Am. J. Hum. Genet. 77:127–131, 2005

Report

Disentangling Fetal and Maternal Susceptibility for Pre-Eclampsia: A British Multicenter Candidate-Gene Study

The GOPEC Consortium

The Genetics of Pre-Eclampsia (GOPEC) collaboration aims to identify genetic factors in U.K. families affected by pre-eclampsia. A number of genetic studies have reported associations with pre-eclampsia, but attempts to replicate these findings have yielded inconsistent results. We describe the results of extensive genotyping of seven candidate genes previously reported as conferring susceptibility to pre-eclampsia. Six hundred fifty-seven women affected by pre-eclampsia and their families were genotyped at 28 single-nucleotide polymorphisms in the genes encoding angiotensinogen, the angiotensin receptors, factor V Leiden variant, methylene tetrahydrofolate reductase, nitric oxide synthase, and TNFα. Genotypes were analyzed by the transmission/disequilibrium test. Genotype risk ratios (GRRs) associated with maternal genotypes had a range of 0.70–1.16; GRRs associated with fetal genotypes had a range of 0.72–1.11. No GRR achieved the prespecified criteria for statistical significance (posterior probability >.05). We conclude that none of the genetic variants tested in this large study of strictly defined pre-eclamptic pregnancies confers a high risk of disease. The results emphasize the importance of conducting rigorously designed studies of adequate size to provide precise genetic risks with narrow confidence intervals, if overreporting of false-positive results is to be avoided.

Pre-eclampsia (MIM 189800), identified clinically by maternal hypertension and proteinuria occurring after the 20th wk of gestation, affects ~3% of pregnancies in Western populations (Lie et al. 1998). Both maternal and fetal genes appear to play an etiological role; a recent analysis of 700,000 pregnancies from the Swedish Birth Registry estimated the heritability conferred by maternal genes as 0.35 (95% CI 0.33–0.36) and that due to fetal genes as 0.20 (95% CI 0.11–0.24) (Pawitan et al. 2004).

Genes that have been implicated in pre-eclampsia include angiotensinogen (*AGT*) (Ward et al. 1993), the type 1 and type 2 angiotensin receptors (*AGTR1* and *AGTR2*) (Morgan et al. 1998; Plummer et al. 2004), tumor necrosis factor α (*TNF*) (Chen et al. 1996), endothelial nitric oxide synthase (*NOS3*) (Yoshimura et al. 2000), methylene tetrahydrofolate reductase (*MTHFR*) (Sohda et al. 1997), and the Leiden variant of coagulation factor V (*F5*) (Dizon-Townson et al. 1996). Results have not been consistently reproducible, and few studies have addressed the role of the fetal genotype. It is therefore important to establish the contribution of maternal and fetal genes in a large study with adequate statistical power to detect modest genotype relative risks with narrow CIs.

The Genetics of Pre-Eclampsia (GOPEC) study, a consortium of researchers from 10 U.K. universities, recruited women affected by pre-eclampsia and their families from 2000 to 2003. We used transmission/disequilibrium testing (TDT) to distinguish between maternal- and fetal-gene effects (Mitchell 1997) and to eliminate confounding due to population stratification (admixture). All volunteers gave informed consent for the study, which was approved by the Trent Multicentre Research Ethics Committee. White western European women with pre-eclampsia, recruited at the time of diagnosis, were eligible for participation in the study if, after the 20th wk of pregnancy, their systolic blood pressure rose to $\geqslant$140 mm Hg and their diastolic blood pressure rose to $\geqslant$90 mm Hg on two occasions measured within 24 h and if they had proteinuria >500 mg per 24 h or 2+ (1 g/liter) on dipstick testing of urine. Women who were hypertensive or had proteinuria prior to the 20th wk of pregnancy were excluded, as were those with essential hypertension, diabetes, renal or cardiac disease, or a cur-

Received March 8, 2005; accepted for publication April 19, 2005; electronically published May 11, 2005.

Address for correspondence and reprints: Dr. Linda Morgan, Institute of Genetics, Clinical Chemistry Division, Queen's Medical Centre, Nottingham, NG14 7GZ, United Kingdom. E-mail: linda.morgan@nottingham.ac.uk

0002-9297/2005/7701-0013$15.00

Table 1

Clinical Features of 627 Index Pregnancies

Feature	5th Percentile	Median	95th Percentile
Maternal age (years)	19.9	29.7	38.4
Maternal BMI (kg/m^2)	20.37	27.01	38.60
Highest SBP[a] (mm Hg)	140	161	199
Highest DBP[b] (mm Hg)	98	110	126
Highest proteinuria (g/24 h)[c]	.50	1.67	9.59
Gestation at delivery (wk)	28	37	41
Infant birth weight (kg)	.90	2.56	3.89
Birth weight percentile[d]	0	11	89

[a] SBP = systolic blood pressure.
[b] DBP = diastolic blood pressure.
[c] 24-h protein results were available for 364 women.
[d] Calculated using GROW freeware (West Midlands Perinatal Institute Web site).

rent multiple pregnancy. Pre-eclampsia was defined as unresolved if hypertension or proteinuria persisted 13 wk after delivery. Details of recruitment protocols are available at the GOPEC Web site.

DNA was extracted from venous blood from adult participants by use of Promega Wizard DNA extraction kits. Fetal DNA was extracted from umbilical cord tissue by use of Nucleon HT kits (Amersham Biosciences). Haplotype tagSNPs with minor-allele frequencies (MAF) >0.05 were selected from bins in linkage disequilibrium ($r^2 > 0.64$) with data from the SeattleSNPs database or published data (Nakajima et al. 2002; Plummer et al. 2004). tagSNP maps generated by complete-gene resequencing were available for *AGT, TNF,* and *NOS3*; this strategy for tagSNP selection is expected to capture >80% of common haplotype diversity in these genes (Carlson et al. 2004). tagSNP maps for *AGTR1* and *AGTR2* were less complete and included flanking and exonic regions only. Two SNPs in *MTHFR* and one SNP in *F5* were selected on the basis of their functional effects (Bertina et al. 1994; Frosst et al. 1995; van der Put et al. 1998). Genotyping was undertaken using *Taq*Man 5′ exonuclease probes; assay details are available on request. Nineteen percent of samples were genotyped in duplicate, and genotyping was confirmed by DNA sequencing of a random selection of samples. The genotyping failure rate was 0.8%, and the genotyping replication rate was 99.4%.

An affected woman and her parents, or one parent and one or more siblings, formed a maternal triad for TDT of maternal genes. An affected woman, her partner, and baby formed a fetal triad for testing of fetal genes. Mendelian segregation inconsistencies, identified using PedCheck (O'Connell and Weeks 1998), may be due to either miscalling of marker alleles or incorrect information about family structure (e.g., nonpaternity or unreported ovum donation); incorrect information about family structure will reduce the power of detecting true genetic effects (Gordon et al. 1999). The information from the 28 segregating markers was used to exclude relationships on the basis of two or more discrepant genotypes (Chakraborty and Stivers 1996). Under the assumption that the probability of detecting a relationship inconsistency with one typically informative biallelic marker is .15 (Jamieson and Taylor 1997) and that the prior probability of nonpaternity/nonmaternity is ⩽10%, then the posterior probability of failing to detect these inconsistent relationships using this "two strikes and you are out" rule is ⩽0.7%. Maternal or fetal triads with a single SNP showing inconsistencies with Mendelian inheritance were not included in the statistical analysis of this specific marker; we assume that isolated segregation inconsistencies can be attributed to occasional genotype miscalls. From 657 families, 14 maternal and 26 fetal triads, which had more than one SNP genotyping result that was inconsistent with Mendelian inheritance, were excluded from further statistical analysis. Six hundred twenty-seven families remained, comprising 2,504 individuals, including 398 maternal triads and 536 fetal triads. Clinical features are shown in tables 1 and 2.

No deviations from Hardy-Weinberg equilibrium in founder individuals were detected using PedStats software (Center for Statistical Genetics Web site). To analyze phase-uncertain data and to study single markers or extended marker haplotypes for linkage in the presence of gene association, the TRANSMIT program was used (Clayton 1999), which implements a score test statistic that omits terms that are sensitive to population stratification. The bootstrap option was used to empirically evaluate the significance of the test statistics. Haplotypes with an estimated frequency of ⩽1% were pooled for statistical analysis. Estimates of the genetic-effect size, expressed as genotype risk ratios (GRRs), (and standard errors of these estimates) were calculated from the proportion of transmitted risk alleles (Kazeem and Farrall 2005).

The results of genotyping are shown in table 3. In

Table 2

Comparison of 627 Index Pregnancies

Feature	No. (%) of Index Pregnancies
Maternal parity:	
Nulliparous	496 (79.1%)
Parous	131 (20.9%)
Sex of infant:	
Male	320 (51.0%)
Female	307 (49.0%)
Resolution[a]:	
Resolved	558 (89.0%)
Unresolved	69 (11.0%)

[a] Resolution = diastolic blood pressure <90 mm Hg and no proteinuria 13 wk postpartum.

Table 3

Results of Analysis of Maternal and Fetal SNP Genotypes by TDT

Gene, GenBank Reference Sequence, and HGVS[a] Name	Alternative Name	Location	Maternal Genotype				Fetal Genotype			
			MAF	GRR	95% CI	*P*	MAF	GRR	95% CI	*P*
AGT:										
AY436323:										
g.1718G→T	−1074G→T	5′ flanking	.11	.97	.74–1.27	.815	.11	1.04	.79–1.37	.785
g.2963C→T	172C→T	Intron 1	.08	1.16	.83–1.63	.392	.08	.82	.60–1.11	.197
g.3467G→A	676G→A	Intron 2	.37	.99	.82–1.19	.913	.36	.90	.75–1.08	.261
g.3826G→A	1035G→A	Intron 2	.25	1.00	.81–1.23	.997	.24	.91	.75–1.12	.375
g.6679C→T	174Thr→Met	Exon 2	.12	.84	.64–1.09	.185	.12	.92	.71–1.19	.519
g.6862T→C	235Met→Thr	Exon 2	.40	.94	.78–1.13	.505	.40	.92	.77–1.10	.349
g.8854C→A	6066C→A	Intron 3	.20	1.06	.84–1.33	.625	.19	.95	.77–1.18	.649
g.14321C→A	11535C→A	3′ UTR	.31	.95	.78–1.15	.583	.32	.99	.82–1.18	.882
AGTR1:										
AF245699.1:										
g.4955T→A	−810T→A	5′ flanking	.20	1.10	.88–1.38	.410	.19	1.08	.87–1.35	.495
g.5245C→T	−521C→T	5′ flanking	.35	.91	.75–1.09	.309	.32	.88	.73–1.05	.144
g.49465C→T	573C→T	Exon 5	.45	.98	.82–1.18	.848	.46	.95	.80–1.13	.569
g.50058A→C	1166A→C	3′ UTR	.31	1.01	.83–1.22	.957	.31	1.01	.85–1.21	.883
AGTR2:										
AY324607.1:										
g.2184A→T	333A→T	Intron 2	.28	***.72***	***.52–.98***	***.039***	.26	1.05	.79–1.40	.720
g.4673G→T	2812G→T	3′ UTR	.22	***.70***	***.48–1.00***	***.054***	.22	***.72***	***.52–.98***	***.038***
g.4679G→A	2818G→A	3′ UTR	.46	1.09	.80–1.47	.590	.44	1.06	.82–1.37	.655
F5:										
NM_000130:										
c.1691G→A	Leiden	Exon 10	.02	1.09	.59–2.07	.779	.02	.85	.47–1.48	.561
MTHFR:										
AY338232:										
g.8747C→T	677C→T	Exon 4	.33	1.04	.85–1.26	.723	.34	1.05	.88–1.25	.609
g.10649A→C	1298A→C	Exon 7	.30	.92	.76–1.12	.419	.29	.87	.72–1.05	.141
NOS3:										
AF519768:										
g.450T→A	−1474T→A	5′ flanking	.39	1.12	.93–1.35	.248	.38	.98	.82–1.17	.824
g.3497G→A	…	Intron 2	.14	1.13	.87–1.48	.355	.15	1.11	.86–1.43	.431
g.7164G→T	298Glu→Asp	Exon 7	.35	.93	.77–1.12	.455	.35	1.06	.88–1.28	.529
g.9932G→A	…	Intron 11	.46	1.03	.86–1.23	.724	.43	.87	.73–1.03	.116
g.13834C→A	…	Intron 13	.36	.96	.80–1.16	.676	.37	1.05	.88–1.26	.557
g.17971A→G	…	Intron 19	.24	.88	.71–1.08	.220	.23	.88	.72–1.08	.215
TNF:										
AY066019:										
g.282G→A	−308G→A	5′ flanking	.20	1.01	.81–1.26	.954	.18	.92	.73–1.14	.437
g.1893A→G	…	Intron 3	.08	.86	.62–1.19	.362	.08	***.73***	***.53–.99***	***.044***
g.4101G→A	…	3′ flanking	.15	.98	.76–1.25	.860	.12	.85	.66–1.10	.220
g.4765G→T	…	3′ flanking	.08	1.16	.82–1.65	.402	.08	1.04	.75–1.43	.833

Note.—Results with statistical probability ⩽.05 are shown in bold italics.

[a] HGVS = Human Genome Variation Society.

maternal triads, two SNPs in *AGTR2,* g.2184A→T and g.4673G→T, demonstrated marginally decreased transmission of the minor allele ($P = .039$ and $P = .054$, respectively). Maternal GRRs associated with minor variants at the remaining 26 SNPs fell within the range of 0.84–1.16. Analysis of fetal triads demonstrated weak evidence of transmission disequilibrium at g.4673G→T in *AGTR2* ($P = .038$) and at g.1893A→G in *TNF* ($P = .044$). GRRs associated with fetal genotype at the remaining 26 SNPs were in the range of 0.82–1.11.

The results of haplotype analysis are shown in table 4. There was weak support for transmission disequilibrium in fetal triads with *TNF* haplotypes ($P = .026$) and with the *AGT* haplotypes defined by g.6862T→C (235Met→Thr) and g.3467G→A, with the common 235Met-g.3467G allele being overtransmitted ($P = .027$).

We have analyzed our gene-association results within a Bayesian framework (Colhoun et al. 2003; Wacholder et al. 2004), which interprets the statistical significance of the results in the context of the prior probability that a candidate gene encodes disease susceptibility. The study was designed to detect moderately sized gene associations; the cohorts of 398 maternal trios and 536 fetal

Table 4

Results of Analysis of Maternal and Fetal Haplotypes

	Haplotype Analysis					
	Maternal			Fetal		
Gene	χ^2	df	*P*	χ^2	df	*P*
AGT[a]	3.56	3	.217	9.41	3	***.027***
AGTR1	6.78	9	.773	11.11	9	.305
AGTR2	6.12	3	.106	4.77	3	.189
MTHFR	2.30	3	.280	3.22	3	.173
NOS3	13.95	11	.232	19.86	11	.237
TNF	1.64	5	.898	10.49	5	***.026***

Note.—*P* values are empirical values generated using the bootstrap option of the TRANSMIT software. Results with statistical probability ≤.05 are shown in bold italics.

[a] The computational complexities of estimating 8-marker haplotypes precluded the use of all markers for *AGT* in a single analysis. All possible pairs of markers for this gene were examined, and the most significant associations are shown. Markers are g.2963C→T and g.8854C→A (maternal haplotypes) and g.6862T→C (235Met→Thr) and g.3467G→A (fetal haplotypes). Addition of a third marker did not increase statistical significance.

trios have 85% power to detect common (MAF = 0.5) susceptibility genes with GRRs of 1.6 and 1.5, respectively, with the assumption of a type 1 error rate of 0.0005. This nominal level of significance is sufficiently stringent for detection at the 5% level of noteworthy effects of candidate genes with modest prior probabilities (≥.01). The power of the study will inevitably be reduced for smaller GRRs, rarer SNPs, low levels of linkage disequilibrium between markers and susceptibility allele(s), or reduced prior probabilities (<.01) of gene association.

None of the examined individual SNPs or haplotypes achieved statistical significance by use of these criteria (posterior probability >.05). Genotypes and haplotypes were examined in the subgroup of nulliparous pregnancies in which hypertension and proteinuria had resolved by 13 wk postpartum, comprising 296 maternal triads and 383 fetal triads. GRRs were similar to those in the full data set, and none achieved statistical significance (posterior probability >.05).

This study of a large and very precisely phenotyped group of pregnancies excludes major risks associated with a number of SNPs in candidate genes that have dominated studies of the genetics of pre-eclampsia. The challenge remains to identify susceptibility genes that will provide greater understanding of the pathogenesis of pre-eclampsia. It is essential to study large numbers of affected women and their babies if misleading results are to be avoided. The formation of national or international consortia is one way forward. In addition, the adoption of clear definitions of phenotype and inclusion and exclusion criteria will facilitate meta-analysis of replicated studies.

Acknowledgments

We thank patients and their families, midwives, and doctors, whose support made the study possible. We are grateful to the British Heart Foundation for their generous funding. L. Morgan (Institute of Genetics, University of Nottingham) and M. Farrall (Wellcome Trust Centre for Human Genetics, Oxford) wrote the article, which was approved by all collaborators: P. N. Baker (University of Manchester); F. Broughton Pipkin, N. Kalsheker, S. O'Malley, M. Henfrey, S. Arulkumaran, and I. Symonds (University of Nottingham); A. Cameron, A. Dominiczak, M. McDade, W. Kwong Lee, and J. McCulloch (University of Glasgow); M. Caulfield (Bart's and the London, Queen Mary's School of Medicine and Dentistry); M. Habiba and C. Dodd (University of Leicester); M. Kilby and L. Davies (University of Birmingham); S. Macphail (University of Newcastle); P. M. S. O'Brien (University of Keele); K. O'Shaughnessy, B. Newcombe, and P. de la Salle (University of Cambridge); C. Redman and P. Jarrett (University of Oxford); M. de Swiet, C. Williamson, E. Byford, and F. Cheng (Imperial College London); J. J. Walker and L. Samwiil (University of Leeds); G. Chapman (University Hospital of North Staffordshire); E. Dennehy (Derby Hospitals Trust); R. Keys and S. Bjornsson (Glasgow Princess Royal Hospital); C. Mercer and M. Mohajer (Royal Shrewsbury Hospital); G. Thompson (Newcastle Royal Victoria Hospital); M. N. Fitzgibbon (Wordsley Hospital); G. Hackett (Cambridge Rosie Maternity Hospital); K. Hinshaw (Sunderland Royal Hospital); B. Lim (Hinchingbrooke Hospital); D. T. Y. Liu (Nottingham City Hospital); W. Mackenzie (Birmingham Heartlands Hospital); M. Selinger (Royal Berkshire Hospital); I. Scudamore (Leicester General Hospital); C. Sparey (Leeds General Infirmary); D. Tuffnell (Bradford Royal Infirmary); S. Ward (Kings Mill Hospital); J. Waugh (Leicester Royal Infirmary); and D. Williams (Chelsea and Westminster Hospital).

Web Resources

The URLs for data presented herein are as follows:

Center for Statistical Genetics, http://www.sph.umich.edu/csg/abecasis/PedStats/ (for PedStats)
GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for the reference sequence numbers included in table 3)
GOPEC, http://www.gopec.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for pre-eclampsia)
SeattleSNPs, http://pga.gs.washington.edu/
West Midlands Perinatal Institute, http://www.perinatal.nhs.uk/ (for GROW freeware)

References

Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369:64–67

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA (2004) Selecting a maximally informative set of single-

nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 74:106–120

Chakraborty R, Stivers DN (1996) Paternity exclusion by DNA markers: effects of paternal mutations. J Forensic Sci 41:671–677

Chen G, Wilson R, Wang SH, Zheng HZ, Walker JJ, McKillop JH (1996) Tumour necrosis factor-alpha (TNF-α) gene polymorphism and expression in pre-eclampsia. Clin Exp Immunol 104:154–159

Clayton D (1999) A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. Am J Hum Genet 65:1170–1177

Colhoun HM, McKeigue PM, Davey Smith G (2003) Problems of reporting genetic associations with complex outcomes. Lancet 361:865–872

Dizon-Townson DS, Nelson LM, Easton K, Ward K (1996) The factor V Leiden mutation may predispose women to severe pre-eclampsia. Am J Obstet Gynecol 175:902–905

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 10:111–113

Gordon D, Matise TC, Heath SC, Ott J (1999) Power loss for multiallelic transmission/disequilibrium test when errors introduced: GAW11 simulated data. Genet Epidemiol Suppl 17:S587–S592

Jamieson A, Taylor SS (1997) Comparisons of three probability formulae for parentage exclusion. Anim Genet 28:397–400

Kazeem G, Farrall M (2005) Integrating case-control and TDT studies. Ann Hum Genet 69:329–335

Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie-Nielsen E, Irgens LM (1998) Fetal and maternal contributions to the risk of pre-eclampsia: population based study. BMJ 316: 1343–1347

Mitchell LE (1997) Differentiating between fetal and maternal genotypic effects, using the transmission test for linkage disequilibrium. Am J Hum Genet 60:1006–1007

Morgan L, Crawshaw S, Baker PN, Brookfield JFY, Broughton-Pipkin F, Kalsheker N (1998) Distortion of maternal-fetal angiotensin II type 1 receptor transmission in pre-eclampsia. J Med Genet 35:632–636

Nakajima T, Jorde LB, Ishigami T, Umemura S, Emi M, Lalouel J-M, Inoue I (2002) Nucleotide diversity and haplotype structure of the human angiotensinogen gene in two populations. Am J Hum Genet 70:108–123

O'Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63:259–266

Pawitan Y, Reilly M, Nilsson E, Cnattingius S, Lichtenstein P (2004) Estimation of genetic and environmental factors for binary traits using family data. Stat Med 23:449–465

Plummer S, Tower C, Morgan L, Alonso P, Baker P, Broughton-Pipkin F, Kalsheker N (2004) Haplotypes of the angiotensin II receptor genes in women with normotensive pregnancy and women with pre-eclampsia. Hum Mutat 24:14–20

Sohda S, Arinami T, Hamada H, Yamada N, Hamaguchi H, Kubo T (1997) Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. J Med Genet 34:525–526

van der Put NMJ, Gabreëls F, Stevens EMB, Smeitink JAM, Trijbels FJM, Eskes TKAB, van den Heuvel LP, Blom HJ (1998) A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 62:1044–1051

Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N (2004) Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 96:434–442

Ward K, Hata A, Jeunemaitre X, Helin C, Nelson L, Namikawa C, Farrington PF, Ogasawara M, Suzumori K, Tomoda S, Berrebi S, Sasaki M, Corvol P, Lifton RP, Lalouel J-M (1993) A molecular variant of angiotensinogen associated with preeclampsia. Nat Genet 4:59–61

Yoshimura T, Yoshimura M, Tabata A, Shimasaki Y, Nakayama M, Miyamoto Y, Saito Y, Nakao K, Yasue H, Okamura H (2000) Association of the missense Glu298Asp variant of the endothelial nitric oxide synthase gene with severe preeclampsia. J Soc Gynecol Investig 7:238–241