Project Details	
Project Code	MRCIIAR26Br Rivino
Title	Elucidating genetic and immune signatures of severe dengue
Research Theme	IIAR
Project Type	This project is cross-disciplinary and includes both wet and dry lab work (approx 60% wet lab and 40% dry lab).
Summary	Dengue is a mosquito-borne virus infection spreading globally for which there is no cure. It remains unclear why some people develop severe disease from dengue infection, while others have only mild symptoms. Genetic variations in two specific genes -important for the function of Natural killer (NK) cells and $CD8^+$ T cells- are linked with susceptibility to developing severe dengue. Using immunology and genetic approaches, you will investigate how these genetic variations affect immune cells and whether these defects are causal for severe dengue. You will perform cutting-edge immunology and genetic epidemiology methodologies and work in containment level-2 and level-3 laboratories.
Description	Dengue virus infections are spreading dramatically from tropical/subtropical regions to new areas including Europe, due to warming climates and increased urbanization and travel. Infections cause a range of clinical features from uncomplicated fever to life-threatening severe dengue (SD). Despite the global burden of dengue there are no therapies and the mechanisms driving pathogenesis remains unclear, although host immunity is known to play a central role. Our recent work shows that NK and T-cell dysfunction discriminates patients that progress to SD from those who don't(1). Host genetics are a known a risk factor for SD, although the underpinning mechanisms remain unclear. A genome-wide association study (GWAS) showed an association of SD with single nucleotide polymorphisms (SNPs) in the major histocompatibility complex class-I polypeptide-related sequence B (MICB) and Phospholipase C epsilon 1 (PLCE1) genes(2). MICB is up-regulated in virus-infected cells and binds NKG2D, a transmembrane glycoprotein expressed in NK and CD8+T-cells, thereby triggering their cytotoxic function. PLCE1 is involved in T-cell activation/migration. We recently identified two SNPs in KLRK1, encoding for NKG2D, which associate with SD(3) and lead to decreased NK-cell cytotoxicity (unpublished). Collectively, these findings suggest that NK/CD8+T-cells play a critical role in dengue pathogenesis. However, the functional impact of the MICB and PLCE1 SNPS on NK/T-cells and their causal role for SD remain unclear. We hypothesize that MICB and PLCE1 SNPs cause impaired anti-viral function of NK/CD8+T-cells resulting in poor viral clearance and increased viremia/inflammation typical of SD. The student will test this hypothesis through development of MICB and PLCE1 engineered cell lines and analysis of samples from dengue patients and healthy volunteers. Epidemiological analyses using Mendelian Randomization (MR), a technique that enables examining causal relationships between phenotypes using evidence from a genetic epidemiological context, will test causality of these SNPs for SD. The student will also use bioinformatic approaches to interrogate the broader links of these SNPs

	with susceptibility to other infections using population/patient genomic datasets. Knowledge gained in this study will provide insights into the mechanisms driving SD, inform the design of therapeutics and potentially identify biomarkers for SD. This work may inform mechanism of pathogenesis for other inflammatory viral infections (e.g., Influenza, SARS-CoV-2, Ebola). Key research questions 1. What is the functional impact of the MICB and PLCE1 SNPs on NK and CD8+ T-cells? 2. Do these SNPs play a causal role for SD? 3. Do these SNPs associate more broadly with susceptibly to other infections? Research plan Objective 1: Impact of SNPs on protein expression. Relevant cell lines will be engineered using CRISPR-Cas9 to express the SNP of interest (e.g. MICB: K562, THP-1; PLCE1: Jurkat, NK-92). Expression of the corresponding mRNA and protein will be evaluated using flow cytometry, RT-PCR and ImageXpress Pico Cell Imaging microscopy. Objective 2: Impact of SNPs on NK/CD8+ T-cell function will be evaluated in the engineered cell lines using a range of methods established in the Rivino lab (e.g., T/NK-cell cytokine production, NK/T-cell killing assays using spectral flow cytometry). NK/CD8+ T-cells will be analyzed for expression of novel cytotoxic molecules critical for NK-cell killing that the Humphreys' laboratory (Cardiff) has recently identified. Objective 3: Genotype to phenotype. MICB, PLCE1, KLRK1 and other relevant SNPs will be assessed in Vietnamese dengue patient samples using TaqMan and/or DNA genotyping. SNP expression will be correlated to the phenotypic/functional NK and T-cell profiles we recently described for these patients(1). Objective 4: Causal inference of relevant mechanisms for SD. MR requires discovery of genetic factors for the causal phenotype of interest, which are then used to examine the phenotype's influence on relevant outcomes such as SD within an instrumental variables analytical framework. Analysis of SD will be enabled using summary statistics from the dengue GWAS(2) and, where available, individual-level datasets containing genotype and infectious disease phenotype information, within an MR framework. As conventional MR methods may not fully accommodate the complexities of infectious disease dynamics, we will explore/adapt emerging genetic epidemiology approaches that may be better suited for application in these contexts (Power, collaboration with Hemani). Objective 5: Impact of SNPs for other infections. The impact of common SD-associated SNPs in MICB, KLRK1, and PLCE1 on a broad spectrum of disease phenotypes, including other viral infections, will be assessed using phenome-wide analysis of publicly available GWAS datasets. In addition, associations involving high-impact variants in these genes will be explored within rare disease cohorts, including the NIHR BioResource for Rare Diseases and the 100,000 Genomes Project, to investigate potential links with severe immunodeficiency states (Burley). The student will take ownership of this cross-disciplinary project across all objectives working closely with experts in viral immunology, genetic

	epidemiology and dengue pathogenesis. An additional impact of this project will be in examining the correspondence of lifetime exposure of risk factors estimated from genetic epidemiology with acute interventions made in cell line experiments. References 1. https://doi.org/10.1038/s41467-025-60941-9 2. http://doi.org/10.1038/ng.960 3. https://doi.org/10.1093/infdis/jiac093
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