Understanding the Implications of Mitochondrial DNA Variation in the Health of Black Southern African Populations: The 2014 Workshop

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Knowledge concerning the prevalence and severity of disease related to/associated with variations in mitochondrial DNA (mtDNA) in Black African populations lags behind that of other groups from a global perspective. Developing an understanding of the variants that cause mitochondrial disease in this population is clearly in-teresting from a scientific perspective, and is also of importance to health services in Africa and nations outside of the African continent with appreciable populations of African origin. In ad-dition to identifying mtDNA mutations causing mitochondrial dis-ease, unravelling the role of mtDNA variants in complex disease is of great relevance and will require the coming together of many emerging specialities in southern Africa. This effort will be aided if conducted in a wider collaborative manner in the context of forums such as the South African Human Genome Programme (SAHGP; http://sahgp.sanbi.ac.za), H3 Africa, and the Human Var-iome Project (HVP; http://www.humanvariomeproject.org).

This report describes the outcomes of a workshop that dealt with the role of mtDNA variation in the health of Black African populations, which was held in November 2014 at Potchefstroom, South Africa, and supported by the National Research Foundation of South Africa and the Royal Society of the United Kingdom. The meeting aimed to review our understanding of mitochondrial variation and disease and considered inherited mitochondrial disorders and the role of mtDNA variants in common disease in Black African populations, particularly in southern Africa. It was hosted by the North-West University, Potchefstroom campus (http://www.nwu.ac.za/) and invitees were based in either a South African or British University. Although small in size, with 20 attendees (15 from South Africa and 5 from the UK), the core of the South African mitochondrial community was present along with some of those in the UK with overlapping academic interests. Those interested in mtDNA from a clinical perspective were well represented including clinical geneti-cists, clinical pharmacologists, pediatric and adult neurologists, and diagnostic scientists. Additionally, scientists specializing in genetic susceptibility and pharmacogenomics, as well as scientists involved in fundamental biochemical and molecular research on mitochon-drial function and disease, were present. The meeting considered both the identification of pathogenic mutations causing mitochon-drial disease in patients, and the importance of conducting mtDNA association studies in southern African populations.

There were two keynote lectures, which provided a setting for the discussion that followed. Professor Himla Soodyall gave an overview of mtDNA variation in African populations within the broader context of population genetics. Professor Michael Pepper presented an overview of the Southern African Human Genome Programme (SAHGP), and a detailed consideration of the ethical framework governing genetic research in the southern African context. Southern Africa is home to some of the world's oldest populations, being inhabited by people who have migrated to the subcontinent from within Africa and more recently from outside the continent. This region is therefore believed to represent the greatest richness in terms of genetic diversity on the planet. As a result, a great deal of interest has been shown in obtaining and analyzing DNA from individuals from the southern African region. However, respect for the

dignity of communities and individuals in this region is paramount, and several important issues need to be considered, two of which are the need for two-tiered informed consent and the notion of genomic sovereignty. With regard to the former, it is imperative that researchers first approach the community and its leaders before approaching individuals for consent. Only once buy-in has been obtained from the community, which requires skilful explanation of complex issues, should researchers be permitted to approach individuals. The question of benefit sharing was raised and should be dealt with up-front before consent is obtained. Benefit sharing is not necessarily monetary in nature, but does involve feedback to the community of important findings that may impact on the health and wellbeing of individuals within the community. With regard to genomic sovereignty, there is an increasing aversion to the wholesale and unregulated export of precious DNA samples to countries with greater analytical capacity. Export permits are required, tied to which are material transfer agreements that once again require consideration of benefit sharing arrangements. In recognition of the need to consolidate genomic research on the subcontinent, and in order to create a vehicle for much needed capacity development, the SAHGP was launched in Irene (near Pretoria) in January 2011 with funding from the Department of Science and Technology. Funding has allowed for the sequencing of southern African genomes and for the development of much needed bioinformatics analytical skills.

Diagnostic and Clinical Perspectives

One of the main objectives of the seminar was to obtain a critical overview of existing capacities for identification of patients with mitochondrial disease in southern African populations, and the discovery of the variants causing disease in these patients. Among the issues considered were current diagnostic best practice, ethical issues, and the complexity arising from the diverse population genetics found in southern Africa. It was noted that phenotypes frequently associated with mtDNA point mutations and deletions have been identified in all the populations of South African in both adult and pediatric patients. The clinical and diagnostic approach in the context of mtDNA disease in the United Kingdom was considered, including the common challenges of providing diagnostic excellence to all sections of diverse communities. This was followed by an overview of the biochemical investigations required to link genotype to phenotype in the context of mtDNA mutations, including a review of the current capacity in South Africa to conduct such investigations. Unlike some countries such as the United Kingdom, an established capacity to routinely conduct such extensive investigations does not currently exist in South Africa, but initiatives such as the SAHGP look set to build capacity. The two main South African centers represented at this meeting see pediatric patients from the most Northern provinces (Limpopo, Gauteng, and Mpumalanga) and the most Southern provinces (Western and Eastern Cape), thus serving different population groups. The sources of referrals and diagnostic setup of these centers differ slightly, but both screen for known pathogenic variants and have the capacity to perform full mtDNA sequencing where a clinical mtDNA disease phenotype is suspected. In the Black African patient population of the Northern provinces, (whose MtDNA background comprises L0 ~36%, L2/3 ~29%, L1 ~5%), it has been reported that an exclusively muscle phenotype is more prevalent in contrast to Caucasian patients who more frequently exhibit neurological complications in addition to a muscle phenotype [Smuts et al., 2010]. Furthermore, in this Northern Province cohort study of >200 patients (61% Black, 32% Caucasian, 7% other), next-generation sequencing was conducted on the mtDNA of all patients. The prevalence of known mtDNA pathogenic variants was exceptionally low (<1%), suggesting that a search for novel mtDNA mutations, in addition to nuclear mitochondrial gene and other nongenetic factor involvement, should be undertaken [van der Walt et al., 2012].

At the National Health Laboratory Services in the Western Cape, using a targeted mutation screening and limited nuclear gene sequencing approach, mutations were identified in 6% of over 1000 unscreened referrals. As in the Northern Province, patients of African ancestry rarely had previously identified point mutations, but were found to have mtDNA deletions. It was clearly recognized in the discussions at the meeting that a coordinated national approach is required to further define mitochondrial disease causing pathogenic variants in southern African populations. Critically the work to date suggests that the variants most frequently seen to cause mitochondrial disease in Caucasian European are not those most frequently causing disease in the Black populations of southern Africa. While it was recognized that inherited mitochondrial disease is not a major governmental health priority, considering other pressing health issues, if the region wishes to offer treatment for mtDNA disease to all of its diverse population groups (once these treatments are available), an understanding of variants causing disease in all populations is essential.

Mitochondrial Association Genetics in Southern Africa

The second major objective of the meeting was to review the role of mtDNA population variants in common disease in Black African populations. There are many who feel that clinically manifesting mutations are not the only impact that mtDNA variation has on human health. Many studies have reported that mtDNA population variants play a role in common complex disease either by altering susceptibility, or perhaps more likely the course of disease. Associating mtDNA variation with common disease is a complex task, which requires a detailed understanding of the nuances of mitochondrial genetics. Currently, the mtDNA association literature is replete with conflicting reports. Part of the reason for this is that mtDNA variation is more prone to population substructure than autosomal nuclear DNA variants as mtDNA has one quarter the effective population size. Additionally, in the context of Southern Africa there is still much to learn about population mtDNA variation. Despite these challenges, work to investigate the possible role of mtDNA variants in complex traits in southern Africa has begun and was reviewed at this seminar.

Pioneering work in the context of HIV/AIDS was presented at the meeting. HIV/AIDS is a major health burden in populations of southern and central Africa. Over 30 antiretroviral drugs have been approved for use resulting in a dramatic decline in deaths from HIV/AIDS, while effective in suppressing HIV these drugs have a number of adverse side effects. Among the adverse effects associated with antiretroviral therapy (ART) are peripheral neuropathy, lipodystrophy, and lactic acidosis. These conditions have been associated with defective mitochondrial function. In an mtDNA sequencing study of 215 HIV/AIDS patients from Malawi, nine mtDNA major subhaplogroups were observed. The patients were on ART regimens that included stavudine (d4T), a drug known to disrupt mitochondria function. Furthermore, it was observed that 25% of the patients developed peripheral neuropathy and 16% lipodystrophy after ART initiation. On correlating haplogroups with peripheral neuropathy, the L2a subhaplogroup was associated with significantly reduced risk while the subhaplogroup

L0a2 was associated with increased risk [Kampira et al., 2013a]. With respect to lipodystrophy, the L3e mtDNA subhaplogroup appeared to be protective against lipodystrophy as none of the subjects with L3e presented with lipodystrophy [Kampira et al., 2013b]. Although additional studies are required to replicate these results, they suggest understanding mtDNA variation could have importance beyond inherited disease in southern Africa. Work is also being conducted into the role of mtDNA variation in hypertension in the Black African population, which supports the aims of the H3Africa initiative (http://www.h3africa.org/). Discussions included consideration on how to construct sound association studies, and how best to overcome the additional barriers found in the southern African setting as mentioned above.

Future Perspectives

This meeting provided investigators from South Africa an important opportunity to meet and discuss national issues, and to have dialog with investigators from the United Kingdom who, although working in a different context, face some of the same challenges. This allowed for constructive discussions and opened up clear collaborative opportunities in both the context of clinical and association genetics. It was decided among the South African investigators that they should form a national network to facilitate setting up the procedures and sharing capacities to identify the mtDNA variants involved in mitochondrial disease as well as common complex disease, present in Black African populations. Furthermore, with regard to mitochondrial disease, the search for nuclear gene mutations in these populations is of paramount importance, which could be better investigated through a network of collaboration. Once established, this network linking key national centers in South Africa will also be able to collectively participate better with initiatives that share similar aims such as the SAHGP, H3Africa, and the HVP.

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