Next-generation sequencing in families with inherited cardiac arrhythmias

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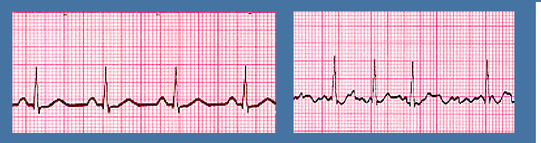
Aim: To use whole-exome capture and next-generation sequencing to identify novel mutations in genes causing familial life-threatening arrhythmia syndromes.

Introduction

Heart arrhythmias can cause abnormal heart rhythm to occur in seemingly healthy people. Physiologically, the patient's heart appears normal but at the genetic level, mutations in key genes cause irregular disturbances to the heart's electrical conductance, or, heart beat.

Mutated genes can be inherited when a carrier, or affected individual, has children and so the disease can be traced through families in a Mendelian fashion. When a patient is found to have an arrhythmia syndrome, it is essential that genetic testing of other family members occurs, in order to identify carriers and non-carriers of the mutated gene/s.

Many types of arrhythmia syndromes have been characterised, but not all of the disease causing genes are yet known. My project focuses on using a technique called next generation sequencing to identify novel mutations in a family where heart arrhythmia has been confirmed.



Far left: Diagram shows the normal sinus rhythm of the heart, as seen on an ECG (Electrocardiogram)
Middle: Normal sinus rhythm
Right: Atrial fibrillation, the most common manifestation of a cardiac arrhythmia, note P waves missing[1]

Techniques Used

Next generation sequencing is a high throughput technique which is changing the face of genetic testing. It uses a sequencing-by-synthesis approach to determine the exact sequence of DNA and thus, shed important insight into the inheritance of rare Mendelian diseases[2].

Sequencing of the whole genome still remains an extremely expensive technique, which is why we aimed to sequence the exome. The exome contains all of the coding regions found in our DNA and consists of exons.

Exons are the regions of the genome that are translated into amino acids, the building blocks used to make proteins. Approximately 180,000 exons can be found in the human genome and the whole exome accounts for just 1.5% of the total genome[3].

Methods Used

Sample Preparation

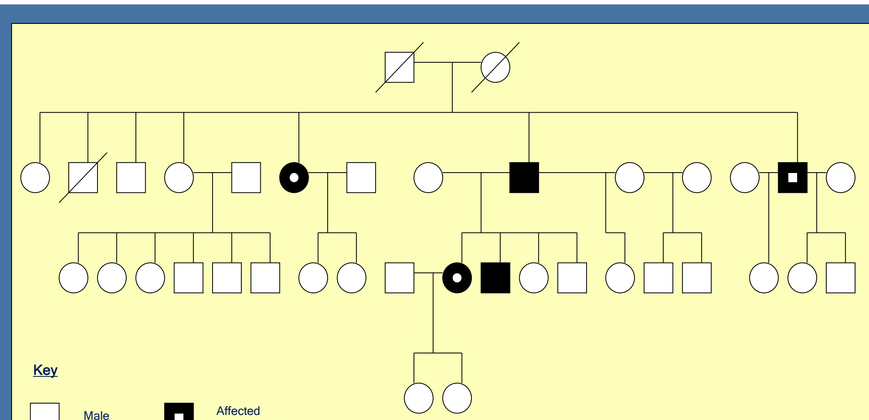
- DNA extracted from blood
- DNA sheared and transformed to blunt ends
- 3' Poly(A) tail added
- Adapter molecules ligated to ends

Target amplification

- Complementary DNA strands added and hybridize to their target DNA
- Hybridized DNA amplified using PCR (polymerase chain reaction)

Sequencing

- After the DNA is checked for contaminants the sequences are run on the Next Generation Sequencer
- Data provided is analysed to identify common variants (mutations of a single base pair common to each sample)



A family tree diagram to show the relationship of the affected family members sequenced in my project,
Above right: Working in the lab, Right: With the Next Generation Sequencer

Results

- 17 mutations in 14 genes were found to be common among all 3 samples
- Of these, 5 are of particular interest because of their related functions to the heart
- 1 is a known cardiomyopathy associated protein coding gene

All of the disease-causing mutations identified map to either chromosome 1 or 2. Each human cell contains 23 pairs of chromosomes, so these finding suggest that the chromosome position of the disease causing genes is important. When the chromosome position of each gene is taken in to account, the results suggest there may be a linked region of chromosome 1 that is associated with cardiac arrhythmias.

These results are consistent with the relationship of the 3 samples (family members who are related to one another) as we would expect certain gene regions to follow similar segregation patterns during cell division.

Future Work

The next step in this work will be to sequence the remaining affected family members. This will help to further validate the results I have found and minimise the list of common variants (mutated genes), making it easier to identify the novel disease causing gene. Experiments can then be done to characterise the gene of interest and to determine the function of the protein for which it encodes.

Any gene identified can then be used in genetic screening of the remaining family members to establish whether clinical intervention is required, such as the insertion of a pace-maker or the administration of drugs. Without genetic validation such as this, the remaining family members have to live in uncertainty, which makes this work, and work like it, extremely worthwhile and important.

References and contacts

[1] http://www.ambulancetechnicianstudy.co.uk/rhythms.html
[2] http://www.illumina.com/technology/sequencing_technology.ilmn
[3] 'Initial sequencing and analysis of the human genome' http://www.nature.com/nature/journal/v409/n6822/full/409860a0.html
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