Concordant pairs}) + \text{Discordant pairs}]$.

Results

We focused on the strategy of screening the twin cohorts for preeclampsia and eclampsia, because preliminary screening of hospital patients with preeclampsia and

Table I. Final diagnostic classification of twins by birth cohort

	Cohort 1			*Cohort 2*		
Diagnosis performed	*With records*	*Without records*	*Total*	*With records*	*Without records*	*Total*
Definite preeclampsia or eclampsia	10	1	11	9	0	9
Probable preeclampsia or eclampsia	6	11	17	5	4	9
Possible preeclampsia or eclampsia	7	76	83	4	17	21
Mild preeclampsia	63	15	78	52	7	59
Pregnancy-associated hypertension	4	6	10	6	0	6
None of these	47	40	87	32	19	51
TOTAL	137	149	286	108	47	155

their partners found few twins who could be contacted and whose co-twins had also reproduced. The two birth cohorts of twins were kept separate for analyses. Reasons for separate consideration were, first, that modern antenatal care and obstetric interventions may have created different circumstances for possible expression of preeclampsia or eclampsia in the two cohorts and, second, that their screening criteria were different.

Twin pair data

Cohort 1. Of the 344 individuals selected for interview from cohort 1, 220 (64%) participated fully, 39 (11%) were interviewed but refused consent for access to medical and hospital records, and 17 (5%) were interviewed but on the basis of the interview no consent form was sent. Of the 68 nonrespondents (20%), 3 were overseas, 28 could not be located, 1 had died, and 1 was unable to participate; 35 refused to participate in the interview. Records were requested for 225 full participants (5 of whom were co-twins who had not been originally selected on the basis of the questionnaire): In 29 cases (12.9%) we received 3 records, in 77 (34.2%) 2 records were received, in 98 (43.6%) 1 record was received, and in 21 (9.3%) no records were received. Diagnostic classification was performed for 286 twins (Table I).

Cohort 2. Individual numbers investigated increased to 224 with the inclusion of 2 twins referred by their co-twins as having had symptoms resembling preeclampsia or eclampsia, and a further 17 were subsequently included for special investigation because of their co-twin's definite or probable preeclampsia or eclampsia. Of the 224 individuals identified from cohort 2, 112 (50%) participated fully, 38 (17%) were interviewed but refused consent for records, and 5 (2%) were interviewed but, on the basis of the interview, no consent form was sent. Of the 69 nonrespondents (31%) in the identified sample, for 23 the interview was not applicable (eg, no pregnancies continued beyond 20 weeks), 22 could not be located, and 24 refused to participate in the interview.

Participation numbers were greater when a female twin rather than a male twin was the proband. Of the identified individual male twins (n = 64), the female partners of 18 (28%) participated fully, 14 (22%) refused, 9 (14%) did not give consent to obtain access to records, 12 (19%) could not be contacted, and the interview was not applicable to 11 (17%) (eg, no female partner or no pregnancies). In comparison, of the 160 identified cohort 2 female subjects, 94 (59%) participated fully, 10 (6%) refused participation, and 29 (18%) were interviewed but did not give consent for records retrieval. Records were requested for 112 full participants. In 36 cases (32.1%) we received all 3 types of records, in 47 (42.0%) we received 2, and in 28 (25.0%) we received 1 record. In only 1 case were no records received at the study's cutoff date in response to our request. Record availability was better in this younger cohort of twins because dates of confinements were more recent. Classification was performed for 155 cohort 2 twins (Table I).

Analysis of twin pair data. Classification categories were treated according to 3 protocols. In protocol A only twins who were classified as definitely having preeclampsia or eclampsia or as probably having preeclampsia or eclampsia (only where records were available for the latter) were considered to have positive results. All other classifications, plus having negative results on screening criteria in the first instance, were counted as negative. In protocol B all definite, probable, and possible cases of preeclampsia or eclampsia were considered positive; all other classifications, plus failure to meet screening criteria in the first instance, were considered negative. Protocol C followed protocol B except that positive cases included those classified as having mild preeclampsia; these were excluded from the negative cases. Concordances and discordances according to each protocol are shown in Table II.

Some pairs do not appear in Table II because co-twins (or the male twins' partners, if they had partners) were nulliparous or because they did not participate in the interview, although they had been selected on the basis of screening criteria. A further reason for exclusion of cohort 2 twin pairs was that the co-twin had not yet responded to the screening interviews, which had not been completed before this study had to be finalized. An example from Table II demonstrates its interpretation. "Twin 1" and "Twin 2" simply refer to a twin and co-twin in a pair, irrespective of birth order. The third and fourth columns of the first and second rows in Table II show the number of monozygotic female twins in the second co-

Table II. Twin pair concordances and discordances according to 3 protocols (A, B, and C) for collapsing categories of diagnoses

		Cohort 1		*Cohort 2*		*Combined cohorts*	
		Twin 2		*Twin 2*		*Twin 2*	
Zygosity	*Twin 1*	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
Monozygotic female pairs							
Protocol A*	Positive	0	3	0	1	0	4
	Negative	3	545	3	232	6	777
Protocol B†	Positive	2	19	0	3	2	22
	Negative	22	508	5	228	27	736
Protocol C‡	Positive	8	30	1	8	9	38
	Negative	33	481	10	217	42	698
Pairs (No.)		551	—	236	—	787	—
Dizygotic female pairs							
Protocol A	Positive	0	6	0	0	0	6
	Negative	1	298	3	199	4	497
Protocol B	Positive	0	21	0	5	0	26
	Negative	8	276	5	192	13	468
Protocol C	Positive	1	33	1	13	2	46
	Negative	11	260	11	177	22	437
Pairs (No.)		305	—	202	—	507	—
Monozygotic male pairs§							
Protocol A	Positive	—	—	0	0	—	—
	Negative	—	—	0	66	—	—
Protocol B	Positive	—	—	0	1	—	—
	Negative	—	—	0	65	—	—
Protocol C	Positive	—	—	0	3	—	—
	Negative	—	—	2	61	—	—
Pairs (No.)		—	—	66	—	—	—
Dizygotic male pairs§							
Protocol A	Positive	—	—	0	0	—	—
	Negative	—	—	0	42	—	—
Protocol B	Positive	—	—	0	0	—	—
	Negative	—	—	0	42	—	—
Protocol C	Positive	—	—	0	0	—	—
	Negative	—	—	0	42	—	—
Pairs (No.)		—	—	42	—	—	—
Dizygotic opposite sex pairs‖							
Protocol A	Positive	0	1	0	1	0	2
	Negative	0	0	1	113	1	113
Protocol B	Positive	0	1	0	2	0	3
	Negative	0	0	1	112	1	112
Protocol C	Positive	0	1	0	7	0	8
	Negative	0	0	3	105	4	105
Pairs (No.)		1	—	115	—	—	116
TOTAL pairs		857	—	661	—	—	1518

*Protocol A: Only classification categories of definite preeclampsia or eclampsia and probable preeclampsia or eclampsia (the latter only where records were available) were considered positive, and the remainder, including those whose screening results were negative, were considered to be negative for preeclampsia or eclampsia.

†Protocol B: Positive classification categories were definite, probable, or possible preeclampsia or eclampsia (with or without records); categories were negative if classified as mild preeclampsia, pregnancy-associated hypertension, or definitely none of these, no reported high blood pressure or toxemia for first pregnancy or reported for >1 pregnancy in 1988-1990 survey (cohort 1, female probands only), or negative response to preeclampsia question at interview (cohort 2, including male twin answering for partner).

‡Protocol C: Positive categories were classified as definite, probable, or possible preeclampsia (with or without records) or mild preeclampsia; negative categories were classified as pregnancy-associated hypertension, definitely none of these, or no reported high blood pressure or toxemia for first pregnancy or reported for >1 pregnancy in 1988-1990 survey (cohort 1) or negative response to preeclampsia question (cohort 2, self or female partner if male twin).

§Female partner of male twin.

‖Female twin and female partner of male co-twin.

hort, with preeclampsia strictly defined (protocol A). There were no pairs with both women affected. In 1 pair the first twin was affected and the second unaffected, in 3 pairs the first was unaffected and the second affected, and in 232 pairs both were unaffected.

The prevalence of a history of preeclampsia in this sample of monozygotic and dizygotic female pairs according to protocol A (the most stringent categorization) was 0.008 in both cohort 1 and cohort 2. Protocol B prevalences were 0.043 in cohort 1 and 0.021 in cohort 2.

Protocol C prevalences were higher at 0.072 for cohort 1 and 0.053 in cohort 2.

With protocol B used for cohort 1 monozygotic female pairs, probandwise concordance was $(2 \times 2)/[(2 \times 2) + (19 + 22)] = 0.089$. Probandwise concordance for dizygotic pairs would be 0. The genetic risk ratio λ for monozygotic female pairs in cohort 1 was 2.17, on the basis of a prevalence in monozygotic female pairs of 0.041; for dizygotic female twins it was 0, because no concordant pairs were identified. The tetrachoric correlation for monozygotic female pairs in the combined cohorts with the use of protocol B was $r = 0.19 \pm 0.17$. Creating 1 concordant dizygotic female pair to allow a tetrachoric correlation to be estimated resulted in a dizygotic female pair correlation of $r = 0.07 \pm 0.22$. In both cohort 1 concordant monozygotic pairs (with protocol B), 1 twin had probable and 1 had possible preeclampsia or eclampsia, but no medical records could be obtained for the twins. There were no concordant cohort 2 female pairs.

Under protocol C collapsing of diagnostic categories, 4 of the 8 cohort 1–positive concordant monozygotic female pairs (Table II) were concordant for mild preeclampsia, whereas in 2 pairs 1 twin had probable preeclampsia and the co-twin had possible preeclampsia. In 1 pair there was possible preeclampsia in 1 twin and mild preeclampsia in her co-twin, and in another pair there was probable preeclampsia in 1 twin and mild preeclampsia in her co-twin. In the concordant cohort 1 dizygotic female pair, 1 twin had definite preeclampsia, whereas her co-twin had mild preeclampsia.

When we included mild preeclampsia (protocol C), we obtained tetrachoric correlations for cohort 1 of $r_{MZ} = 0.36 \pm 0.12$ and $r_{DZ} = -0.14 \pm 0.23$, where subscript MZ is monozygotic and subscript DZ is dizygotic. For cohort 2 $r_{MZ} = 0.22 \pm 0.27$ and $r_{DZ} = 0.05 \pm 0.42$, but standard errors were again high. For the combined cohorts, the tetrachoric correlation coefficients were $r_{MZ} = 0.35 \pm 0.11$ and $r_{DZ} = -0.03 \pm 0.17$. There were no positive concordant pairs when this categorization scheme was used for female partners of monozygotic and dizygotic male pairs and dizygotic opposite sex pairs. Numbers of cases in monozygotic male, dizygotic male, and dizygotic opposite sex pairs were too low for conclusions to be drawn from these data. Potential numbers were reduced substantially because of no partners, no consent, and nulliparity.

Comment

Concordance results from female twin pairs do not suggest the operation of clear-cut, solely maternal genetic influences on preeclampsia and eclampsia. Population prevalence estimates with which to compare those in our twin cohorts are difficult to find. Our screening procedures may have missed some cases, or the twin sample may not have been representative of the female population. However, the relative differences between the cohorts are sensible given the ages of the two cohorts and the increased likelihood of preventive interventions for the younger twins.

The low prevalence of cases created difficulty for pairwise analysis. This was exacerbated in the younger sample, in which clinical interventions may have averted or ameliorated the outcome in potential cases. Undetected false-negative determinations may have resulted, or our screening tools may have been inadequate. Attrition of twins who had preeclampsia or eclampsia from the twin cohorts is a possibility, but it is unlikely because of the broad range of health issues studied. Maternal death from preeclampsia or eclampsia may have had a small effect on prevalence rates in this volunteer twin sample. Determinations may have been falsely negative, for example, in cohort 1 twins who were not selected for interview if they had reported high blood pressure or toxemia for >1 pregnancy.

We could not compute concordance statistics on the basis of our original diagnostic criteria (protocol A) because of a lack of concordant pairs. We first used a very stringent definition of preeclampsia and eclampsia in our study (protocol A) and failed to identify one concordant twin pair, either monozygotic or dizygotic. Relaxing the classification (as in protocols B and C) still resulted in modest monozygotic correlation coefficients and ratios of monozygotic to dizygotic twin pair correlations. The genetic recurrence risk to cohort 1 monozygotic co-twins of affected twins (with our intermediate classification system, protocol B) of 2.23 was not as high as expected in the case of a condition with strong (maternal) genetic influences. This risk coefficient was more of the order that one would expect for dizygotic twins or siblings in conditions proving amenable to gene searching.

Our exploratory twin study was conducted with constraints. A complete follow-up cohort study was too expensive, so we used the approach described. The initial size of the cohorts was large, but estimated prevalence rates were low. This finding was salutary in that an even larger twin sample would be required to detect genetic effects at this apparently low magnitude. We were unable to glean useful information about paternal transmission of a genetic predisposition to preeclampsia and eclampsia from consideration of opposite sex or male pairs. Numbers were reduced by the younger age of the cohort (which implies lower parity), by less accurate male response to the screening question, and by reduced rates of consent to participation from female partners who could be ascertained only by consent from their male (twin) partners. Male twins and dizygotic twins are commonly underrepresented in volunteer twin studies relative to the general population.[20] This was the case in our sample and contributed to the low level of data on male twins' partners. Although recruitment bias and response bias are potential problems in twin research, they are less

threatening when (un)cooperativeness is not correlated with the trait being analyzed.[20] Involving male respondents remains a difficult area for studies of the genetic features of preeclampsia and eclampsia.

The implications of our findings depend on the extent to which the twin samples are representative of the general female population. Cohort 1 female twins have been shown to be representative of the Australian population on a variety of indicators, including age, general level of education, and marital status.[21] Comparative data are not available for subjects in cohort 2. Community prevalence estimates for preeclampsia and eclampsia have not been found for comparison. There is limited ethnic diversity in the volunteer twin samples. Twins have volunteered to participate in medical research in general. Some few deaths from preeclampsia or eclampsia may have occurred, precluding or removing female twins from the Register, but we have no reason to consider selection bias to be a significant problem. We raise the possibility that being a twin mother may confer some protection against preeclampsia, which is a novel alternative explanation of the low prevalence of preeclampsia in these twin cohorts.

Our results led to conclusions similar to those of Thornton and Macdonald[8] in their study of twin mothers. An earlier, smaller British study of 99 monozygotic parous twins also found no pairs concordant for proteinuric preeclampsia in first pregnancy, although some pairs concordant for nonproteinuric hypertension in pregnancy were reported.[22] A previous report had also found no concordant monozygotic pairs.[23] However, the zygosity assignment protocol in the former study and the loose definition of preeclampsia in the latter have been criticized.[7] Sample sizes were not large in these studies, and concordant pairs may not have been detected for this reason. Pairwise discordance per se does not negate the pertinence of genetic influences,[24] as implied by some earlier reports.[25] The 3 reported concordant monozygotic pairs with proteinuria and preeclampsia[7] were identified in a sibling pair sample rather than a twin pair sample, so no conclusions regarding concordance and discordance rates could be drawn.

Ours was a complex epidemiologic study that used clear strategies to maximize case finding and followed strict protocols for follow-up and diagnosis. If strong, purely maternal genetic effects were present, we would have expected to find at least one monozygotic twin pair that was concordant for preeclampsia or eclampsia when the most stringent clinical criteria were used. The 2 concordant monozygotic pairs we identified could not be confirmed as having had definite preeclampsia because records were unavailable. Like Thornton and Macdonald,[8] we found evidence of negligible heritability for proteinuric preeclampsia. We found higher monozygotic concordance by extending the definition of affected to include mild preeclampsia.

Overall, the results achieved by this epidemiologic approach to the question of the cause or causes of preeclampsia and eclampsia do not support a strong maternal genetic effect, although they do not exclude the involvement of maternal susceptibility genes. We can exclude neither genetic conflict (differential imprinting of either paternal or maternal genes, or both) nor the involvement of purely paternal genetic factors. Although our sample of reproducing male twin pairs was too small to detect any effect, when we take into account other preeclampsia and eclampsia genetic data, we can conclude that fetal contributions must be important for any genetic basis of preeclampsia and eclampsia.

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