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 study[3] of 158 women with severe pre-eclampsia, a mutation frequency of 8.9% was reported compared with a control group frequency of 4.2% ($\chi^2 = 4.686$, $P = 0.03$). All patients were heterozygous for the mutation.[3] 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.[6] 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 unmasks this condition earlier in susceptible patients.

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Disease-association studies are usually more significant when carried out on well-stratified patients from a homogeneous

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

Age (yrs)	Race	History	Mutation	Complication	Cigarette smoker	Gestation at delivery (weeks)	Birth mass (g)
36	C	G4P2	A1691G	PE	No	36	3 794
26	C	G3P2	A1691G	PE	Yes	40	3 260
30	C	G2P1	A1691G	AP	No	30	1 394
20	C	G1P0	A1691G	AP	No	34	1 250
28	C	G1P0	A1691G	PIH	No	41	2 306
17	C	G1P0	G20210A	Severe PE	Yes	30	1 208
19	B	G1P0	G20210A	Severe PE	No	32	1 722

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

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.

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1. Bertina RM, Koeleman BP, Koster T, *et al*. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994; **369:** 64-67.
2. Dekker GA, de Vries JIP, Doelitzsch PM, *et al*. Underlying disorders associated with severe early-onset-eclampsia. *Am J Obstet Gynecol* 1995; **173:** 1042-1048.
3. Dizon-Townsen DS, Nelson LM, Easton K, Ward K. The factor V Leiden mutation may predispose women to severe pre-eclampsia. *Am J Obtet Gynecol* 1996; **175:** 902-905.
4. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3' untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996; **88:** 3698-3703.
5. Kupferminc MJ, Eldor A, Steinman N, *et al*. Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med* 1999; **340:** 9-13.
6. Rubinstein R, Koila F, Novitzky N. Prevalence of factor V Leiden in three ethnic groups of patients with deep vein thrombosis in the Western Cape province of South Africa. *Br J Haematol* 2000; **65:** 78-79.526

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.[1] A similar argument applies to African trypanosomiasis, where bites from the tsetse fly can transmit the *Brucei gambiense* from western 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[4] was obtained and commenced within 24 hours of the diagnosis. Further literature also confirmed usefulness of pentamadine[5-8] 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

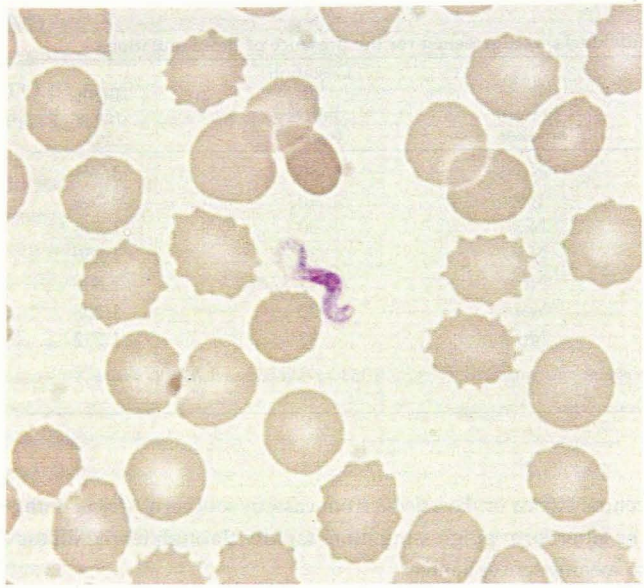

Fig. 1. Trypanosoma. Peripheral blood, May-Grünwald stain, × 1 000 oil.

treatment with IV suramin but to introduce plasma exchange[9,10] 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 (CVVHD) 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/l, 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.

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1. Kirchhoff LV. American trypanosomiasis (Chagas' disease) — a tropical disease now in the United States. *N Engl J Med* 1993; **329:** 639-644.
2. Murray HW, Pépin J, Nutman TB, Hoffman SL, Mahmoud AAF. Tropical medicine. *BMJ* 2000; **320:** 490-494.
3. Barrett MP. Problems for the chemotherapy of human African trypanosomiasis. *Curr Opin Infect Dis* 2000; **13:** 647-651.
4. Burri C, Nkunku S, Merolle A, Smith T, Blum, J, Brun R. Efficacy of new, concise schedule for melarsoprol in treatment of sleeping sickness caused by *Trypanosoma brucei gambiense*: a randomised trial. *Lancet* 2000; **355:** 1419-1425.
5. Loiseau PM, Dreyfuss G, Daulouede S, Lachatre G, Vincendeau P, Craciunescu DG. Trypanocidal effect of Ir-(COD)-pentamidine tetraphenylborate on *Trypanosoma brucei* and *T.b gambiense* rodent models and serum kinetics in sheep. *Trop Med Int Health* 1997; **2:** 19-27.
6. Pepin J, Khonde N. Relapses following treatment of early-stage *Trypanosomas brucei gambiense* sleeping sickness with a combination of pentamidine and suramin. *Trans R Soc Trop Med Hyg* 1996; **90:** 183-186.
7. Bronner U, Gustafsson LL, Doua F, *et al*. Pharmacokinetics and adverse reactions after a single dose of pentamidine in patients with *Trypanosomas gambiense* sleeping sickness. *Br J Clin Pharmacol* 1995; **39:** 289-295.
8. Berger BJ, Carter NS, Fairlamb AH. Polyamine and pentamidine metabolism in African trypanosomes. *Acta Trop* 1993; **54:** 215-224.
9. Pan AA, Winkler MA. The treat of Chagas' disease in transfusion medicine: the presence of antibodies to *Trypanosoma cruzi* in the US blood supply. *Lab Med* 1997; **28:** 269-274.
10. Spinazzola F, De Felici A, Paglia MG, *et al*. Plasmapheresis for late-stage trypanosomiasis. *Lancet* 1989; **1:** 1200.

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

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[1] 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 cyto- and haemotoxic and in *Latrodectus indistictus* O P-Cambridge it is neurotoxic so that a bite may be fatal to humans,[1,2] although no fatalities have been reported yet. The effects of the venom of cytotoxic spiders have been extensively studied,[1,3,4,5] while the venom characteristics and symptoms of the button spiders and their relatives have also received attention.[1,2] 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

Fig. 1: A male Araneus apricus spider on a leaf (photo courtesy of John Leroy).

very aggressive and bit the collector, a healthy 21-year-old male, dorsally on the knuckle of the thumb of the right hand. She kept her fangs locked and was allowed to bite for 27 seconds as araneid spiders are not known to be venomous to humans. After relaxing her fangs the spider was removed and preserved in 70% ethanol for identification. It must be noted that the victim is severely allergic to bee stings, but had undergone a course of antivenom to develop immunity.

The reactions to the venom were as follows. The bite was not especially painful, but the pressure of the fangs could be felt while the bite was inflicted, as well as mild pain in the thumb and surrounding areas immediately following envenomation. Soon afterwards local swelling and red dermal colouration around the bite site developed, with the thumb and forefinger area becoming numb. Within the first hour sharp pain developed in the bicep, armpit, shoulder, lateral chest and pectoral muscles, after which discomfort levels decreased. Lymph glands in the armpit also started to swell during this time. Intense pain began developing in the thumb and index finger an hour after envenomation, and continued during the subsequent hours.

Local swelling around the bite site gradually increased to an area approximately 2.5 cm in diameter, which was bright red in colour and extremely itchy. Swelling of the hand and armpit glands receded within 8 hours of the bite. A small scar formed at the site of the bite marks, which healed after 16 days (without any treatment), and no tissue necrosis occurred.

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*,[2] 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* C L Koch[2] 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.

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1. Newlands G, Atkinson P. Review of the southern African spiders of medical importance, with notes on the signs and symptoms of envenomation. *S Afr Med J* 1988; **73:** 235-239.
2. Müller GJ. Black and brown widow bites in South Africa. A series of 45 cases. *S Afr Med J* 1993; **83:** 399-405.
3. Newlands G, Atkinson P. Behavioural and epidemiological considerations to necrotic araneism in southern Africa. *S Afr Med J* 1990; **77:** 92-95.
4. Newlands G, Atkinson P. A key for the clinical diagnosis of araneism in Africa south of the Equator. *S Afr Med J* 1990; **77:** 96-97.
5. Croucamp W, Veale R. The venom of the sac spider *Cheiracanthium furculatum* Karsch, 1879 — à three year study. Abstracts of the Sixth International Colloquium on African Arachnids, Swakopmund, Namibia, 19 - 23 April 1999.
6. Müller GJ, Kriegler AB, van Zyl JM, van der Walt BJ, Dippenaar AS, van Jaarsveld PP. Comparison of the toxicity, neurotransmitter releasing potency and polypeptide composition of the venoms from *Steatoda foravae, Latrodectus indistictus* and *L. geometricus* (Araneae: Theridiidae). *South African Journal of Science* 1992; **88:** 113-116.