Targeted resequencing of coding and cardiac non-coding regulatory regions related to genes implicated in cardiomyopathy

Authors:
E Nagyova¹, M Harakalova¹, V Tragante¹, G Kummeling¹, A Sammani¹, JAJ Verdonschot², A Baas¹, J Van Den Velden³, M Mokry⁴, FW Asselbergs¹, ¹University Medical Center Utrecht, Department of Cardiology, Division Heart & Lungs, Utrecht University - Utrecht - Netherlands, ²Maastricht University Medical Centre (MUMC), Department of Cardiology and Clinical Genetics - Maastricht - Netherlands, ³VU University Medical Center, Department of Physiology - Amsterdam - Netherlands, ⁴University Medical Center Utrecht, Department of Pediatric Gastroenterology, Wilhelmina Children's Hospital - Utrecht - Netherlands,

Background:
In dilated cardiomyopathy (DCM), mutations in over than 40 genes encoding crucial elements of cardiomyocytes have been detected. However, the coding variants in DCM genes explain inheritance in only a third of DCM patients. It has been described that regulatory sequences of genes, promoters and enhancers, regulate the time, location and levels of gene expression programs. Variants in those regulatory elements can alter the binding affinity of transcription factors, thereby changing or even diminishing the expression of a regulated gene even though the gene itself is not mutated. The regulatory processes that mediate biological mechanisms of DCM remain incompletely understood and in genetic diagnostics of cardiomyopathies regulatory sequences have largely been ignored.

Methods and Results:
We have performed a custom-designed targeted next generation sequencing of 113 genes previously linked to dilated cardiomyopathy, including their coding sequences, untranslated regions and cardiac-specific cis-regulatory elements (promoters and enhancers) in 25 Dutch DCM patients. Regulatory elements were designed based on a full promoter sequence spanning 1kb from transcription start site of each gene, including 5’UTR sequence and known cardiac DNAse hypersensitivity sites within a window of +/-30kb from gene start. Variants were prioritized using various filters, mainly based on overlap with known (cardiac) transcription factor-binding sites and regulatory signals from other types of chromatin methods (e.g. H3K27ac ChIPseq). Using various filters, we have narrowed down the list of potential disease causing non-coding variants to 0-3 per patient. In addition, we have used genotype data from 499 GoNL controls to test the mutational load in DCM cohort.

Conclusion:
For the first time we have created lists of variants in regulatory elements (promoters, enhancers) of gene involved in dilated cardiomyopathy. We have performed an important exercise before moving to whole genome sequencing analyses of diagnostic panel and exome sequencing negative DCM patients.