Fainting and weakness: novel approaches to solve common problems

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Objective

My project aimed to analyse the DNA of patients suffering rare diseases to find a cause of their disease.

Understanding more about the cause of disease within these patients may suggest better ways to treat their condition.

Introduction

DNA sends codes to our body to induce the production of specific proteins. If the DNA is faulty, it can result in abnormal protein production leading to disease. DNA is normally divided into groups, known as genes.

The DNA of three patients was searched for any abnormalities that may be causing irregular protein production. In particular, only DNA within the protein-coding regions of genes – **exons –** were examined.

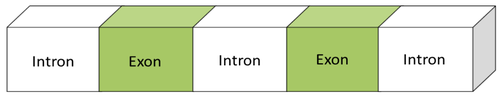
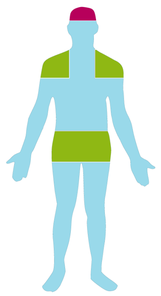


Figure 1: Structure of a single gene, showing both exons (green) and other non-protein-coding regions (white).

The first patient suffered **limb-girdle muscular dystrophy** (LGMD), which is a disease characterised by muscle weakness particularly in the shoulder and hip regions. The other two patients were related to one another and suffered **vasovagal syncope** (VVS), a condition characterised by an abnormal lowering of blood pressure leading to fainting.

Figure 2: Areas of the human body particularly affected by LGMD (green) and VVS (red).



Methods

DNA was extracted from the three patients. A computer **Ingenuity Variant Analysis program** was used, which compares DNA sequences of patients against DNA sequences of unaffected subjects. This allows any DNA abnormalities **(mutations)** in the patients to be identified.

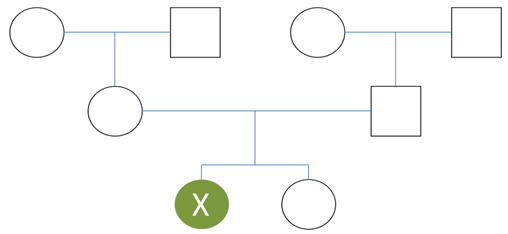
From the mutations identified by the program, mutations were excluded if they did not meet the criteria listed in my **filter parameters**. These parameters were chosen to match the presentation of the patient, the inheritance pattern of the disease through the patient's family tree and the rare nature of the disease.

Filter Parameters

Mutations were filtered to keep only the mutations which:

- Fit the disease inheritance patterns from the patient's family tree
- Showed an association to the disease from previously published articles
- Showed a prevalence in the population of less than 1%

1)



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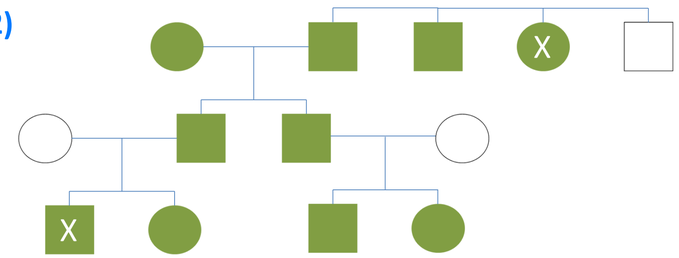

Key

= Unaffected Male

= Unaffected Female

= Affected Male

X = Patient being studied

Figure 3:
(1) Family tree of LGMD patient, inferring a random mutation occurred in the patient.

(2) Family tree of VVS patients, inferring only one affected parent is required to transmit the disease.

After filter parameters were applied, any remaining mutations were further researched to assess if they were likely to play a role in the cause of the disease.

Confirming and Analysing Mutations

A **Sanger Sequencing** method was used to further confirm the mutations detected by the Ingenuity program were present in the patient of interest. The procedure allows one to image the affected gene of the patient and compare it to the gene in a control patient.

Each of the considered mutations were also run through two programs: **Sorting Intolerant from Tolerant (SIFT)** and **Polymorphing Phenotyping v2 (PolyPhen-2)**. These programs assess if the mutations are likely to cause the encoded proteins to be defective, potentially leading to disease.

Results

LGMD Patient

Filter Parameters	Remaining mutations
None	113951
Disease Inheritance Pattern	1471
Association to the disease	9
≤1% Prevalence	4
Likely exonic candidate mutations	1

Figure 4: Ingenuity Variant Analysis results for LGMD patient after filter parameters are added.

A mutation in the **KCNN3 gene** was found in the muscle DNA.

The KCNN3 gene plays a role in skeletal muscle development and has been shown to be upregulated in other dystrophy types, e.g. myotonic dystrophy.[1]

Sanger Sequencing was unable to be performed with the KCNN3 gene . SIFT and PolyPhen-2 also implied the mutation was unlikely to produce a defective protein.

VVS Patients

Filter Parameters	Remaining mutations
None	407637
Disease Inheritance Pattern	7737
Association to the disease	4813
≤1% Prevalence	2784
Likely exonic candidate mutations	4

Figure 5: Ingenuity Variant Analysis results for VVS patients after filter parameters are added.

A mutation was found in the **ACE, WNK4, EPAS1** and **PLCG2 genes** in the blood DNA of both patients.

The ACE and WNK4 genes are involved in the kidney regulation of blood pressure. The EPAS1 gene is important for maintaining adequate blood oxygen levels. The PLCG2 gene usually produces proteins involved in maintaining blood pressure.[2,3,4,5]

Sanger Sequencing confirmed the presence of the ACE, EPAS1 and PLCG2 mutations. However, SIFT and PolyPhen-2 showed only the ACE mutation was capable of producing a defective protein.

Conclusion

The results support the involvement of the mutation of the ACE gene in the cause of VVS in the two related patients. This may point towards a current target for treatment within these patients.

On the other hand, the cause of the LGMD patient's disease is still unclear. This may indicate the non-protein-coding parts of the LGMD patient's DNA need to be examined as well for mutations to learn more about the cause of their disease.

References: **1.** Kimura T, Takahashi M, Fujimura H, Sakoda S. Expression and distribution of a small-conductance calcium-activated potassium channel (SK3) protein in skeletal muscles from myotonic muscular dystrophy patients and congenital myotonic mice. Neuroscience Letters. 2003;347(3):191-195. **2.** Genetics Home Reference. ACE gene [Internet]. 2015 [cited 5 August 2015]. Available from: http://ghr.nlm.nih.gov/gene/ACE. **3.** Genetics Home Reference. WNK4 gene [Internet]. 2015 [cited 5 August 2015]. Available from: http://ghr.nlm.nih.gov/gene/WNK4. **4.**Genetics Home Reference. EPAS1 gene [Internet]. 2015 [cited 5 August 2015]. Available from: http://ghr.nlm.nih.gov/gene/EPAS1. **5.** Genetics Home Reference. PLCG2 gene [Internet]. 2015 [cited 5 August 2015]. Available from: http://ghr.nlm.nih.gov/gene/PLCG2.

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