Scientific Abstract

Background

Whole exome sequencing (WES) has risen to the forefront of genetic testing based on its potential to uncover genetic causes for inherited conditions. We wish to apply this technology to discover underlying disease causing mutations in families with Mendelian conditions affecting cardiac and skeletal muscle.

The sarcomere is the functional unit of both cardiac and skeletal muscle contraction and mutations in sarcomeric proteins are known to cause an increasing number of cardiac and skeletal muscle diseases. The range of clinical and phenotypic manifestations in patients with myopathies is wide. Variability in cardiomyopathy patients ranges from a benign course with minimal symptoms to progressive disease and development of heart failure and sudden death. Muscle myopathies represent a large group of diseases that result in weakness of the skeletal muscles. Many result in the degeneration of skeletal muscle fibres. The skeletal myopathies also vary in severity from paralysis at birth to mild conditions compatible with normal life span. Although many disease genes have been identified for these conditions, many patients remain without a clear genetic diagnosis, suggesting that additional causes are yet to be uncovered.

The overall objective for this project is to provide new insight into the molecular basis of these rare conditions. This will advance our knowledge of their genetic basis, and improve prediction, prevention, and treatment options. Once a new causal mutation is confirmed, it is immediately reported back to the clinic, with great benefit for the patients who are given an accurate diagnosis and recurrence risks for siblings and offspring.

Methods/analysis

By working with both national and international clinical colleagues we will investigate families with cardiac/skeletal myopathies for which there is no current genetic diagnosis. All families have been recruited under local research ethics approval, with full patient/parental consent; and conform to the Declaration of Helsinki. We will particularly focus on families where gene panel testing for known genes has not proved successful in identifying the underlying molecular cause of their condition.

We will carry out whole exome sequencing on DNA from affected individuals, and their parents, using the Illumina HiSeq® 2500, with the SureSelect XT Target Enrichment system at a read depth of 50X. Reads will be aligned to genome assembly hg19 with the Burrows-Wheeler Aligner (BWA,V.0.5.87.5). High quality indel and single nucleotide variant calling and annotation will be performed using the latest version of GATK with standard filtering criteria (read depth >=10%, genotype quality score >=30). Candidate genes will be prioritized according to a series of filtering criteria dependent on the anticipated pattern of inheritance in the families. These criteria will include variant zygosity, minor allele frequency, absence in variant databases such as dbSNP, 1000 Genomes and ExAC, and biological function of candidate gene pathway. Potential candidate variants will be validated in the affected individual, and the family investigated for co-segregation of the variant with disease status by Sanger Sequencing.

Projected outcomes and future studies/clinical efforts this data will facilitate.

Future work will involve functional validation to identify the biochemical mechanisms associated with these disorders and begin to explore pathogenesis. In our group we routinely develop disease models for cardioskeletal muscle diseases in zebrafish. We use human skeletal muscle cultured cells and induced pluripotent stem cell derived cardiac cells to verify mutations as being pathogenic and to study functional and structural consequences of such mutations.

The results will no doubt reveal new molecular mechanisms for these conditions and define the normal functions of the genes we identify, and novel pathways which will be open to targeting for preventive medical therapy.

Be sure to describe how you and your team are well positioned to successfully complete this study.

As PI on this project I have over 20 years of experience in human genetics, and in particular human genetic variation discovery and analysis. I currently lead the Genomics Centre at St George's University of London, which includes 6 academic genetic research groups. My own research team are a dedicated group of scientists including postgraduate students, post-docs, clinical colleagues and tenured staff interested in cardiac and skeletal muscle development and disease. Key members of the group that will be involved in this project include Reza Maroofian (PhD student) and Dr Dan Osborn (Lecturer).

Our research institute is co-located with St George's Hospital and Congenica Ltd who are a global genome-based medicine company, founded on pioneering work from the Deciphering Developmental Disorders project at the Sanger Institute. Their onsite translational research laboratory focuses on development and translation of assays for rare, human disease diagnosis using next generation sequencing.

Recent successes using our in house disease gene discovery platform have been disseminated to the scientific community and public through publications in peer reviewed journals, invitations to speak at national and international conferences, and comments in the general media. Some examples are provided below:

<u>Selected publications:</u>

1) Petropoulou E, Soltani M, Firoozabadi AD, Namayandeh SM, Crockford J, Maroofian R, Jamshidi Y**.** ***Digenic inheritance of mutations in the cardiac troponin (TNNT2) and cardiac beta myosin heavy chain (MYH7) as the cause of severe dilated cardiomyopathy.*** Eur J Med Genet. 2017 Sep;60(9):485-488.
2) Osborn D, Pond HL, Mazaheri N, Dejardin J, Munn CJ, Mushref K, Cauley ES, Moroni I, Pasanisi MB, Sellars EA, Hill RS, Partlow JN, Willaert RK, Bharj J, Malamiri RA, Galehdari H, Shariati G, Maroofian R, Mora M, Swan LE, Voit T, Conti FJ, Jamshidi Y*, Manzini CM*. ***Mutations in the inositol phosphatase INPP5K cause a congenital muscular dystrophy syndrome overlapping the dystroglycanopathies and Marinesco-Sjögren Syndrome.*** American Journal of Human Genetics 2017 Mar 2;100(3):537-545.
3) van der Harst P*, van Setten J*, Verweij N*, Vogler G*, Franke L*, Maurano MT*, Wang X*, Leach IM*, (…), Chambers JC*, Jamshidi Y*, Visel A*, Christoffels VM*, Isaacs A*, Samani NJ*, de Bakker PIW*. ***Fifty-two Genetic Loci Influencing Myocardial Mass*****.** J Am Coll Cardiol. 2016 Sep 27;68(13):1435-48.

4) Muggenthaler M, Petropoulou E, Omer S, Simpson MA, Sahak H, Rice A, Raju H, Conti FJ, Bridges LR, Anderson LJ, Sharma S, Behr ER, Jamshidi Y. ***Whole exome sequence analysis reveals a homozygous mutation in PNPLA2 as the cause of severe dilated cardiomyopathy secondary to neutral lipid storage disease.*** Int J Cardiol. 2016 Feb 13; 210:41-44.
5) UK10K Consortium, Walter K, Min JL, Huang J, Crooks L, Memari Y, McCarthy S, Perry JR, Xu C, Futema M, Lawson D, Iotchkova V, Schiffels S, Hendricks AE, Danecek P, Li R, Floyd J, Wain LV, Barroso I, Humphries SE, Hurles ME, Zeggini E, Barrett JC, Plagnol V, Richards JB, Greenwood CM, Timpson NJ, Durbin R, Soranzo N. ***The UK10K project identifies rare variants in health and disease.*** Nature. 2015 Oct 1; 526(7571):82-90.
6) Behr ER, Savio-Galimberti E, Barc J, Holst AG, Petropoulou E, Prins BP, Jabbari J, Torchio M, Berthet M, Mizusawa Y, Yang T, Nannenberg EA, Dagradi F, Weeke P, Bastiaenen R, Ackerman MJ, Haunso S, Leenhardt A, Kaab S, Probst V, Re don R, Sharma S, Wilde A, Tfelt-Hansen J, Schwartz P, Roden DM, Bezzina CR, Oleson M, Darbar D, Guicheney P, Crotti L, UK10K Consortium, Jamshidi Y. ***Role of common and rare variants in SCN10A: Results from the Brugada syndrome QRS locus gene discovery collaborative study.*** Cardiovascular Research 2015 Jun 1; 106(3):520-9.
7) Magnani JW, Brody JA, Prins BP, Arking DE, Lin H, Yin X, Liu C-T, Morrison AC, Zhang F, Spector TD, Alonso A, Bis JC, Heckbert SR, Lumley T, Sitlani CM, Cupples LA, Lubitz SA, Soliman EZ, Pulit SL, Newton-Cheh C, O'Donnell CJ, Ellinor PT, Benjamin EJ, Muzny DM, Gibbs RA, Santibanez J, Taylor HA, Rotter JI, Lange LA, Psaty BM, Jackson R, Rich SS, Boerwinkle E, Jamshidi Y, and Sotoodehnia N, the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE), the NHLBI's Exome Sequencing Project (ESP) and the UK10K. ***Sequencing of SCN5A identifies rare and common variants associated with cardiac conduction.*** Circulation: Cardiovascular Genetics 2014 Jun; 7(3):365-73.
8) Jamshidi Y, Nolte IM, Dalageorgou C, Zheng D, Johnson T, Bastiaenen R, Ruddy S, Talbott D, Norris KP, Snieder H, George AL, Marshall V, Shakir S, Kannankeril PJ, Munroe PB, Camm AJ, Jeffery S, Roden DM, Behr ER. ***Common variation in the NOS1AP gene is associated with drug-induced QT prolongation and ventricular arrhythmia.*** JACC 2012 Aug 28; 60(9):841-50.

Speaker invitations:

European Society for Human Genetics Conference, Copenhagen, May 2017. Oral presentation: "Inactivation of KLHL24 is associated with hypertrophic cardiomyopathy and abnormal glycogen storage in heart and skeletal muscle".

Sixth Biennial Cardiff International Clinical Cardiovascular Genetics Conference, Cardiff, October 2017. Oral presentation.

Abstract accepted for Oral presentation at the ICHG2016, "Exome-chip meta-analysis identifies novel associations of low-frequency and rare coding variants with cardiac conduction in 85,593 adults of European and African descent from the CHARGE Consortium." April 2016.

Invited speaker at the 10th Workshop on Cardiomyopathy and Contractility, Imperial College, November 2015.

20 years of Cardiogenetics in the Netherlands, December 2015."Exome chip meta analysis identifies novel associations of coding variants with cardiac conduction"