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Thrombophilic polymorphisms in pre-eclampsia: altered frequency of the functional 98C>T polymorphism of glycoprotein IIIa

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EDITOR—Pre-eclampsia is a heritable endothelial disorder, which is unique to pregnancy¹ and characteristically associated with haemostatic and thrombophilic abnormalities. This association has led to the identification of a number of gene variants that might confer thrombophilic risk in pre-eclampsia. An increased carrier rate for two of these polymorphisms, factor V Leiden² and the thermolabile variant of methylenetetrahydrofolate reductase (MTHFR),³ has been reported in some women with pre-eclampsia. However, we have been unable to replicate these findings in our own East Anglian population.⁴

We have now looked at two further candidate thrombophilic polymorphisms in our pre-eclampsia cohort that are involved in the regulation of vascular thrombosis. The first is the 20210G>A polymorphism in the 3' UTR region of the prothrombin (PT) gene that causes a modest rise in plasma prothrombin levels,⁵ and is reportedly associated with severe pre-eclampsia in an Israeli cohort.⁶ The second is a coding variant (98C>T) in exon 2 of the *GPIIIa* gene that provides the common beta subunit for several β 3-integrins including the platelet fibrinogen receptor. This polymorphism causes a 33Leu>Pro substitution and the existence of two antigenically distinct forms of the mature GPIIb/IIIa antigen on platelets (the Pl(A) antigens 1 and 2). Loss of functional GPIIIa is associated with a rare bleeding disorder (Glanzmann's thrombasthenia) and the 33Pro variant itself has been associated with risk of premature acute coronary syndromes and stroke in young white women.⁷

Materials and methods

The method of recruitment and the definition of pre-eclampsia used in our cohort have been published previously.⁴ In brief, they all had proteinuric pregnancy related hypertension as defined by the criteria of Redman and Jefferies.⁸ The local ethics committee approved the study and all patients gave written informed consent before taking part. At the time of this study, we had genomic DNA available from 356 women with pre-eclampsia. All the women were white northern Europeans. The 200 normotensive control samples were those

reported in our earlier study⁴ and had no history of pregnancy induced hypertension or pre-eclampsia.

Genomic DNA was obtained from venous blood using standard methods and genotyped for the two polymorphisms using PCR/RFLP as follows.

GPIIIa C1565T POLYMORPHISM

Exon 2 of the *GPIIIa* gene was amplified using forward (5' TCT GAT TGC TGG ACT TCT CTT) and reverse (5' TCT CTC CCC ATG GCA AAG AGT) intronic primers (Genbank accession No AH00311). The 266 bp product was digested with 10 U of *NciI* (New England Biolabs) overnight and fragments resolved on a 3% Metaphor[®] gel (FMS Bioproducts, Rockport, USA).

GENOTYPING OF THE *PT* G20210A POLYMORPHISM

The 3' UTR region of the *PT* gene was amplified using previously reported forward (5' TCT AGA AAC AGT TGC CTG GC) and reverse (5' CAA CGC CTG GTA TCA AAT GG) primers.⁵ The 345 bp PCR product was digested overnight with 10 U of *HindIII* (New England Biolabs). Fragments were again resolved using a 3% Metaphor[®] gel.

The genotype and allele frequencies were compared by χ^2 analysis. Hardy-Weinberg equilibrium was tested using the HWE program of the LINKUTIL package (downloaded from <http://linkage.rockefeller.edu/ott/linkutil.htm>). Odds ratios and confidence intervals were calculated by the method of Wolf.⁹ A p value of <0.025 (0.05/2) was considered significant to reflect a necessary Bonferroni correction.

Results

The genotyping results for the two polymorphisms are shown in table 1. Both polymorphisms were in Hardy-Weinberg equilibrium in both groups of subjects. The carrier rate for the prothrombin A20210 variant was not significantly different between our control and pre-eclamptic groups and no subjects in either group were homozygous for the A20210 variant. There was no evidence that the

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G20210A polymorphism was a severity marker within the pre-eclampsia group, 5/149 (3.4%) delivering before 37 weeks of gestation and 2/42 (4.8%) with severe disease (defined as having HELLP syndrome or an eclamptic seizure) were A20210 carriers. These were not significantly different frequencies from the pre-eclampsia group as a whole.

For the glycoprotein IIIa polymorphism, there was a significant excess of C98 homozygotes in the pre-eclampsia group (18/356) versus controls (1/200, $p < 0.01$) with a corresponding excess of the C98 allele ($p < 0.02$). Expressing the relative risk as odds ratios: for C98 versus T98 homozygosity it was 11.3 (95% CI 1.5–85.5), and for carriage of at least one C98 allele it was 1.4 (95% CI 1.0–2.1). In the pre-eclampsia group, 9/149 (6.0%) delivering before 37 weeks and 3/42 with severe disease (see above) were C98 homozygotes (7.1%). Again, these were not significantly different frequencies from the pre-eclampsia group as a whole.

Discussion

In our population, the carrier frequency for the A20210 prothrombin gene variant was within the range previously reported for white populations of 0.7–4%.¹⁰ However, our results contrast sharply with previous reports that carriage of the A20210 variant is commoner in women with pre-eclampsia.^{6,7} These studies found a three to four-fold increase in the frequency of this thrombophilic variant, although the numbers of subjects studied were small, consisting of just 34 subjects with pre-eclampsia compared to over 350 in our current study group.⁶ Differences in the severity of pre-eclampsia between studies is unlikely to be important since the carrier rate for A20210 within our own sample was not affected by severity, expressed either as premature delivery, or the presence of HELLP syndrome, or eclamptic seizure as used in the study of Kupferminc *et al.*⁶ Interestingly, the most recent report from Kupferminc's group, using an increased sample size, has failed to replicate the original findings in patients with severe pre-eclampsia.¹² This work is in keeping with our current negative results and suggests that the original publication may have represented a type I error. Even supposing the G20210A is a susceptibility gene variant for pre-eclampsia, its role would be restricted to certain ethnic groups; it is virtually absent, for example, in non-white populations.¹⁰

In contrast, we did see an excess of homozygotes for the T98 allele of *GP1IIa* (17 in the pre-eclamptics versus 1 in the controls, table 1)

and overall the frequency of the T98 allele was significantly increased in our pre-eclamptic subjects (20.1% versus 14%, $p < 0.01$). This raises the intriguing possibility that the *GP1IIa* C98 polymorphism may predispose women to pre-eclampsia. Previous association studies with this polymorphism have modelled its effects as a dominant allele, largely reflecting the bias of early studies that could type on the basis of the presence or absence of the Pl(A)1 and Pl(A) 2 surface antigens. Expressing our data in this fashion gives an odds ratio for women carrying at least one T98 allele of 1.4. Although this appears a rather modest effect, it is similar to that previously published for the factor V Leiden and MTHFR variants in women with pre-eclampsia.^{2,3}

The *GP1IIa* gene codes for the $\beta 3$ subunit of the $\beta 3$ integrin subfamily; this consist of $\alpha IIb\beta 3$ (glycoprotein IIb/IIIa) and $\alpha v\beta 3$. The glycoprotein IIb/IIIa receptor is confined to platelets and megakaryocytes and has a key role in platelet aggregation by binding fibrinogen and von Willebrand factor. In contrast, the $\alpha v\beta 3$ integrin is a widely expressed receptor for ligands such as vitronectin and fibronectin. It appears to be an important player in tumour angiogenesis, but is also expressed by invading trophoblast suggesting a role in placentation.¹³ Indeed, mice knockouts in which the $\beta 3$ integrin is deficient have normal implantation but develop a characteristic placental defect.¹⁴ The $\beta 3$ integrin has also been implicated in the failure of the cytotrophoblast to adopt a vascular phenotype in pre-eclampsia.¹⁵

Previous epidemiological work on the C98T *GP1IIa* polymorphism has focused on its role as a risk allele for arterial thrombosis, specifically for acute coronary syndromes. Weiss *et al.*¹⁶ initially reported that carriage of the T98 variant of *GP1IIa* was a risk factor for myocardial infarction in young patients. This has been confirmed in a large cohort of sib pairs with premature heart disease and probably explains why some studies have failed to replicate the original work of Weiss *et al.*¹⁶ because they used elderly patient cohorts.¹⁷ Clear evidence now exists that the T98 *GP1IIa* allele that carries the thrombotic risk is functionally distinct from the C98 wild type receptor. This is apparent both in terms of the level of IIb/IIIa expression on activated platelets as well as in the adhesion properties of cells expressing $\alpha v\beta 3$.^{18,19} Given the importance of platelet and trophoblast function in the pathogenesis of pre-eclampsia, the *GP1IIa* gene is a very attractive biological candidate gene for pre-eclampsia and the functional C98T polymorphism in particular. Our findings here that the thrombotic risk variant, T98, is commoner in pre-eclamptic women suggests that we may have identified a new risk allele for the disease.

In summary, we have not been able to show any association of the prothrombin polymorphism, G20210, with pre-eclampsia. There is significant excess, however, of the thrombotic variant of the $\beta 3$ integrin protein *GP1IIa* in the same population. This result needs confirmation in other populations, preferably using robust family based association studies, but it

Table 1 Comparison of the frequencies of the genotypes and alleles for the TP 20210 and *GP1IIa* C98T polymorphisms in subjects with pre-eclampsia versus controls

	No (% frequencies) of individual genotypes			No (% frequencies) of alleles	
<i>TP 20210A</i>	<i>GG</i>	<i>GA</i>	<i>AA</i>	<i>G</i>	<i>A</i>
Pre-eclampsia	342 (96.1)	14 (3.9)	0 (0)	698 (98.0)	14 (2.0)
Controls	197 (98.5)	3 (1.5)	0 (0)	397 (98.9)	3 (1.1)
<i>GP1IIa C98T</i>	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>
Pre-eclampsia	231 (64.9)	107 (30.0)	18 (5.1)	569 (79.9)	143 (20.1)
Controls	145 (72.5)	54 (27.0)	1 (0.5)*	344 (86.0)	56 (14.0)**

* $p < 0.01$, ** $p < 0.02$.

suggests that integrin polymorphisms might be an important new focus for studies on genetic risk factors for pre-eclampsia.

- This study investigated the role of two thrombophilic gene polymorphisms, prothrombin 20210G>A and the 98C>T variation in the $\beta 3$ integrin glycoprotein IIIa (GPIIIa), as risk factors for pre-eclampsia in an East Anglian cohort of 356 affected women.
- In contrast to an earlier report, we found no excess of carriers for the prothrombin 20210A variant in our cohort. However, for the GPIIIa 98C>T polymorphism there was an excess of 98T homozygotes in our pre-eclampsia group (18/356) versus controls (1/200, $p < 0.01$). The odds ratio for 98T homozygotes was 11.3 (95% CI 1.5–85.5) and for carriage of at least one T98 allele, 1.4 (95% CI 1.0–2.1).
- These data suggest that carriage of the 98T polymorphism of GPIIIa may be a previously unidentified risk factor for pre-eclampsia.

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Cystic fibrosis patients with the 3272-26A>G splicing mutation have milder disease than F508del homozygotes: a large European study

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EDITOR—Cystic fibrosis (CF, MIM 219700) is a common, severe, autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene cloned in 1989.^{1–3} The disease, characterised by chronic

lung disease which is the main cause of morbidity and mortality, pancreatic dysfunction, raised electrolyte levels in sweat, and male infertility, is caused by altered chloride (Cl[−]) secretion across the apical membrane of epithelial cells.⁴ There