University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship 2019

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: maureen.bain@glasgow.ac.uk **within four weeks of the end of the studentship**.

1. Student

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   E-mail address: 2298130M@student.gla.ac.uk

2. Supervisor:

   Surname: Davies Forename: Eleanor

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3. Research Project Report

   3.1 Project Title (maximum 20 words):

   Identifying Polymorphisms on Exon 3 of the MRAP Gene as a Predisposing Factor to Hypertension

   3.2 Project Lay Summary (copied from application):

   High blood pressure affects one in three adults increasing risk of heart attack and stroke[1,2]. Its causes are poorly understood but involve genes, stress and excess production of the hormone aldosterone. Recent studies suggest a large proportion of people with high blood pressure are super sensitive to another hormone called ACTH which causes excess production of aldosterone[3]. We believe this is due to genetic differences and so intend to analyse genes associated with ACTH response. This may enable tests to identify the hyper-responders so they can be treated quickly and appropriately and reduce their cardiovascular risk.

3.3 Start Date: 05/08/2019 Finish Date: 06/09/2019

3.4 Original project aims and objectives (100 words max):

The aim of this project is to identify any polymorphisms present in the third exon of the gene coding for the MRAP protein.

MRAP is an accessory protein for the ACTH receptor (MC2R) that is required for ACTH to bind to the receptor and elicit a response. It is hypothesised that potential common polymorphisms have significant functional effects on the expression of the receptor and may lead to an increased response to ACTH. This 'hyper-responsiveness' may increase aldosterone secretion, which in turn can increase blood pressure and progress into essential hypertension.

3.5 Methodology: Summarise and include reference to training received in research

methods etc. (250 words max):

53 DNA samples from previously identified hypertensive patients were amplified using polymerase chain reaction. All samples were taken from the MRC BRIGHT study, who were selected according to set criteria[4]. Each sample was purified and DNA concentration measured using spectrophotometry. Gel electrophoresis was then used on the purified samples and compared with a DNA ladder to ensure the samples were of the correct length and that there were no other amplified sequences.

The purified samples were sent for Sanger sequencing to Eurofins MWG Operon (Germany) and the sequencing results were returned by email. Initially the samples were sent using a forward sequencing primer only. UGENE software[5] was used to align the samples against a consensus sequence of the human MRAP gene. The sequences were analysed by eye against a key which identified abnormalities in the sequence through comparison of the samples against the consensus and looking at the associated chromatogram which indicated measured levels of each base at a specific location. The samples were filtered if their sequence matched less than 50% of the DNA consensus. Samples containing suspected polymorphisms were sent to Eurofins using a reverse sequencing primer to establish whether the changes were genuinely polymorphic or caused by a misreading of the sequencing. The genuine polymorphisms were then compared against known polymorphisms on the Ensembl Genomic Database to analyse their frequency in populations and phenotypic outcomes.

All techniques were taught at the Institute of Cardiovascular and Medical Sciences (ICAMS) and were practised on DNA samples from different studies.

3.6 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

Spectrophotometry showed that there was a wide range of DNA concentrations present in the sample from 4.4 to 63.8 ng/µL. However, the majority of the samples were of sufficient quality and quantity to provide reliable sequencing results could be adequately interpreted.

Gel electrophoresis showed that all of the samples were of the correct length and that the primers were not amplifying any other region of the genome (see appendix figure 1.1).

Using only the sequences from the forward sequencing primers, 4 potential polymorphisms were identified (see table 1.1 and appendix figures 1.2 to 1.5).

Supposed Polymorphism	**Location (Base Number)**	**Frequency in Samples (no. out of 61)**
Intronic polymorphism replacing a C with a T	607	4
Insertion of T	After 258	6
Deletion of up to 3 bases (CCC)	290 to 292	11
Intronic polymorphism replacing a T with either an A or C	352	4

Table 1.1 – Initial Polymorphisms Suspected Based on Forward Primer Sequencing Samples

Upon analysis and comparison of the samples together with the reverse sequencing primers, only one of these supposed polymorphisms was present in both the forward and reverse primer sequences, which was the missense mutation at base number 607. This was detected by clear peaks in the associated chromatogram. The remaining three were only present in the forward strand, indicating that secondary structure in the forward sequence had produced spurious results in certain of the study samples.

The Ensembl database showed that the confirmed polymorphism (rs2254251) has already been identified with a minor allele frequency (MAF) of 0.118 in European populations. However, there is no information of any phenotypic consequence of this polymorphism and there is no evidence that this will affect ACTH response.

There was no variation found within the exon that codes for MRAP, only in the intronic region immediately outside of the exon (out with base numbers 400 and 516; see figure 1.6). Three samples did appear to have variation in the exon, but this was dismissed as an artefact of the sequencing process, following further confirmatory sequencing.

3.7 Discussion (500 words max):

This project aimed to identify the presence of common polymorphisms in the third exon of the gene coding for MRAP as part of a wide initiative to sequence the entire MRAP and MC2R genes. The results have identified a polymorphism at base number 607 which has an MAF of 0.033.

The majority of samples used came from the BRIGHT study, a predominantly white European population, which has an MAF of 0.12. However, this polymorphism is more common in African and East Asian populations[6]. With there being a greater degree of hypertension in Africa than in Europe[7], future studies should aim to obtain a group of samples that shows greater ethnic variation to obtain whether the polymorphism is of any significance.

63 samples were deemed to be sufficient in order to achieve statistically significant results. Previous studies have shown that the estimated detection rate for polymorphisms with a MAF of >5% is 99%, with the detection of all polymorphisms (i.e. with a MAF of >1%) being an estimated 87%[8].

As the polymorphism is found to a greater degree in the European population as whole compared to the sample group, this indicates an inadequate representation of the general population and that a larger sample size is required for future studies if the findings are to have a more significant impact.

Despite this polymorphism already being recognised, there is no data on its phenotypic outcome in affected patients, creating the theory that it may result in no significant changes to normal physiological function. This warrants further investigation by recreating these genetic sequences in cells and monitoring any notable differences in their phenotype.

The large variation in the spectrophotometry readings affected the ability to interpret the sequencing and the chromatograms, due to the lines from the chromatograms overlapping leading to a misreading in the sequence. This may have been caused by a problem with DNA amplification or the overall quantity of DNA found in the original, unamplified samples.

The MRAP gene is composed of 5 exons and the gene coding for MC2R has 2 exons, the longest of which is 3,603 bases long. Whilst a polymorphism was found in MRAP exon 3, it is still possible that other, more significant polymorphisms are still to be discovered. This is still to be carried out by other researchers in the near future.

The only polymorphism was found in intronic material, with none being found in the exon. With some evidence showing that introns may have more functions than initially thought[9], it is possible that the polymorphism could affect the structure of MRAP. However, this is unlikely to be to as great a degree as a polymorphism in an exon.

References

1. Chonabanian AV (1992) 'Vascular effects of systemic hypertension', *American Journal of Cardiology,* 69(13), pp. 3E - 7E.
2. Rapsomaniki E. et al. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1·25 million people. *The Lancet* 2014; 383(9932): 1899-1911.
3. Markou A. et al (2015) 'Stress-induced Aldosterone Hyper-Secretion in a Substantial Subset of Patients With Essential Hypertension', *Journal of Clinical Endocrinology and Metabolism,* 100(8), pp. 2857-2864
4. Caulfield M. et al (2003) 'Genome-wide mapping of human loci for essential hypertension', *The Lancet,* 361(), pp. 2118-23.
5. UGENE (2019) *Unipro UGENE,* Available at: *http://ugene.net/* (Accessed: 26th August 2019).
6. Ensembl (2019) *rs2254251 SNP,* Available at: *https://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=21:32298668-32299668;v=rs2254251;vdb=variation;vf=293800064* (Accessed: 26th August 2019).
7. World Health Organisation *Raised blood pressure,* Available at: *https://www.who.int/gho/ncd/risk_factors/blood_pressure_prevalence_text/en/* (Accessed: 3rd September 2019).
8. Crawford D. et al. (2004) 'Haplotype Diversity across 100 Candidate Genes for Inflammation, Lipid Metabolism, and Blood Pressure Regulation in Two Populations', *American Journal of Human Genetics,* 74(4), pp. 610-622.
9. Bong-Seok J, Choi SS (2015) 'Introns: The Functional Benefits of Introns in Genomes', *Genomics and Informatics,* 13(4), pp. 112-118.

4. Reflection by the student on the experience and value of the studentship (300 words max):

I feel that the time I spent in ICAMS for 5 weeks across summer has been an invaluable experience. On the medical curriculum there is not a large amount of laboratory work in comparison to life sciences degrees, so this placement has given me a very informative introduction into what a career in research is like and the various difficulties that are involved.

I was taught various techniques that are seen as standard throughout most research projects of this nature (e.g. PCR and gel electrophoresis) as well as using bioinformatics, which has made me appreciate the large role that computers have to play in the field of medical research. I also learned that even with all the positive aspects, if you make a small mistake, for example labelling a sample incorrectly, it can be incredibly frustrating to try and rectify your mistake, which can hold you back in the progress you are making.

I have had an interest in endocrinology since I began to learn about it in my 2nd year of medical school. This placement has both consolidated my knowledge of the various endocrinological mechanisms behind the control of blood pressure and also made me seriously consider following a career in the field.

Working with various experts in this field has been a pleasure as they have always been highly informative when I have any queries regarding the research and always made sure that there was someone there for me if I needed any assistance.

I would strongly recommend to anyone on the Head of College Scholar's List Scheme to apply for the summer studentship as an experience like this will help shape your future if you are considering a career in research.

5. Dissemination: (note any presentations/publications submitted/planned from the work):

Upon completion of the project, I will collate my findings and those already obtained from other parts of the project to make a poster that will be presented at the University of Glasgow Medical Research Conference later this year.

6. Signatures:

Supervisor	Date	Student	Date
Eleanor Davies	25/9/19	C. Mooney	25/9/19
PROF ELEANOR DAVIES		CALUM MOONEY	

Appendix

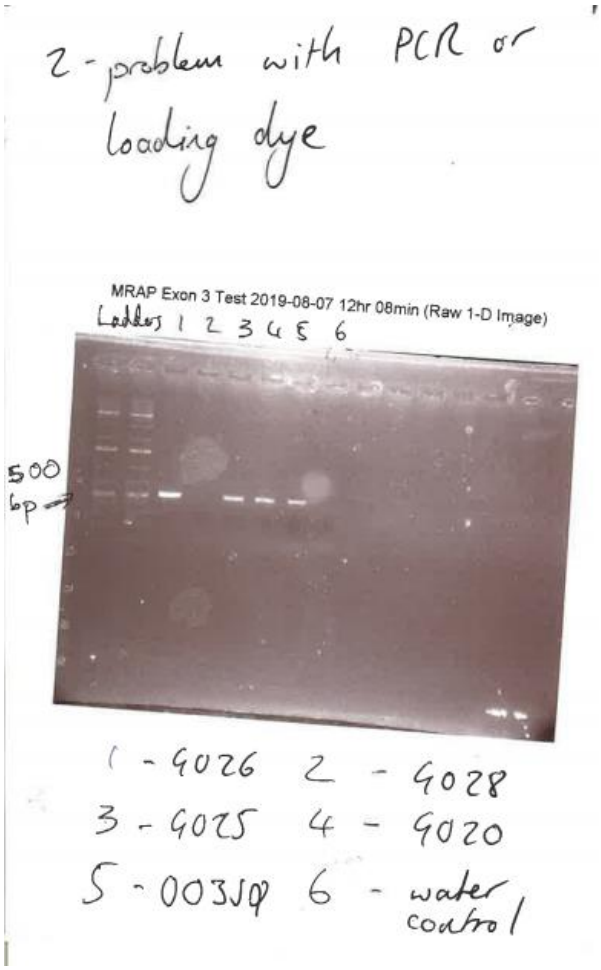

Figure 1.1 – Gel Electrophoresis Displaying Length of the Region Being Amplified in Selected Samples

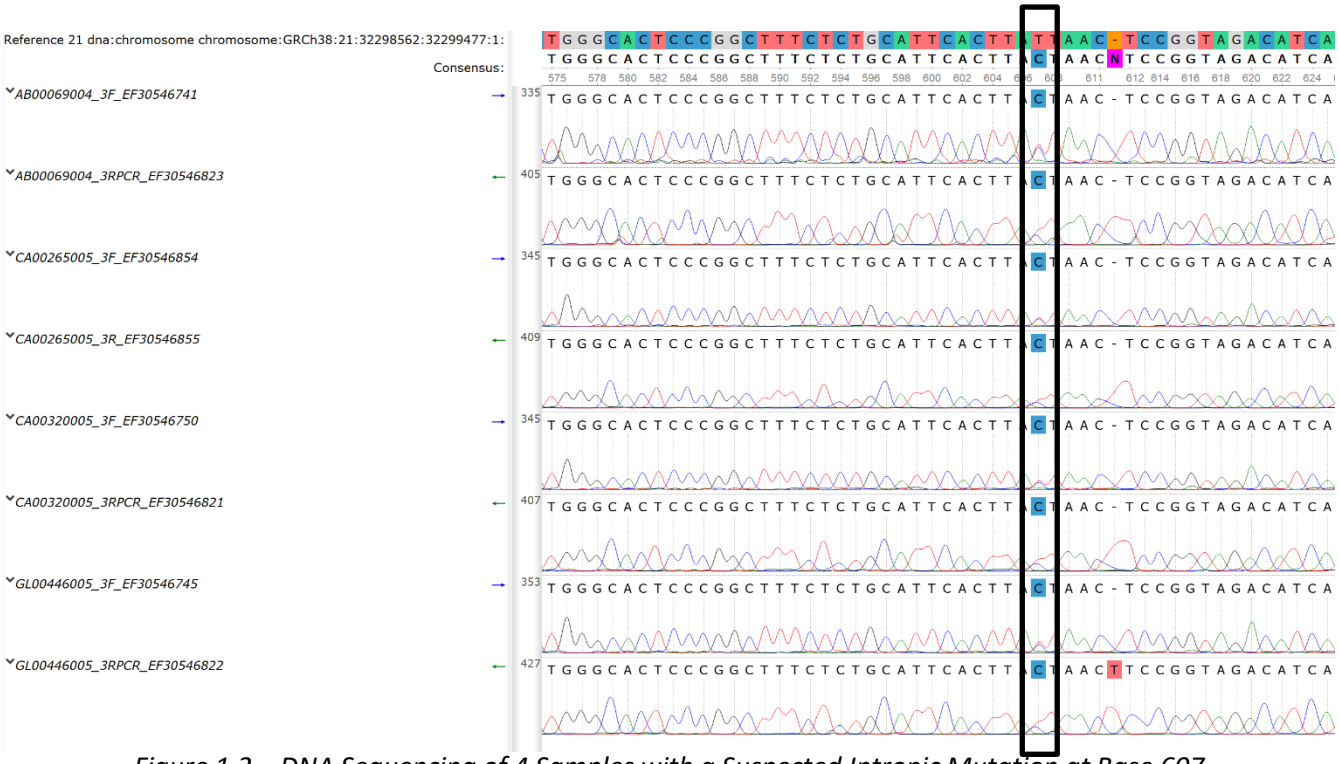

Figure 1.2 – DNA Sequencing of 4 Samples with a Suspected Intronic Mutation at Base 607

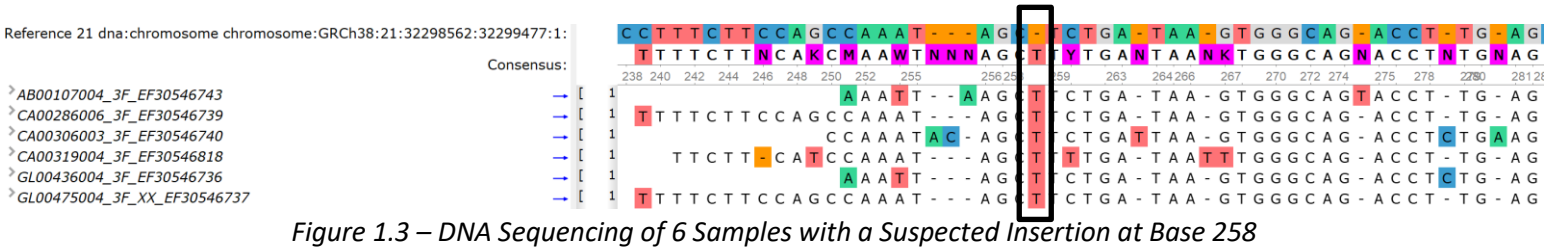

Figure 1.3 – DNA Sequencing of 6 Samples with a Suspected Insertion at Base 258

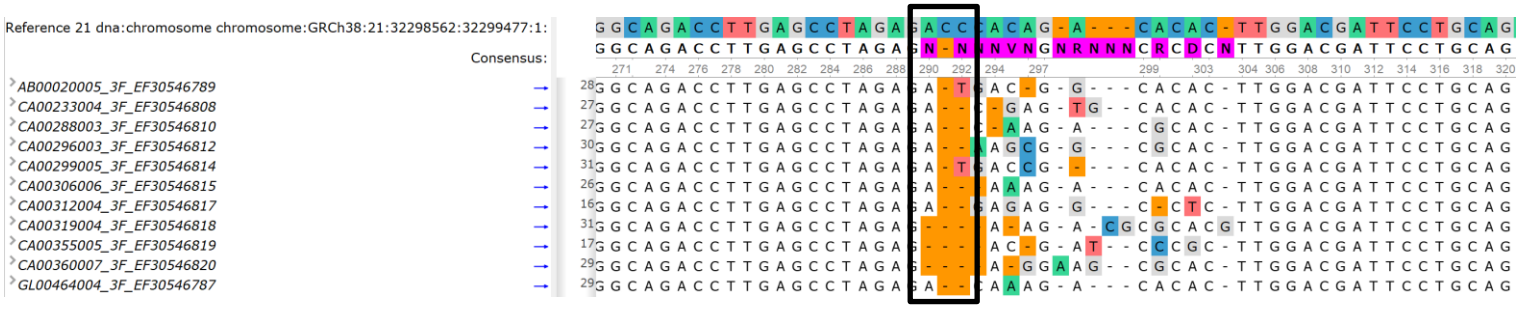

Figure 1.4 – DNA Sequencing of 11 Samples with Suspected Deletions from Bases 290 to 292

Figure 1.5 – DNA Sequencing of 4 Samples with a Suspected Intronic Mutation at Base 358

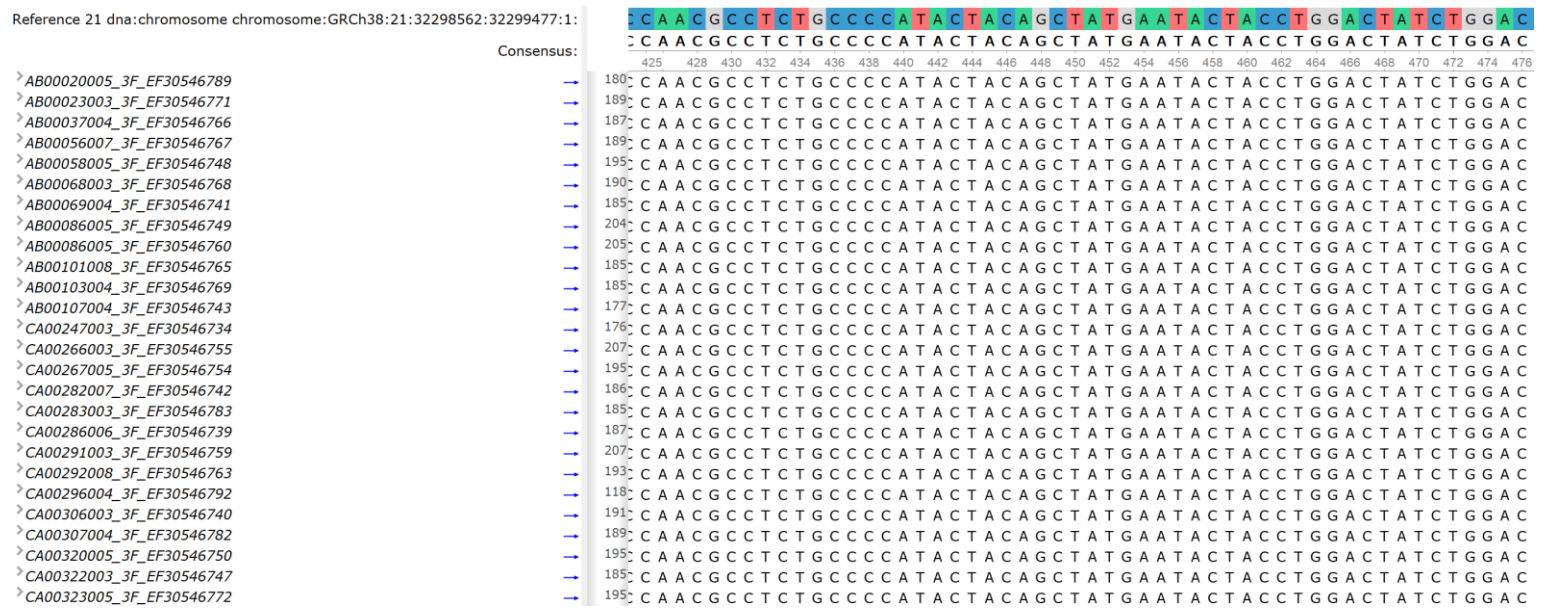

Figure 1.6 – DNA Sequencing Showing the Absence of Variation within the 3rd MRAP Exon