REVIEW ARTICLE

The molecular basis of South African genetic porphyria established at last!

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Human geneticists throughout the world know South Africa because of the presence here (in high frequency) of a genetic disorder of haem biosynthesis, which is rare elsewhere. The condition, known as South African genetic porphyria or variegate porphyria (VP), was recognised independently by workers in Cape Town and Johannesburg over 50 years ago, but it was a young immigrant physician, Geoffrey Dean, practising in Port Elizabeth in the late 1940s, who realised that the disease had an unusually high prevalence in his patients who were of Afrikaans origin and, over the ensuing 20 years, carried out a large-scale genealogical study which explained why there were so many cases of VP in South Africa.

Dean had hit upon a remarkable example of random genetic drift (the operation of chance factors resulting in the high (or low) frequency of a gene in a population) which is known as founder effect. During the early years of the founding of the 'new' population, when small numbers of individuals came to the Cape of Good Hope from Western Europe, in particular Holland, Germany and France, it happened that one individual was heterozygous for (or a 'carrier' of) the gene for VP. During the 18th century the population underwent a rapid expansion in size, from just over 1 000 to 17 000 in 85 years. This was largely due to natural population increase, and as a result of isolation there was relatively little immigration or gene flow into it. The number of individuals with the gene for VP therefore increased enormously. Dean estimated that in the 1960s there may have been as many as 20 000 individuals with the VP gene in the white Afrikaans population of South Africa; however, because of the mixing with the more recent immigrants from Britain and other countries which has undoubtedly occurred, the VP gene might now be expected to be found in members of these populations too. Cases are also encountered in the so-called 'coloured' population (even though we do not know the prevalence rate or gene frequency), bearing testimony to the fact that gene exchange is not prevented by social customs or even by the strict apartheid laws which were only recently repealed. According to Dean, all the VP patients in South Africa are 'blood relations', carrying the same gene for this condition that was considered to be rare in other parts of the world.

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By demonstrating that all the VP patients whose lineages could be traced were descended from a couple of Dutch origin, 'Gerrit Jansz from Veldcamp, free burgher at Stellenbosch, young man with Ariaantje Adriaansse from Rotterdam, young woman', who married at the Cape in 1688, Dean hypothesised that the VP mutation was introduced into the proto-Afrikaans population by one of them. Gerrit had been at the Cape 3 years before Ariaantje arrived; she was one of eight orphan girls who arrived on the ship *China* and were to be the brides of young men, in her case Gerrit Jansz, within weeks of their arrival. The marriage produced eight children, four of whom inherited the gene for VP (from whichever parent carried it) and, in turn, passed it on to approximately 50% of their children.

The gene for VP could not have had a significant detrimental effect on those who possessed it, because it has remained at high frequency throughout the intervening 300 years. The skin lesions resulting from sun exposure, although bothersome, could not have affected fitness (in the genetic sense), and the relatively small proportion of individuals who in the 20th century have experienced acute neurological crises, sometimes resulting in death, when given certain drugs, have probably not significantly reduced its gene frequency. It is possible, of course, that individuals carrying the gene may be at a selective *advantage,* but there is no evidence to suggest that this is the case.

Contrary to what modern-day researchers might think, Dean did not spend all his time working on porphyria. In addition to his work as a general physician in a busy provincial hospital (not attached to a medical school) and his porphyria research, which must have consumed an enormous amount of his 'spare' time, Dean also contributed to our understanding of the epidemiology of multiple sclerosis,[1-3] poliomyelitis[4] and lung cancer,[5] and among his published papers is one on the causes of death of South African doctors and dentists.[6]

Dean was able to carry out his porphyria researches because of the collaboration he forged with a South African-born scientist, who worked at the South African Institute for Medical Research in Johannesburg from 1919 until he retired and left the country in about 1960. His name was Hubert Dixie Barnes and he was born in Bedford, Cape Province, in 1900. He entered the employ of the SAIMR as an apprentice (there were no trainee medical laboratory technologists in those days), and in 1925 he was chosen to assist Dr F W Fox in creating a biochemistry department. He became fascinated by porphyria pigments, completing an MSc degree (before 1936); for a thesis entitled 'The incidence of the various types of porphyria in South African Bantu as compared with that in the white population with a chemical-pathological study of representative cases' he was awarded the PhD by London University in 1956.

Barnes had been making the laboratory diagnosis of porphyria (called porphyrinuria) in the mid-1930s[7] and reported a series of 11 cases in the mid-1940s, including both white and African patients.[8] In his paper, Barnes referred to a case reported by Kooy from Groote Schuur Hospital, Cape Town, in 1939,[9] but he was apparently unaware of a much more detailed description[10] of the same patient in *I'nyanga*, the University of Cape Town medical students' journal, probably owing to the fact that the SAIMR library did not subscribe to it. The *I'nyanga* report, 'A case of

acute idiopathic porphyria' by Eales and Chait, is a fine description of what became known as VP. Noteworthy is the fact that the *I'nyanga* paper is the first contribution to the subject by Lennox Eales, at the time a 5th-year medical student, who subsequently became a leading authority on the porphyrias and after whom the porphyrin research laboratories in Cape Town have been named — from which have come the recent breakthrough paper by Meissner *et al.*[11] and the important review paper by Hift *et al.*[12] published on p. 718 of this issue of the *SAMJ*.

Eight enzymes are involved in the haem biosynthetic pathway and all have been recognised for at least 15 years. The gene coding for protoporphyrinogen oxidase (PPO), the penultimate enzyme in the pathway, deficiency of which results in VP, is the last to be cloned even though the enzyme defect has been known since 1980.[13] Human PPO has been difficult to purify, and success in cloning the gene only followed the recognition by Meissner, collaborating with workers in Georgia, USA,[14] of a bacterial PPO gene which enabled Japanese workers to exploit the properties it shared with the eukaryotic enzyme to isolate the human gene.[15] Researchers in Wales[16] then assigned VP to chromosome 1, a finding at variance with the earlier claim by Pretoria workers[17] that the locus was on chromosome 14, and the Welsh team also mapped the human PPO gene to chromosome 1q23.

The Cape Town group, in collaboration with colleagues in Georgia and Cardiff, Wales, identified the common mutation in Afrikaners, a substitution of tryptophan for arginine at position 59 (R59W), and found, in addition, another 'milder' mutation which, when co-existing with Arg168Cys in a child under their care, caused a severe congenital form of VP.[11] This compound heterozygote had previously been reported as having homozygous VP.[18] The clarification of this child's genotype lends support to the view, long held by clinicians in South Africa, that homozygosity for the common Afrikaner mutation is probably lethal.

Since the publication of the paper by Meissner *et al.*[11] in the May issue of *Nature Genetics*, a paper by researchers in the Department of Genetics, University of Stellenbosch, in collaboration with Meissner and Hift of UCT,[19] reported failure to confirm the linkage of VP to chromosome 14q32. Another paper from the Stellenbosch researchers,[20] submitted to *Human Molecular Genetics* some 6 weeks after Meissner *et al.*'s[11] paper was submitted to *Nature Genetics*, reports the identification of R59W in 15 of 17 unrelated South African patients with VP, while one had the Arg 168 Cys (R168C) mutation and the other had a novel mutation, histidine 20 proline (H20P). Two different mutations have been identified in 4 patients of French Caucasoid origin: in 1 patient a point insertion of a G at position 1022 produced a frameshift resulting in a premature stop codon; in the 3 other patients, from two unrelated families, there was a missense point mutation (G232R).[21]

It is fitting that South African workers should have played a crucial role in the elucidation of the molecular lesion(s) responsible for VP. It was in this country that the disease was first described and differentiated from acute intermittent (or Swedish) porphyria[22] at a time when many experts in Europe claimed they were the same condition. Dean and Barnes[23] christened South African genetic porphyria VP and, by an amazing feat of genealogical research, Dean[24] traced hundreds of cases back over 300 years to the particular founder couple who introduced the gene into the small European community at the Cape of Good Hope. With important contributions from Eales in Cape Town, Joubert in Durban and Kramer in Johannesburg (as well as from their collaborators and successors), understanding of VP deepened and the stage was set for the identification of the specific mutations responsible for VP in South Africa. For this final assault on the problem, the UCT group have collaborated (and apparently competed) with workers at the University of Stellenbosch.

The stage is now set for establishing why VP is so rarely (if ever) expressed before puberty and why there should be so many 'silent' carriers of the gene. The majority remain 'silent' even when they are exposed to drugs or chemicals which are porphyrogenic in others with the same mutation. Accurate diagnosis in the vast majority of sufferers and 'silent' carriers in South Africans now becomes possible by means of the relatively simple and inexpensive polymerase chain reaction and restriction enzyme analysis. Likewise, individuals at risk of inheriting the gene which has been demonstrated in the family, can be tested and, if found to be negative, can be reassured. But the new molecular technology cannot substitute for the biochemical investigation of a patient with the signs and symptoms of VP. The absence of mutations common in the Afrikaner does not mean that VP is absent — only biochemical investigations can prove that to be the case.

If Dean's estimate of the number of heterozygotes for VP in South Africa is correct, more than 80% of such individuals remain undetected but are potentially at risk of an acute neurological attack if suitably challenged. Do the unlucky individuals carry another predisposing gene? This could be at the PPO locus (i.e. they are compound heterozygotes) or at an entirely different locus, perhaps a locus in the haem biosynthetic pathway or at loci that determine the way in which the porphyrinogen drugs are metabolised. The results of investigations along these lines will be awaited with keen interest.

The applications of molecular genetics are far-reaching and extend well beyond the practice of medicine and the elucidation of the causes of genetic disease. In a fascinating book, *Carpet of Silver* by Phillip Playford,[25] we read about the last voyage of the *Zuytdorp,* a great ship of the Dutch East India Company, which disappeared in 1712 *en route* from Cape Town to Batavia (Jakarta) carrying a rich cargo of silver coins. Playford was co-discoverer of the wreck of the *Zuytdorp* on the western coast of Australia in the Shark Bay area, some 400 miles north of Perth. He is of the opinion that some of the crew survived the disaster and became assimilated by the Aboriginal people living in that part of the island continent. He claims that some present-day descendants of those men suffer from VP. If molecular studies were to show that they have one of the Afrikaner mutations, this would be good indirect evidence that among the survivors of the wreck of the *Zuytdorp* was a man who had been recruited as a crew member in Cape Town. When the ship docked there in 1711 it had a very depleted complement of sailors because of the unusually high mortality suffered on the first leg of the voyage from the Netherlands to the Cape of Good Hope. Is it possible that Australia's first European settlers were sailors from South

Africa who scrambled ashore in the Shark Bay area in 1712, rather than the British colonists who arrived in New South Wales some 76 years later?

Playford[25] reports (p. 229) that Dean (the same indefatigable Geoffrey Dean who gave the initial impetus to the study of variegate porphyria in South Africa about 50 years ago, and who is alive and well and living in Ireland) has carried out archival research in Cape Town and, although no listing of the new crew of the *Zuytdorp* has survived, perhaps 80 or 90 persons joined the ship at Cape Town. Gerrit Jansz had two sons at that time and the one born to his wife, Ariaantje, did not leave the Cape; the other son, who was extramarital, and possibly born to a Khoi or Malay slave woman, was aged 25 years in 1712, and may well have enlisted as a crew member of the *Zuytdorp*. Other theories explaining how the gene for VP got from Cape Town to Australia aboard the *Zuytdorp* have been propounded by Geoffrey Dean, and interested readers will find them in Playford's new book. Long may Dr Dean retain his interest in VP. If ever researchers deserved to have a disease named after them they must be Geoffrey Dean and Hubert Barnes, whose names would certainly grace variegate porphyria. But eponymous diseases are no longer in fashion!

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