Investigating Rare Mutations in Genetic Male Infertility

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Aims

- To analyse data from a sample of infertile patients
- To identify specific genes with a high risk of causing infertility
- To assess whether these genes are likely to be responsible for each patient's infertility

Introduction

Infertility is clinically defined as a couple being unable to conceive a child after 1 year of unprotected intercourse, being the fault of the man in about half of all cases. Infertility can be caused by a wide variety of factors, but is often due to genetic mutation(1).

Prior to the start of this project, DNA samples were collected from a large cohort of Estonian men that could produce little to no sperm. The project itself centres around analysis of the data obtained from sequencing these samples.

Methods

To start, all of the patient data was compiled into a spreadsheet and a number of light filters were used to ensure high quality data that could be trusted to be reliable. After this, further and more rigorous filtering was used to identify specific mutant genes that may be responsible for the infertility of the patients.

Following this, the properties of each mutation and the gene they occurred in was investigated, making use of the standardised classification method published by Oud *et al.*, 2019.

As per this method, each gene containing a mutation was checked for how strongly it is involved in spermatogenesis, the process of producing sperm cells. This gives a measure of how likely it is that a mutation in the gene would be detrimental to spermatogenesis. Genes were classified as either "unlikely causative", "unclear" or "possibly causative".

Additionally, each mutation was checked against a number of database scores to give an estimation of how badly the mutation might affect the protein product of the gene. Mutations were classified as either "likely benign", "unknown significance" or "likely pathogenic"(2).

Figures

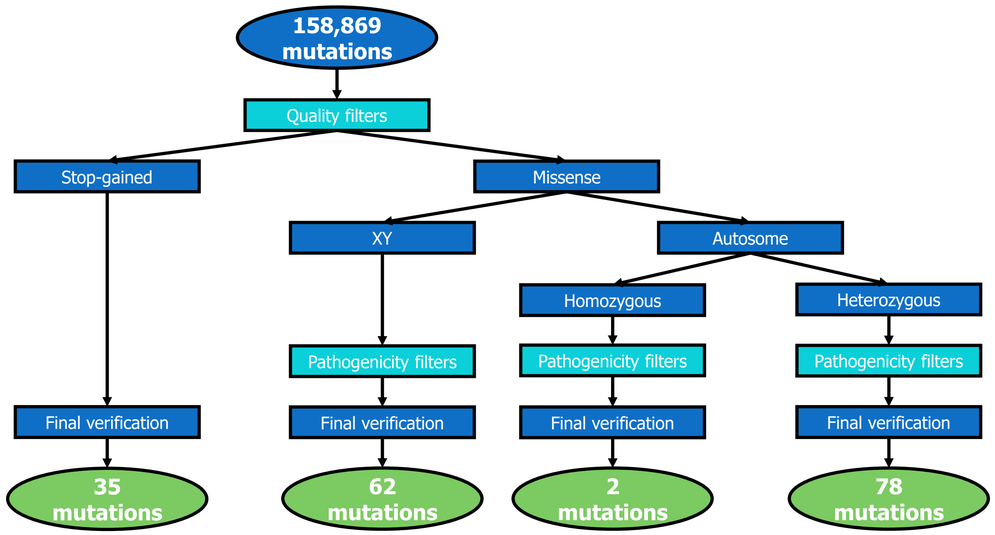

Figure 1
Filtering steps required to narrow down the number of mutations to investigate. The exact filters used for quality and pathogenicity have been omitted for clarity.

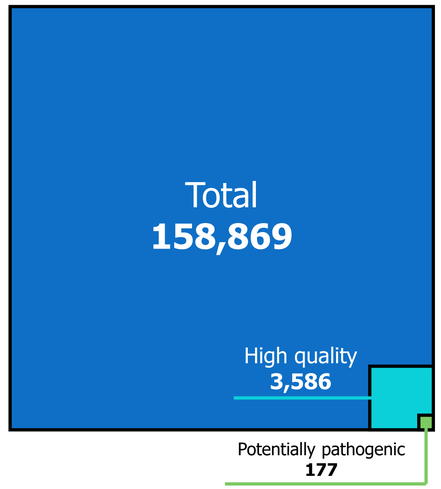

Figure 2
Relative proportions of mutations included after passing the data through quality and pathogenicity filters. Of the initial 158,869 mutations, only 3,586 were of high enough quality to be reliable data. Of these, only 177 were identified as being potentially pathogenic.

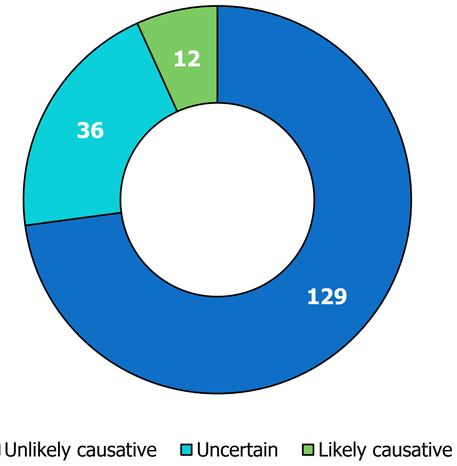

Figure 3
Relative proportions of mutation classifications. The vast majority of mutations were predicted not to be causative and a very small proportion were predicted to be responsible for the patient's infertility. A larger proportion did not have enough data to make a prediction.

Findings

Of the 158,869 mutations initially found, 53 mutations were classified as likely pathogenic. However, many of these mutations had to be discarded due to a number of factors.

These factors included pathogenic mutations found in unlikely causative genes and recessive mutations in a heterozygous state.

After this consideration, 9 mutations were left, in the APOB, CHD2, DNAH6, GRP64 and ZFY genes.

For these 9 patients, it can be strongly suspected that the mutation is the reason for their infertility, but further testing will be needed to confirm this.

What next?

Continuing on from this project, the next step is to validate and confirm the findings. This would involve replicating and resequencing the sections of patient DNA containing the mutations, to be certain they are real.

After this, it is possible to compare the DNA of the patient with that of their parents to ascertain which parent the mutation came from, or if it is a *de novo* mutation.

Acknowledgements

I would like to thank Prof. Joris Veltman and Dr Miguel Xavier for their supervision of my project, as well as Amrik Virdi, Bilal Alobaidi, Kumara Mastrorosa, Giles Holt, Hannah Smith, Sam Cheers and Maia Ozdemir for their help and support throughout.

1) Zegers-Hochschild F, Adamson GD, De Mouzon J, Ishihara O, Mansour R, Nygren K, Sullivan E, Van der Poel S. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology. Human Reproduction. 2009;24(11):2683-2687.

2) Oud M, Volozonoka L, Smits RM, Vissers LELM, Ramos L, Veltman JA. A systematic review and standardized clinical validity assessment of male infertility genes. Human Reproduction. 2019;34(5):932-941.