Perspective Piece

The Impact of Prior Flavivirus Infections on the Development of Type 2 Diabetes among the Indigenous Australians

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Abstract. It is estimated that 5% of Australians over the age of 18 have diabetes, with the number of new cases increasing every year. Type 2 diabetes (T2D) also represents a significant disease burden in the Australian indigenous population, where prevalence is three times greater than that of non-indigenous Australians. Prevalence of T2D has been found to be higher in rural and remote indigenous Australian populations compared with urban indigenous Australian populations. Several studies have also found that body mass index and waist circumference are not appropriate for the prediction of T2D risk in indigenous Australians. Regional and remote areas of Australia are endemic for a variety of mosquito-borne flaviviruses. Studies that have investigated seroprevalence of flaviviruses in remote aboriginal communities have found high proportions of seroconversion. The family Flaviviridae comprises several genera of viruses with non-segmented single-stranded positive sense RNA genomes, and includes the flaviviruses and hepaciviruses. Hepatitis C virus (HCV) has been shown to be associated with insulin resistance and subsequent development of T2D. Flaviviruses and HCV possess conserved proteins and subgenomic RNA structures that may play similar roles in the development of insulin resistance. Although dietary and lifestyle factors are associated with increased risk of developing T2D, the impact of infectious diseases such as arboviruses has not been assessed. Flaviviruses circulating in indigenous Australian communities may play a significant role in inducing glucose intolerance and exacerbating T2D.

DIABETES MELLITUS TYPE 2 IN INDIGENOUS AUSTRALIANS

Chronic disease is the epidemic of the new millennium, surpassing infectious disease and injury as the dominant health concern currently facing humanity. It is estimated that, by 2020, 75% of deaths in Australia will be due to chronic diseases, such as diabetes.¹ A systematic review of type 2 diabetes (T2D) studies surveying both rural and urban indigenous Australian populations found higher prevalence of T2D in the former compared with the latter.² This result differs from prevalence surveys conducted with indigenous peoples in North America and the Pacific.² The onset of T2D in non-indigenous Australians generally occurs in the mid-forties, with prevalence peaking in the age of 75 years and older cohort.² For indigenous Australians, prevalence is highest in the 35- to 55-year old group.² The prevalence of T2D within the indigenous Australian community is three times higher than non-indigenous Australians.³ Development of T2D is predominantly associated with metabolic changes due to obesity, and the increase in T2D in indigenous Australians is primarily attributed to changes in nutrition. However, urban-living indigenous Australians have similar access to a nontraditional diet and it would be expected they would have similar prevalence of T2D. In addition, several studies on the outcomes of educational and dietary intervention initiatives in remote communities have demonstrated little impact on diabetes prevalence and body mass index (BMI).^{4,5} In one of these studies, diabetes prevalence increased by 80%.⁴ Other studies of T2D prevalence in indigenous Australians and Torres Strait Islanders have found BMI and waist circumfer-

ence (WC) are not appropriate measures for prediction of T2D risk.^{6,7} One of these studies, performed in remote communities in far northern Queensland, found similar T2D development rates in both Australian Aboriginal and Torres Strait Islanders despite large differences in baseline BMI and WC.⁶ Although dietary and lifestyle factors are well-described associations with increased risk of developing T2D, other factors such as the impact of arbovirus infection on the development of insulin resistance and subsequent diabetes mellitus have not been assessed.

HEPATITIS C VIRUS HAS BEEN IMPLICATED IN THE DEVELOPMENT OF DIABETES MELLITUS TYPE 2

The family Flaviviridae comprises several genera of small, enveloped viruses with non-segmented single-stranded positive sense RNA genomes.⁸ Of these genera, the flaviviruses and hepaciviruses are particularly important human pathogens. Insulin resistance is a pathological feature of hepatitis C virus (HCV) infection that often leads to the development of T2D.⁹ Meta-analysis of 17 studies ($N = 286,084$) comparing HCV-infected individuals with uninfected individuals found an increased risk (odds ratio [OR] 1.68) of T2D in HCV-infected cases.¹⁰ An increased risk (OR 1.92) of T2D was also found in HCV-infected individuals compared with hepatitis B virus (HBV)-infected individuals ($N = 51,156$), with the association becoming greater over time. HBV is similar to HCV in targeting the liver and being capable of inducing chronic disease. However, HBV is a DNA virus from the family Hepadnavirus. A study by Mehta and others¹¹ found HCV infection accelerated the onset of T2D, with development of T2D a decade earlier compared with uninfected individuals. Available studies indicate that the HCV core protein is involved in inducing insulin resistance through alteration of insulin receptor substrate-1 pathway signalling.^{10,12} Studies

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have also demonstrated that a significant proportion of insulin resistance in HCV patients originates from extrahepatic sites.^{13,14} As HCV has tropism for the liver, these observations suggest that HCV infection is involved in the production of mediators capable of inducing endocrine effects at extrahepatic sites. The HCV possesses highly structured 5' and 3' untranslated regions (UTRs) that are involved in viral replication and protecting viral RNA from nucleolytic degradation, and have been demonstrated to bind to the HCV core protein and host proteins involved in mediating the host antiviral immune response.^{15–17} HCV UTRs have been detected in peripheral blood cell subsets in HCV-infected individuals and are associated with relapse posttreatment in these individuals.¹⁸

OTHER MEMBERS OF THE FLAVIVIRIDAE MAY BE CAPABLE OF INDUCING INSULIN RESISTANCE

The majority of flaviviruses are mosquito-borne arboviruses. Important members include the dengue viruses, Japanese encephalitis virus, West Nile virus (WNV), and yellow fever virus. Dengue virus is among the most important flaviviruses, in terms of annual infections and morbidity. There is a substantial overlap between endemic regions and regions with increasing prevalence of T2D.¹⁹ Annual infections with dengue virus and other flaviviruses are increasing in the same regions,²⁰ raising the question of their potential role in inducing insulin resistance and exacerbation of T2D. Linear regression analyses comparing dengue burden to T2D prevalence in dengue endemic countries indicates that approximately 10–11.4% of the variation between T2D prevalence can be attributed to dengue burden (Figure 1). A small-scale study conducted in a highly dengue virus–endemic region found 75% of patients developed glucose intolerance during early infection, with 17.5% presenting with persistent impaired glucose intolerance postinfection.²¹

Flaviviruses such as Murray Valley encephalitis virus (MVEV), Kunjin virus, Alfuy virus, Kokobera virus, Stratford virus, and Edge Hill virus are endemic to Australia and have been isolated from mosquitoes throughout Australia and the Torres Strait.^{22–25} Serological surveys of horses and sentinel animal surveys have also demonstrated the circulation of these viruses in Australia.^{26–28} Although these viruses are capable of causing human disease, the majority of infections are asymptomatic or cause only a nonspecific febrile illness. Outbreaks of Murray Valley encephalitis are more common in non-indigenous adults and young indigenous children, indicating regular exposure to the virus results in seroconversion and subsequent protection from severe disease.²⁸ Cross-sectional and longitudinal serological surveys in one aboriginal community demonstrated that over 50% of the population had antibodies to MVEV and a high proportion of the community seroconverted during the 5-year study.²⁹

Like HCV, flaviviruses also possess a core protein and 5' and 3' UTRs. The 3' UTR of flaviviruses has been shown to produce subgenomic flavivirus RNA (sfRNA).³⁰ Studies have found that sfRNA is required for flavivirus pathogenicity.³¹ This sfRNA has also been shown to modulate host responses to infection via subversion of the interferon response,³² inhibition of cellular exoribonuclease,³³ and suppression of the antiviral RNA interference response.³⁴ However, the specific binding partners for sfRNA have not been elucidated.³⁰

FIGURE 1. Correlation between dengue burden and diabetes prevalence in dengue endemic countries. Data for apparent [A] and inapparent [B] dengue burden estimates for dengue endemic countries was obtained from the model developed by Bhatt and others.²⁰ Apparent dengue infection was defined as an infection of sufficient severity to modify an individuals' regular schedule. Data for diabetes prevalence, adjusted for age profile, was obtained from the *International Diabetes Federation Diabetes Atlas*. For some countries, adjusted diabetes prevalence was based on extrapolation from similar countries.

Multiple amino acid sequence alignments of the core protein of members of the Flaviviridae combined with structural analysis demonstrate conservation within the N-terminal region.³⁵ The N-terminal region of core protein is the region that has been found to interact with cellular proteins¹⁷ in addition to the UTRs in HCV.¹⁸ The degree of conservation in the N-terminal region of the core protein between HCV and the flaviviruses raises the possibility for a similar interaction in flavivirus infection. This is particularly important in light of the association between core protein and the induction of insulin resistance in HCV infection.¹² Like HCV, several other flaviviruses, including WNV, have been demonstrated to be capable of producing chronic infection despite rapid production of virus-specific neutralizing antibodies.³⁶ Persistent

infection can induce chronic inflammation, which has been shown to contribute to T2D pathogenesis.³⁷

MicroRNAs (miRNA) are generally involved in epigenetic control of expression in eukaryotes and have been found to be involved in insulin signaling, glucose metabolism, and lipid metabolism.³⁸ The production of miRNA from flaviviral RNA has been predicted by computational methods.³⁹ However, miRNA production has only been observed to date in vitro in mosquito cells lines infected with WNV.⁴⁰ This particular miRNA was derived from the highly conserved distal domain of the subgenomic RNA and was determined to up-regulate expression of a host protein linked to lipid trafficking GATA-binding protein 4 (GATA-4).^{30,38} In human and murine cell lines, GATA-4 has been demonstrated to be associated with the transcriptional regulation of the miRNA, miR-144.⁴¹ Interestingly, miR-144 has been linked to the development of insulin resistance and T2D.⁴² Molecular assays targeting the 3' UTR of dengue virus have demonstrated the ability to detect this target up to 10 days post-onset of fever, with this period the limit of the clinical samples assayed.⁴³ The upper limit for the detection of flaviviral UTRs post-infection has not been elucidated. The sfRNA derived from flaviviral 3' UTRs may be capable of persisting for greater periods due to their resistance to exonuclease degradation.³³ The potential role of flaviviral core protein and UTRs in the development of autoimmune disease and diabetes has not yet been investigated. The stability and resistance of sfRNA to exonuclease degradation may result in its persistence and the continuation of its role in dysregulation of host pathways involving RNA-binding proteins after the virus is cleared.

PROPOSALS FOR TESTING FLAVIVIRAL EXACERBATION OF T2D HYPOTHESIS

An important aspect in investigating the potential role of flaviviral infection in T2D pathogenesis is the determination of the extent of viral UTR and sfRNA persistence post-infection. This could be determined through a combination of screening convalescent sera from clinical samples, sera from individuals with prior diagnosed flaviviral infection, and detection in animal models post-infection. Methods developed for the detection of miRNA could be trialed for the detection of sfRNA.⁴⁴ A variety of computational methods have been developed that could be used to predict host-binding targets for sfRNA.⁴⁵ However, this may be limited to binding targets that have been well-researched to date. A more conclusive method for testing the hypothesis would involve the use of animal models. A polygenic murine model of T2D has recently been developed, with mice developing the etiology and clinical criteria of human T2D based on the development of insulin resistance, concomitant dyslipidemia, and overt hyperglycemia when fed a high fat/high glycemic index diet for 20 weeks.^{46,47} The investigation of the effect of flaviviral infection in these mice prior to the development of clinical prediabetes and T2D, compared with that of uninfected mice, and infected and uninfected mice of the same genetic background on standard murine diet, would determine whether infection is capable of contributing to T2D development. More extensive seroprevalence surveys for flaviviral infection in indigenous Australians living in regional and remote areas, compared with indigenous Australians living in urban areas, would be valuable in investigating the potential relationship between infection and T2D.

CONCLUSION

The prevalence of T2D within the indigenous Australian community is three times higher than that of non-indigenous Australians. Although dietary and lifestyle factors are associated with increased risk of developing T2D, the impact of infectious diseases such as arboviruses has not been assessed. This is particularly important in light of the increased prevalence of T2D for indigenous Australians living in regional and remote areas, compared with indigenous Australians living in urban areas. Regional and remote areas of Australia are endemic for a variety of mosquito-borne flaviviruses. HCV and flaviviruses possess conserved proteins and subgenomic RNA structures that may play similar roles in the development of insulin resistance. Therefore, flaviviruses circulating in indigenous Australian communities may play a significant role in inducing glucose intolerance and exacerbating T2D.

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