Summer Studentship

Background: Each person's genetic 'blueprint' is stored in their DNA. DNA is tightly packaged and stored as chromosomes. In humans, cells normally contain 23 chromosome pairs (**Figure 1**). At the ends of each of these chromosomes, there are protective caps called telomeres. Telomeres protect the coding region of DNA (the section that provides the cells with instructions) from degradation. Each time a cell divides, the chromosomes must be copied; however, the cellular machinery that does this can't reach right to the chromosome ends, so the protective caps (the telomeres) get a little shorter each time (**Figure 1**). Once telomeres reach a critical length, the cell is effectively too biologically old to divide. This is vitally important in chronic kidney disease (CKD), as more older cells are found in the kidneys of individuals with CKD. The number of older cells present in each person increases as the severity of kidney disease progresses.

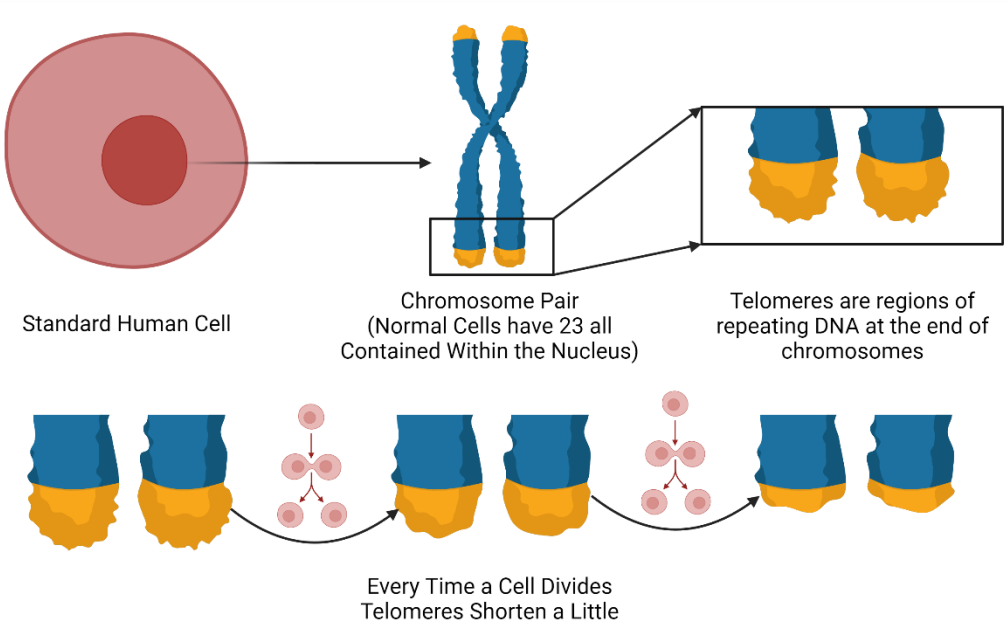

Figure 1: Background information on Telomeres

Measuring the length of telomeres in the past was very difficult as the DNA code was read as short chunks. As the telomeres are composed of many repeating sequences, it was impossible to work out which sections of the telomere these short reads belonged – imagine trying to build a picture with multiple jigsaw pieces of similar appearance. However, new nanopore sequencing technologies allow the genetic code to be read in much larger sections, enabling very accurate measuring of the total telomere length and identification of which telomere is associated with each chromosome.

What will I do: I will work with Claire, AJ and colleagues to develop this cutting-edge method in our laboratories, and for the first time, determine exactly how telomere length varies between individuals with and without kidney disease in a small number of people. Future work may explore how telomere length varies before and after people have a kidney transplant and how they change with age. This

research will deepen our understanding of biological processes that may lead to the onset and progression of kidney disease and will help us understand some of the underlying biological consequences of kidney disease. The workflows established during this studentship, will produce data that will help researchers decide future research directions and contribute towards to development funding applications by the team.

Tiernan hopes that this studentship will provide him with laboratory techniques that will aid his professional development and prepare him for his master's research project. Additionally, through creating this project's workflow alongside other career scientists, Tiernan will be armed with both the theoretical and practical knowledge to provide an excellent foundation in his first steps towards becoming a career scientist.

The Science View:

Tiernan Coulter is an Undergraduate Scientist at Queen's University of Belfast. Tiernan was recently awarded a First-class honours degree in Human Biology and will graduate in July 2023. Tiernan is the first recipient of the Norther Ireland Kidney Research Fund (NIKRF) Janet Greeves Legacy Training Grant and will train under Dr Claire Hill and Professor Amy Jayne McKnight. This 8-week Summer Studentship will develop Tiernan's skills in Oxford Nanopore long-read sequencing, data analysis and will reinforce his skills in scientific writing. Tiernan will follow this with a Master's in Bioinformatics and Computational Genomics at Queen's University Belfast, of which he has been fortunate to have received the North-South Scholarship for his studies and will commence in 2023.

The prevalence of chronic kidney disease (CKD) is rising globally; with CKD projected to become the 5th leading cause of death by 2040 (1,2). Indeed, in June 2023, Kidney Research UK (KRUK) published a report declaring kidney disease a UK public health emergency and highlighted four key interventions that could save more than 10,000 lives in the next ten years (3):

(1) earlier / improved diagnosis,
(2) improved management,
(3) greater use of medications and
(4) increased rate of transplantation.

This project aims to establish novel methods and advance fundamental knowledge to aid the discovery of biomarkers for CKD onset and progression. This research holds potential to contribute towards two of the KRUK proposed interventions: earlier / improved diagnosis and improved management.

The pathogenesis of CKD has a genetic component, with cases commonly being associated with multiple genetic mutations (4). However, genetic variation alone is insufficient to explain individual susceptibilities to CKD (5). This points to additional factors playing a role in CKD onset and progress, such as telomere length, sex chromosomes, epigenetics, copy-number variations, and mitochondrial DNA (5).

Following on from his undergraduate research project with Claire and AJ, Tiernan's studentship will further investigate the role of telomere length in CKD. Telomeres are protective caps found at the ends of chromosomes that protect important coding regions of the genome. Due to the inability of DNA replicating molecular machinery to work on chromosome ends, every cellular division results in chromosomes becoming slightly shorter than the previous generation (6). Repeated telomere truncation gives way to cellular senescence, where the cell is metabolically active but unable to divide (7,8). Whilst telomere shortening and cellular senescence is a natural consequence of ageing, this process may become accelerated during disease, such as CKD. The presence of senescent cells has been found to correlate positively with the progression of CKD (9,10). Furthermore, CKD itself produces physiological circumstances that accelerates the shortening of telomeres, such as hypertension, oxidative stress or inflammation, potentially potentiating a cyclical feedback loop (11,12).

A common consensus on the influence of telomere shortening on kidney function is yet to be reached. Several studies show a significant association between the shortening of telomeres and the progression of CKD (13,14), whilst others were unable to detect this significant association (15,16). Potential reasons for the varied findings may be due to factors such as differences in methodologies, variance in CKD classifications or underpowered cohorts within the study. A major methodology difference can be how telomeres are measured. Previous techniques fell into two main classes: (1) Techniques that provided a relative or average telomere length but were unable to measure telomeres on specific chromosomes (Q-PCR, Southern Blot, Q-Fish or Flow-FISH). (2) Techniques that were able to measure telomeres on specific chromosomes but were limited in either their ability to be utilised on all chromosomes, their ability to measure ultra-long telomeres, throughput, or labour intensity (STELA, Universal-STELA, or TeSLA) (17).

This studentship will involve establishing a novel method of telomere measurement "NanoTelSeq" within the MEPH labs. "NanoTelSeq" is a technique utilising Oxford nanopore sequencing to measure chromosome aligned telomere reads at single base-pair resolution (18). "NanoTelSeq" allows not only the measurement of telomere lengths at each chromosome end, but also facilitates the measurement of ultra-long and ultra-short telomeres, thus bypassing the limitations of previous methods (17,18) (Figure 1). Oxford nanopore sequencing is able to produce reads that are only limited by the size of the DNA fragment provided (19,20). As telomeres are composed of repeating nucleotide sequences, it was impossible to align short-read sequences (21). However, nanopore produced ultra long-reads allow for reads to stretch from telomere ends to the non-repeating sub-telomeric region, enabling the reads to be aligned to specific chromosomes whilst encompassing the full telomere (**Figure 2**). This will be the first time this technique has been utilised for the purpose of measuring telomeres in individual participants with kidney disease.

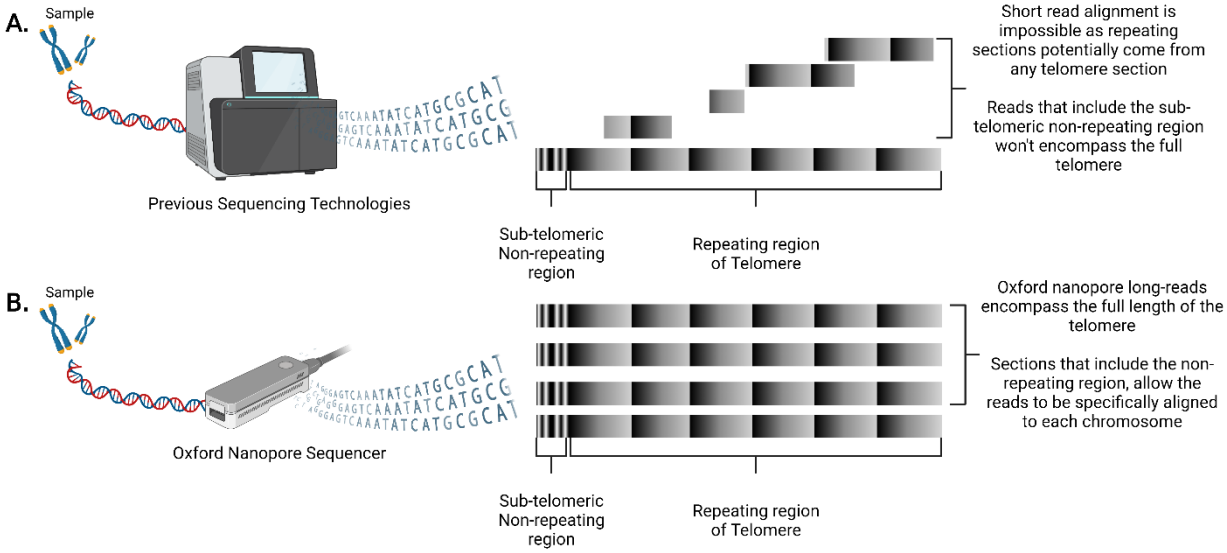

Figure 2A - Difficulties in Telomere Measurement due to Limitations of Short-read Sequencing.

Figure 2B - How Oxford Nanopore Sequencing Enables Telomere Measurement

This studentship first seeks to establish a "NanoTelSeq" workflow within control samples. From this, it is hoped that the workflow can be used post-studentship to investigate kidney disease in the NICOLA cohort (Northern Ireland Cohort for the Longitudinal Study of Aging), as well as the Belfast Renal Transplant Cohort, pending appropriate ethics and governance approvals. Data analysis will enable us, for the first time, to compare telomere length between those with and without kidney disease at chromosome level resolution. The preliminary data generated from this project will be of use for research scientists seeking to upscale the project and will enable grant applications from additional funding bodies. Furthermore, the studentship will utilise the ability of nanopore sequencing to provide

simultaneous epigenetic analyses, facilitating the establishment of multi-omics pipelines vital for future investigations.

The project will not solely focus on establishing a new workflow pipeline. Tiernan also plans to produce material for the purpose of public engagement. For example, through reviewing key literature in the field of telomere biology and kidney disease, Tiernan will co-produce engaging and eye-catching summaries alongside the public and NIKRF, that will have been specially tailored for non-expert audiences.

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