Mutations in prothrombin and factor V genes do not contribute significantly to placental vasculopathy in a high-risk patient cohort in South Africa

To the Editor: During normal pregnancy there are dramatic changes in the coagulation and fibrinolytic systems. There is deposition of fibrin in the uteroplacental walls and fibrinolysis is suppressed. An increase in levels of clotting factors VII, VIII and X and a doubling in the levels of fibrinogen are observed. The end result is the well-described hypercoagulability of pregnancy, protecting the mother against blood loss at delivery, but also predisposing her to possible thrombotic complications. Naturally occurring anticoagulants including antithrombin III and the protein C-thrombomodulin-protein S complex protect against generalised thrombosis. Protein C (with its co-factors protein S and thrombomodulin) inactivates factors V and VIII. Abnormal forms of factor V, such as those arising from DNA mutation, resist such inactivation and thrombosis can result.

The association between placental infarcts and adverse pregnancy outcome, especially midtrimester pregnancy loss and the development of pre-eclampsia, is well described in the antiphospholipid syndrome. Progressive thrombosis and infarcts in the placenta predispose to pregnancy complications such as intrauterine growth restriction (IUGR), pre-eclampsia and abruptio placentae. It is therefore reasonable to anticipate that other forms of thrombophilia could lead to adverse pregnancy outcome.

One such inherited condition is activated protein C resistance (APCR). Coagulation factor V (FV) acts as co-factor for activated factor X to activate prothrombin in the coagulation cascade. FV is normally cleaved by protein C rendering it inactivated. The gene encoding human FV contains 25 exons (ranging from 72 to 2820 base pair (bp) in size) and spans more than 80 kilobase pair (kb). A mutation in exon 10 (A1691G) of the gene was described,1 which results in the substitution of an arginine with a glycine residue and abolishes the site of protein C cleavage. This variant was named the FV Leiden mutation, after the city in which it was discovered.

The association between FV Leiden mutation and pre-eclampsia was first reported in a small patient cohort with severe early-onset pre-eclampsia.2 In a larger study3 of 158 women with severe pre-eclampsia, a mutation frequency of 8.9% was reported compared with a control group frequency of 4.2% (χ² = 4.686, P = 0.03). All patients were heterozygous for the mutation.2 Since then several contradictory reports regarding this apparent association have appeared in the literature.

In 1996, a g → a transition at position 20210 of the 3′ untranslated region of the gene encoding prothrombin was described. This mutation (G20210A) could be associated with elevated plasma prothrombin levels and an increased risk of venous thrombosis.4 Subsequently, independent associations with pre-eclampsia were reported, including a study of 110 women with serious pregnancy complications (abruptio placentae, severe pre-eclampsia, and IUGR). The incidence of the G20210A mutation was reported to be 10% compared with 3% in a control panel (P < 0.03).5 However, globally the frequency of this mutation (and that of FV Leiden) demonstrates strong population specificity.

Within the venous thrombosis patient population of the Western Cape region of South Africa, the frequency of the FV Leiden mutation is 26% among whites, 8% in coloureds (mixed ancestry) and apparently absent in black South Africans.4 The frequency of the prothrombin mutation in unknown.

The broad spectrum of mutation frequency within different populations together with the high incidence of placental vasculopathy in the study cohort prompted an investigation into the contribution of these mutations to pre-eclampsia in the pregnant population in the Western Cape. The three study groups comprised 50 primi- and 50 multigravida women with: (i) early onset severe pre-eclampsia; (ii) late onset pre-eclampsia; or (iii) pregnancy-induced hypertension. A further 50 women with abruptio placentae and a control group (50 individuals with uncomplicated pregnancy outcome) were also included.

Heterozygosity for FV Leiden and the G20210A prothrombin gene mutation was identified in 5 (1.4%) and 2 (0.6%) patients, respectively (Table I). No homozygote mutant status could be demonstrated. No mutation was detected in any individual in the control group. All mutation-positive patients had an uneventful postpartum course and none developed any sign of thrombotic complication.

The incidence of the thrombotic mutations is extremely rare in the population studied suggesting that it is not a significant contributing factor in the development of pre-eclampsia. Antenatal screening for inherited thrombophilia in these patients (coloured and black South Africans) is therefore not warranted. It remains to be seen whether these mutations are more common in white South Africans with pre-eclampsia.

The contribution of inherited thrombophilia to placental infarctions and pre-eclampsia suggests that there may be other, as yet unknown, underlying hypercoagulability factors that play a role in these conditions. It may also imply that the real cause of placental vasculopathy lies elsewhere, and that a tendency to increased clotting only un_masks this condition earlier in susceptible patients.

The authors thank Erika van Papendorp for her diligent and untiring help in recruiting patients for the study. The University of Stellenbosch and the Medical Research Council are gratefully acknowledged for their support. R Hillermann is a recipient of a post-doctoral fellowship at the University of Stellenbosch.

Disease-association studies are usually more significant when carried out on well-stratified patients from a homogeneous
population. Characterising sequence variants in the LDLR gene in the South African Afrikaner population is an example. Pre-eclampsia affects women of all ethnic groups, but for this study mainly coloured (representing San, Khoi, African Negro, Madagascan, Javanese and Western European origins) patients, who fall within our hospital catchment area, were included. Restricting the analysis to a single group should therefore increase the resolution/power of the study.

Renate Hillermann
G Stefan Gebhardt
Rochelle Isaacs
D Wilhelm Steyn
Hein J Odendaal

Medical Research Council Research Unit for Perinatal Mortality
Department of Obstetrics and Gynaecology
University of Stellenbosch
Tygerberg, W Cape

Table I. Demographic details of mutation-positive patients. A total of 400 individuals were screened for the presence of these mutations.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Race</th>
<th>History</th>
<th>Mutation</th>
<th>Complication</th>
<th>Cigarette smoker</th>
<th>Gestation at delivery (weeks)</th>
<th>Birth mass (g)</th>
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<tr>
<td>36</td>
<td>C</td>
<td>G4P2</td>
<td>A1691G</td>
<td>PE</td>
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<td>3794</td>
</tr>
<tr>
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<td>A1691G</td>
<td>PE</td>
<td>Yes</td>
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<td>3260</td>
</tr>
<tr>
<td>30</td>
<td>C</td>
<td>G2P1</td>
<td>A1691G</td>
<td>AP</td>
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<tr>
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<td>AP</td>
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<td>34</td>
<td>1250</td>
</tr>
<tr>
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<td>PIH</td>
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<td>Severe PE</td>
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<td>30</td>
<td>1208</td>
</tr>
<tr>
<td>17</td>
<td>B</td>
<td>G1P0</td>
<td>G20210A</td>
<td>Severe PE</td>
<td>No</td>
<td>32</td>
<td>1722</td>
</tr>
</tbody>
</table>

C = coloured/mixed ancestry; B = black Africans; G = gravida; P = para; PE = pre-eclampsia; AP = abruptio placenta; PIH = pregnancy-induced hypertension; A1691G = factor V Leiden mutation; G20210A = prothrombin mutation.

central Africa or *rhodesiense* from eastern southern Africa, with the latter form progressing more rapidly through febrile illness to meningoencephalitis.\(^2,3\)

A recently encountered case of trypanosomiasis is reported with life-threatening multiple organ dysfunction.

A 56-year-old woman from the USA had been bitten on the lower leg while visiting a game reserve in Tanzania. She had arrived in Africa via Nairobi, and travelled through various game reserves in Kenya and Tanzania. On board a cruise ship from Mombasa to Cape Town she became severely ill with a septic ulcer on her ankle, the site of the bite, and subsequently with pyrexia and a skin rash. The skin lesion was treated topically and the ship’s doctor gave her intravenous (IV) ceftriaxone.

There was no apparent past medical history and she had been on mefloquine for malaria prophylaxis. Her condition deteriorated and on arrival in Cape Town she was admitted to hospital with a septicaemic illness characterised by high fever and a diffuse purpuric rash over her entire body.

Although initially conscious, she rapidly became confused and stuporous and renal function deteriorated, with creatinine rising to 450 μmol/L. Respiratory distress developed, as did clinical jaundice, with biochemical evidence of acute hepatitis.

Even though a tropical disease was suspected the initial diagnosis was a rickettsial illness. Her full blood count showed features of a disseminated intravascular coagulation (DIC) with thrombocytopenia but routine microscopic examination of the peripheral blood slide by the haematologist fortuitously identified large numbers of trypanosomes (Fig. 1).

After extensive consultation suramin sodium\(^1\) was obtained and commenced within 24 hours of the diagnosis. Further literature also confirmed usefulness of pentamidine\(^8\) and this was added to her regimen but discontinued after 5 days.

The patient’s condition deteriorated and she was transferred to another hospital for further management. Because of the notoriously bad prognosis of this disease and from previous experience with severe malaria, it was decided to continue

TRYPANOSOMIASIS — AN UNUSUAL CAUSE OF REVERSIBLE MULTIPLE ORGAN DYSFUNCTION IN SOUTH AFRICA

To the Editor: It is recognised that American trypanosomiasis or Chagas’ disease causes morbidity and mortality among poor people in developing countries, but it is not as widely recognised as it should be in Europe and the USA. A similar argument applies to African trypanosomiasis, where bites from the tsetse fly can transmit the *Brucel gambiense* from western Africa or *rhodesiense* from eastern southern Africa, with the latter form progressing more rapidly through febrile illness to meningoencephalitis.\(^2,3\)

There was no apparent past medical history and she had been on mefloquine for malaria prophylaxis. Her condition deteriorated and on arrival in Cape Town she was admitted to hospital with a septicaemic illness characterised by high fever and a diffuse purpuric rash over her entire body.

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The patient’s condition deteriorated and she was transferred to another hospital for further management. Because of the notoriously bad prognosis of this disease and from previous experience with severe malaria, it was decided to continue
treatment with IV suramin but to introduce plasma exchange to increase clearance of the parasite and toxins from the blood.

Shortly after transfer adult respiratory distress syndrome developed. The patient required ventilation as well as continuous haemodialysis (CVVHHD) for deteriorating renal failure.

After about 10 days she started improving, her lungs cleared and she was weaned off ventilation. Her urine output improved and dialysis was reduced and then stopped. Mental state rapidly returned to normal.

Although a lumbar puncture was not done initially, cerebrospinal fluid (CSF) on day 13 of treatment was abnormal with a protein of 0.73 g/1, 13 lymphocytes and 18 red blood cells per microlitre. No trypanosomes were seen in the CSF.

The ulcer on the leg remained large and purulent with central necrosis and required surgical debridement, but thereafter it began to heal, although final skin closure did require a small skin graft.

It is concluded that there is a need for greater awareness of the risk of serious tropical diseases manifesting after touring in tropical countries.

We acknowledge Dr John O'Brien for referring the patient and initial excellent clinical management. We thank Professor Erna Mansvelt from Tygerberg Hospital for the photomicrographs.

Lucille Wood
Derek Miller
Peter Jacobs

Department of Haematology and Bone Marrow Transplantation Unit
Constantiaberg Medi-Clinic
Cape Town

Erna Mansvelt
Department of Haematological Pathology
Tygerberg Academic Hospital and University of Stellenbosch
Tygerberg, W Cape


SYMPTOMS OF THE BITE OF AN ORB-WEB SPIDER ARANEUS APRICUS (ARANEAE: ARANEEIDAE)

To the Editor: Research on the toxicity of southern African spiders has concentrated mainly on species of known venomous genera, namely the button spiders Latrodectus (Theridiidae), the sac spiders Cheiracanthium (Miturgidae), the violin spiders Loxosceles and the six-eyed crab spiders Sicarius (both Sicariidae).

Spider venom can be categorised into three main types based on the general symptoms effected: cytotoxic venom, which causes swelling and formation of lesions; neurotoxic venom, which affects the transport of neural impulses and consequently cardiovascular, respiratory and muscular functioning; and haemotoxic venom, which causes degradation of blood cells. The venom of some species of the aforementioned genera is severely toxic; in some Sicarius species it is cytotoxic-haemotoxic and in Latrodectus indistinctus O-P-Cambridge it is neurotoxic so that a bite may be fatal to humans, although no fatalities have been reported yet. The effects of the venom of cytotoxic spiders have been extensively studied, while the venom characteristics and symptoms of the button spiders and their relatives have also received attention. Here the symptoms of the bite of an orb-web spider, Araneus apricus (Karsch) (Araneae: Araneidae), are briefly described.

Arboreal spiders were collected from dense subtropical bush in the Ndumu Game Reserve in northern KwaZulu-Natal during July 2000 using the beating and hand-to-jar methods. An adult female A. apricus specimen collected proved to be
The *A. apricus* female collected had a bright lime-green oval abdomen and reddish cephalothorax with cream-coloured setae, and the typical *Araneus* body shape. The spider was deposited as a voucher specimen in the National Collection of Arachnida, Agricultural Research Council (ARC) Plant Protection Research Institute, Pretoria. Orb-weavers collected from northern KwaZulu-Natal fitting the above description are likely to be this particular species. This species has a wide distribution in north-eastern South Africa, and has been collected from Nelspruit, Letaba (where it is common in citrus, macadamia and avocado orchards), Acornhoek, Nylsvlei, Naboomspruit, the Kruger National Park, and in gardens in Pretoria, Johannesburg and Rustenburg (Dr Ansie Dippenaar-Schoeman — personal communication). It is therefore found in close association with humans on farms, in gardens and in rural areas and the possibility of bites occurring does exist.

Observations at Ndumu in July and November/December 2000 showed that the species is much more abundant in summer months and will more likely be encountered at this time. *A. apricus* appears to be largely restricted to tree layers more than 2 m above the ground, but was occasionally collected in orb webs constructed between shrubs at heights of 1.5 m.

The bite of *A. apricus* cannot be regarded as being as severe as that of *L. indistictus,* and no tissue necrosis resulted from the bite. The muscular pains caused by *A. apricus* bites can probably be relieved using painkillers. It seems unlikely that antivenom is needed, owing to the mild reaction to the bite. Many of the signs and symptoms described for *L. geometricus* and *L. kochi* were experienced in this case, but diagnosis can be distinguished by a lack of burning at the bite site and shorter duration and mildness of symptoms in *A. apricus.* However, it is necessary to note that the bite of some araneids can result in severe muscular pains and general discomfort and handling them should therefore be avoided.

My sincerest thanks to Dr Ansie Dippenaar-Schoeman (ARC-Plant Protection Research Institute, Pretoria) for identifying the spider, supplying distribution data and her comments on this report.

Charles R Haddad
Department of Zoology and Entomology
University of the Free State
Bloemfontein