UNIVERSITY OF LIVERPOOL

Spring 2021 | Issue 4

Insider Imprint

The life sciences journal for undergraduate and masters students at the University of Liverpool

Scimos
Our Solution to Science Inaccessibility
Daisy Cookson and Nicole Szekeres-Tap, p. 16

The COVID Crossroads
How a Needle in Your Arm Will Put a Glass in Your Hand
Riley McMahon , p. 52

Wellbeing Reminders
From One Student to Another
Rebecca Court, p. 77

ISSN: 2516-256X

Insider Imprint

Cover picture by Ivan Samkov, available in Pexels.

Free stock images from Pixabay and Pexels by fernando zhiminaicela, lil_foot_, StockSnap, VIN JD, S. Hermann & F. Richter, Please Don't sell My Artwork AS IS, Thanks for your Like • donations welcome and Cottonbro.

Additional images from University of Liverpool image library.

Insider Imprint (Print) **ISSN 2516-256X**

Insider Imprint (Online) **ISSN 2516-2578**

UNIVERSITY OF LIVERPOOL

Published Spring 2021

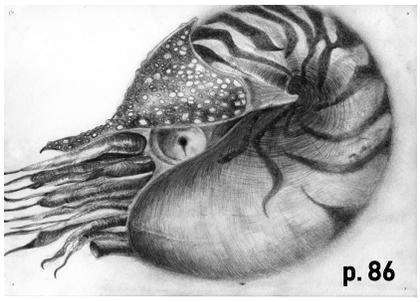
INSPIRE

wellcome

The Academy of Medical Sciences

Contents

Insider Imprint | Issue 4 Spring 2021



Foreword

I am delighted to be asked to write this foreword to the fourth edition of Insider Imprint, although it is impossible to start without recognising the difficult year we have all had adapting our personal and working lives to the challenges of living in a period of a global pandemic. I hope you and your family are well and taking care of one another.

Insider Imprint continues to go from strength to strength and, as you will see from the articles contained within, it contains much to stimulate the little grey cells of anyone with an interest in the life sciences and how the biological world around us works. However, it contains much more too, including employability advice and career paths to consider, insights from our graduates who have been there and done it, and, importantly, how we can support one another in our wellbeing.

This journal gives our undergraduate and postgraduate students an invaluable opportunity to disseminate their research and develop their scientific communication and evaluation skills, whether as contributors, reviewers and/or editors. This year I am particularly pleased to see the launch of the new Ambassador Scheme which includes new opportunities to participate in outreach activities, bringing the research work of the University to an even greater audience and, hopefully, encouraging future generations of contributors.

I am sure that, like me, you will be hugely impressed with the high standard of the journal's production and content; my congratulations to all those involved. There is something here for everyone, from proton NMR Spectroscopy (I spent many a happy hour acquiring proton spectra of oligosaccharides during my PhD) to the very topical design of Covid vaccines. I particularly love the included artwork at the end, for what good is science without culture? Taken as a whole this journal reminds us how dedicated, talented and caring our student community is.

The Covid pandemic has highlighted just how important our universities are to tackling the big global issues of our time, doing research with impact and informing public understanding, debate and policy. This journal is a part of that commitment to educate and communicate. To those of you about to turn the page, enjoy.

Professor Gavin Brown

Pro-Vice-Chancellor for Education, University of Liverpool

The world and how we live in it has changed a lot in the last year, but despite the challenges of the new reality students at Liverpool have continued to submit high quality articles to us for publication making this issue full from cover to cover with a wide range of engaging, insightful and captivating content. We would like to congratulate our issue 4 authors for possibly your first publication; an excellent achievement that you can be very proud of.

At Insider Imprint we have had a particularly exciting year, launching two new enterprises: our Student Ambassador Scheme (see page 8) and our Insider Insights Podcast (see page 9). With these new initiatives we aim to give you extra value content and further enriching opportunities with us.

We hope you enjoy reading issue 4, gaining new knowledge and inspiration.

Stay safe and well.

Insider Imprint Team

Insider Imprint

UNIVERSITY OF LIVERPOOL

STAFF Awards 2019 Innovation of the Year

Meet the Team

Meet the team behind Insider Imprint! We are a dedicated team from across the Faculty coming together with a common goal of creating a space for undergraduate and masters students to showcase their achievements, to inspire the upcoming generation of students and to support students in becoming confident and competent presenting their work, ideas and experiences.

Editorial Team

DR FABIA ALLEN
Managing Editor

Lecturer in the School of Life Sciences

CATARINA CASTANHEIRA
Editor & Artistic Director

PhD student in Musculoskeletal Biology

MANOHAR KODURI
Editor & Co-Peer Review Manager

PhD student in Bioengineering

NATALIE KOCH
Editor & Outreach Manager

PhD student in Animal Biology

HEATHER DAVIES
Editor & Social Media Manager

PhD student in Veterinary Health Informatics

LIAM MCCORMICK
Editor & Co-Peer Review Manager

PhD student

EMILY CLARKE
Editor

PhD student in Musculoskeletal Biology

Student Ambassadors

PRAKRITI SEHGAL

MSc Advanced Biological Sciences

TIERNEY WITTY

4th year MA Classics and Ancient History

AMY MCDERMOTT

Final year Veterinary Sciences

MEGAN KERRIDGE

3rd year Zoology

What are we all about?

Insider Imprint is Liverpool's Life Sciences journal, publishing work from undergraduate and masters students at the University of Liverpool. The journal goals are to:

- Provide a platform for undergraduate and master students to **showcase** their many activities and successes
- To **inspire** the upcoming generation of students through facilitation of transfer of knowledge & experiences between students
- To **support** students in becoming confident and competent presenting their work, ideas and experiences for publication
- **Communicate** the best Life Sciences research from the students of the University of Liverpool

Collaboration • Sharing • Supporting Success

Who can write for us?

If you are an **undergraduate or masters student** in the **Faculty of Health and Life Sciences**, you can write for us. If you have something you want to write about, we want to hear from you!

What do we publish?

Research articles

Such as based on honours, masters projects or internships

Literature reviews

Commentaries such as:

Reflections on work/internship experience

Hot topics and debate articles

Reviews e.g. on a book/seminar/summer school

Reflections on topics related to your studies

Creative pieces such as:

Artwork

Photography

….and more!

Got an idea not listed above?

Great, contact us!

Why publish with us?

Publishing with Insider Imprint is an excellent opportunity to **showcase your achievements** and **enhance your CV**. As an open access online journal, you will gain **visibility for your accomplishments**. Whether you are interested in staying in academia or have alternative career goals, having a published article or two is a sure way to **increase your employability** and impress potential employers.

Publishing with us will give you experience on how you go from an idea to print. We will guide you through the typical publication roadmap, from following author guidelines, submitting your work & receiving feedback to reviewing proofs before publication. You will also gain an insight into the peer-review process that is widely used to validate scholarly work.

Getting your first publication can **be a big step forwards for your career** and will help break down barriers early researchers often face when contemplating their first article submission. It also looks great on your CV for non-academic jobs too!

NEW Student Ambassador Scheme!

Find out more on page 8.

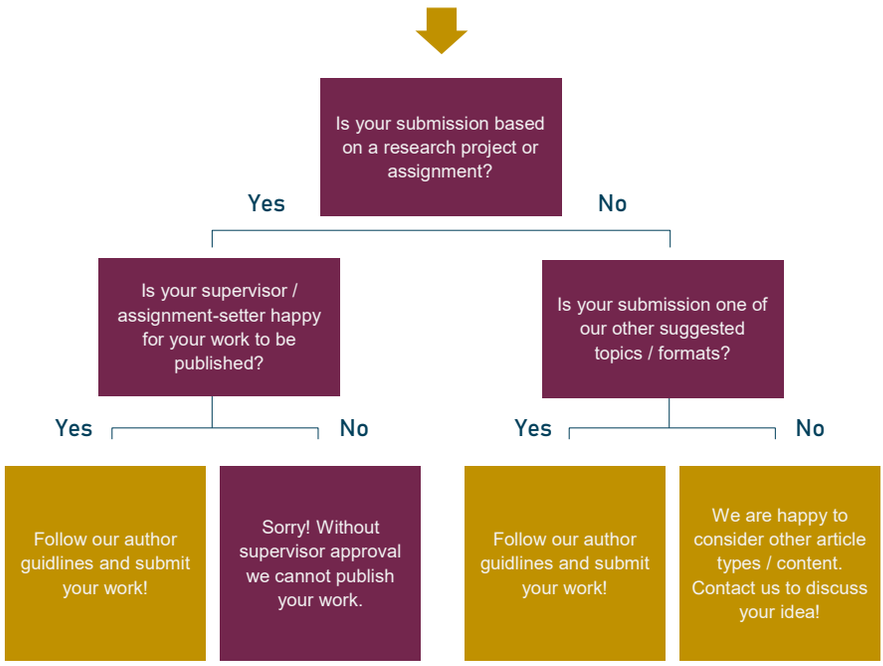
Getting started...

At *Insider Imprint* we accept a wide range of article types. Here are some things to think about to get started:

* If your submission is based on work set as an assignment, or on research you have taken part in, you will need **permission** from the member of staff involved before we can publish your work. So make sure you check this before you put in a lot of effort!

* If you are preparing an article for us, make sure you have a read over our **author guidelines** on our website. Just like other journals, we have certain content and formatting rules we ask you to follow - if you are unsure about anything, get in touch so we can guide you.

* If you have an idea for an article but are not sure if it would be considered for publication, just send us an email so we can advise you.



Motivations to publish

Showcase your achievements

Boost your CV

Inspire other students

Wow your future employer

Your work viewable to everyone online

High quality and peer-reviewed

Gain real-life experience working with a journal to publish your work

Opportunities for PhDs

Editorial Board:

We are a small team of PhD students, and will be periodically recruiting new members; keep an eye out for our calls or drop us a line via our email to express your interest.

Peer Review:

We are looking for enthusiastic and committed individuals to join our team of peer-reviewers. No prior experience is needed; an optional online training course is provided. Please find our application form at insiderimprint.com and send it over to us.

For both of the above, you must be a PhD student in the Faculty of Health and Life Sciences at the University of Liverpool. You must have permission from your supervisor and at least 1 year of your studies left.

The Fine Print

Work involving others, such as a supervisor, needs approval from them before it can be published. Any work previously submitted as an assignment should be re-written to align to our format, and appropriate permissions given from the staff member who set the work. You can find the rest of our journal policies on our website.

Connect with us on:

Email us at: insiderimprint@liverpool.ac.uk

More information at www.insiderimprint.com

NEW!

Ambassador Scheme

We are very excited to have launched our **student ambassador scheme for Insider Imprint** this year! Being a student ambassador is an excellent opportunity, as our ambassadors get to be involved in all the behind the scenes running of a scientific journal. Attending our editorial team meetings and getting involved in our outreach activities, our ambassadors gain a good understanding of the academic publishing process and also develop many skills including communication, organisation and teamwork. This is a HEAR accredited activity.

Student Ambassador Roles:

Attending editorial team meetings

Promoting Insider Imprint

Gathering feedback from our readership

Contributing to our news feed

Participating in our outreach activities

We are happy to welcome new ambassadors to our team. If you are an **undergraduate** or **masters student** at Liverpool, you can be an ambassador for Insider Imprint!

If you are interested in knowing more, please visit www.insiderimprint.com/join-the-team or email us at **insiderimprint@liverpool.ac.uk.**

What are our current ambassadors saying?

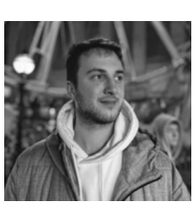
We already have **four wonderful student ambassadors**, Amy, Megan, Tierney and Prakriti. Find out more about the role from their perspective here:

I was always intrigued with science and research, most of the information regarding which is exclusively found in journals. The publishing of an article fascinated me and I want to learn about how the journal works, who are involved in the decisions. Thus, when I heard about the opportunity to apply to be a student ambassador in the Insider Imprint journal I immediately stepped in, and it turns out to be an amazing experience. Fabia and all the team members are incredibly supportive, and the environment is very friendly. I am looking forward to adding my contributions in the journal, and I would highly recommend students to get involved and enjoy the venture of new learning.

Prakriti Sehgal

The process of scientific publishing is something I have always been interested in. Working with Insider Imprint has really opened my eyes to the complexities and nuances of the editorial process. As an undergraduate student the experience has exposed me to a career path I never would have considered before. The whole team have been endlessly welcoming, I would recommend the experience to anyone!

Amy McDermott



Not coming from a life sciences background, I originally thought I wouldn't cope very well as a Student Ambassador at Insider Imprint. However, I applied anyway, and I have become part of an open, welcoming and fun team. The editorial team and Fabia have been there to help support us if we don't know what to do, and they've allowed us to throw ourselves into the journal and contribute. As someone who wanted to gain an insight into academic publishing, this has been an amazing opportunity, and it's given me that chance to develop skills which can be used in job applications. I would definitely recommend any student, no matter their academic background, to consider applying!

Tierney Witty

After publishing in issue 3, I was very excited about the opportunity to join the team and see what goes on behind the scenes of making the journal. The team have been welcoming and allowed us ambassadors to get stuck in with many aspects of the journal which I have found extremely interesting and rewarding! It has been a wonderful and unique experience to be a part of this team, it works well alongside my undergraduate degree, and I would highly recommend students to get involved!

Megan Kerridge

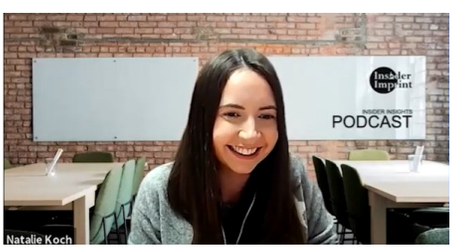
NEW!

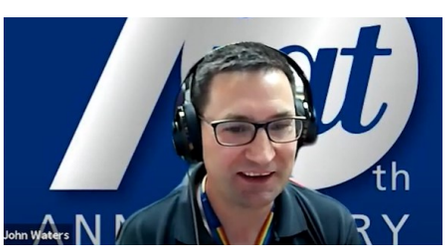
Insider Insights Podcast

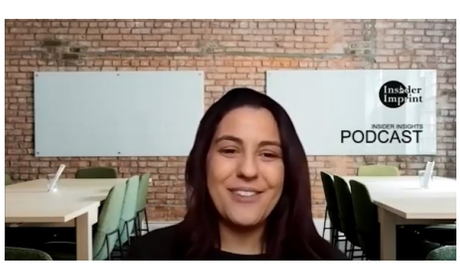
We have been very busy this year! In addition to our new Student Ambassador Scheme, we have launched our podcast **Insider Insights**!

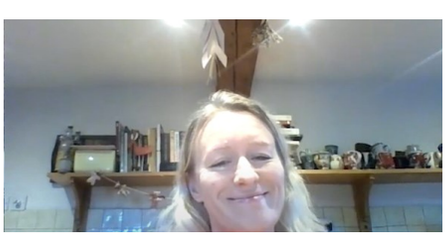
Join our editors as they chat with previous Insider Imprint authors. *Get to know the story behind an article, what was the inspiration, and what publishing has meant from the student's perspective*. We also invite professionals to gain insights into different career paths.

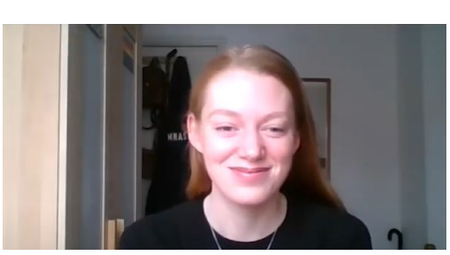
Now you can hear from our student authors themselves as they talk about their articles; you will learn about research students are performing, experiences studying abroad, volunteering opportunities, different careers and so much more!











WATCH ALL EPISODES

You can access all our episodes through our website at insiderimprint.com/podcast or directly through our YouTube channel @insiderimprint.

Reflections

What are your fellow students up to?

Read on to discover what your fellow students have been doing and their advice to you.

Pets and the Pandemic

Hannah Barton-Cooke

3rd year Veterinary Science

On the odd occasion when I've been called 'Crazy Cat Lady' in the past, I've not taken offence; I unashamedly adore my feline babies. In 2020, my two indoor cats - Isla and Lola - remained faithfully by my side, with not a care in the world, as I desperately tried to adjust to COVID life and university being online. Seemingly overnight, our whole understanding of concepts like 'practicals', 'dissections' and 'clinical skills' changed beyond recognition, as we had to adapt to watching, instead of doing these sessions. Then, as zoom calls and team meetings established themselves as the 'new norm' in our lives, it appears my cats have viewed this as a perfect opportunity to get their big break into the media industry. Isla, in particular, seems to have made it her life's mission to make a cameo appearance on tutorials, whenever my camera is turned on. Her typical performance comprises of giving everyone a little 360° spin, offering a quick flash of her back-end to the screen, before trying to find the best place to nestle down on my laptop. At the same time, there's Lola mistaking my slipper socks for a small furry mammal and going all predatory on me, or playing with boomingly loud toys that clatter and smash around my little island of Uni work. Whilst such commotion is arguably not fully conducive to academic success, in all honesty, I quite like the little pockets of entertainment my cats give me during the day.

As I am studying Veterinary Medicine, Isla and Lola have also become useful 'living models', to apply what I learn in lectures to my own cats (please note: this is for diagnostic purposes only; no surgery has taken place in my home). For example, during behaviour lectures, we were taught how cats can display negative social behaviour, such as engaging in quiet stand offs, showing particular body posture, and so on. The lecturer suggested that sometimes the negative behaviour between cats can be so subtle, owners may even struggle to notice it. It was at that precise moment I heard a massive thud, followed by frantic footsteps running down the stairs, before my two cats burst into the room in the midst of a (thankfully, rare) brawl. Clearly, Isla and Lola wanted to make their negative feelings nice and easy for me to interpret.

> "Admittedly, lockdown gerbils may not have the same immediate appeal as the new pet dog (…) "

Having previously only ever had cats, I ventured outside my metaphorical pet box in October 2020 by getting two adorable little gerbils, and am now well on the way to becoming a self-proclaimed 'Raving Rodent Woman'. You may well be thinking, cats and gerbils don't sound like such a sensible mix (cue Tom and sort-of-Jerry jokes), but rest assured Isla and Lola are kept away from them at all times, so the gerbils are perfectly safe! My first challenge was to think of the best names for them. As there are two males (gerbils are social species so you must get at least a pair) I was quite spoilt for choice by the list of famous/infamous dynamic duos to choose from: Caesar and Mark Antony; George and Lennie; Morecambe and Wise; Freddie and Jason; Macbeth and Banquo, to list but a few... (side note: it was disconcerting to compare the abundance of well-known male dynamic duos with renowned female couples, but that's a reflection for another time...). In the end, I thought 'Scrooge and Marley' was perfect, as Scrooge has a nice, 'old man' shade of grey fur and Marley is a beautiful, ghostly white.

Admittedly, lockdown gerbils may not have the same immediate appeal as the new pet dog that gave so many people an excuse to leave the house last year, but I can safely say they come with their own benefits - they're low maintenance, are happy to keep themselves occupied and provide endless free entertainment. When figuring out which cage would be best, I read that gerbils require lots and lots of bedding to allow them to replicate their natural burrowing behaviour in the desert. Having bought what I naively thought was a really decent sized one to prepare

for their arrival, I went off it by the end of the first month and decided ‘my boys’ deserved nothing less than a palace for a cage. Overnight, I became an architect/ interior designer, and got to work on my creation... The final result (for now!) is their original cage placed on top of a whopper of a fish tank, filled with masses of bedding. As for making the tunnels, Scrooge seems to have mastered the art and can create a system of intricate and complex thoroughfares; whereas Marley resembles a bulldozer, spending most of his time and energy figuring how to kick out the bedding!

To non-animal lovers (or possibly, to everyone!) I may be sounding absolutely barmy for writing a whole reflection on how much I love my pets. What I haven’t mentioned - until now - is that 2020 also made me proud owner and ‘mum’ of a packet full of Aqua Dragons (aka sea monkeys, or more scientifically, *Artemia salinas*) which, miraculously, actually hatched! Nor have I admitted - until now - that I have become mildly possessive of the various species of birds that frequent our garden and have, perchance, begun to consider the couple of pigeons that come to visit daily, as ‘part of the family’...

The truth is, throughout lockdown, our pets have provided us with much-needed serotonin to help us through this bizarre time. They are funny, they are cute and, whilst 2020 may have been the ‘Year of Government U-Turns’, our furry friends remain as dependable and constant as ever.

Working as an NHS Medical Laboratory Assistant during the COVID-19 Pandemic

Irene Burke

3rd year Biological Sciences BSc

What is LAMP testing and how has this developed over the course of the SARS-COV-2 pandemic?

RT-LAMP (Reverse transcriptase loop-mediated isothermal amplification) testing is a novel technique used to detect the SARS-CoV-2 virus in saliva samples. As this type of testing does not require thermal cycling at different temperatures, it can produce results faster than standard laboratory PCR tests. However, LAMP still needs to be conducted in a laboratory environment and is not suitable for the large-scale requirements of public testing.

LAMP testing has been offered for asymptomatic testing of NHS patients and staff at the Royal Liverpool University Hospital. The lab has been built up from ten original staff to over 130 colleagues as of March 2021. Our lab provides an essential testing service for frontline workers who are vulnerable to contract and spread SARS-CoV-2.

A Medical Laboratory Assistant is responsible for carrying out general duties around the lab. The specific jobs are specimen reception, sample preparation, and template addition. The main skills we perform are accurate pipetting, preparation of reagents and inputting of patient details.

The first stage for our samples involves booking them into the Laboratory Information Management System known as Telepath. This links the sample to the patient request form and allows the final test result to be issued directly to the patient. The samples arrive at the lab in sample bags that need to be removed, and the primary sample tubes are arranged to match a plate map. Each sample now has a unique barcode identifier that links the entry in Telepath to the sample as it moves along the testing process.

The next stage is sample preparation, where the primary saliva samples are aliquoted with a buffer to deactivate any virus present. The eppendorf tubes are then heated at 98°C for two minutes to begin the reaction. The eppendorf tubes are then added to a template with a reaction mixture which is then run for genomic analysis on an OptaGene Genie HT machine. At this point, the results, either positive or negative, are issued to patients.

> "Working in the lab has allowed me to develop both laboratory-based and interpersonal skills"

LAMP has the potential to be adapted for large-scale testing programs but is still an emerging technique and at time of writing has not yet been approved for mass testing pilots of the general public. The timeframes for processing and result issuing are faster than other types of tests. However, there is still the need for sample processing, reagents, and genomic analysis, all of which require a laboratory setup such as in a clinical or research setting. Therefore, whilst LAMP testing has the potential for larger-scale uptake, it has limits in its potential as a mass testing tool.

As the lab capacity builds up, we will be taking on more hospital and NHS trusts to the point where the Liverpool lab will become the main NHS testing service for the Merseyside region. Since I started in December, the

capacity has increased such that the lab operates 7 days a week and is open twelve hours on weekdays. To match the continual increase in sample volumes, the number of new staff has also increased with approximately 100 Medical Laboratory Assistants working here as of the beginning of March 2021.

How has this role helped me, and what skills have I learned?

When I started at the lab in early December 2020, we were validating equipment and standard operating procedures, accepting samples from only a few departments within the Royal Liverpool University Hospital. Once the lab was “live” we began accepting samples from other hospitals in the Liverpool University Foundation Trust. Since then, we have gradually taken on other NHS trusts so that the volume of samples has increased to approximately 3000 samples per week at the beginning of March. As we take on more trusts across Merseyside, the volume of samples we will process is projected to increase further.

The coronavirus pandemic has meant that my final year undergraduate honours project was adapted to a systematic review. There is limited lab access and, therefore, little possibility for a lab-based project. As I felt that practical skills remain an essential part of being a life sciences student I wanted to take the opportunity to develop my skills despite the impact of the pandemic restrictions.

Working in the lab has allowed me to develop both laboratory-based and interpersonal skills. By learning the processes required for LAMP testing, I have extended the skills base I developed during the first years of my degree. I feel this will be advantageous for my progression onto postgraduate study and possible lab-based careers.

Before taking this role, I had little experience working in a clinical lab. Now, having seen the lab develop in the short time I have been there, I am considering working in the biomedical sciences field, which has influenced my decision for postgraduate study at master's level. I have also become more open to the idea of working in a clinical laboratory in my future career.

> “Overall, this work has helped me develop my skills as a scientist which I feel has been useful preparation for life post-graduation”

Being part of a large workforce has forced me to develop team working skills and good communication to ensure colleagues are comfortable in their tasks and that processes are running smoothly. When I have been given the responsibility of “room lead,” I have been involved in training newer staff and delegating job roles to ensure we are continually processing samples throughout the day. Overall, this work has helped me develop my skills as a scientist which I feel has been useful preparation for life post-graduation.

Scimos

Our Solution to Science Inaccessibility

Daisy Cookson[1] and Nicole Szekeres-Tapp[2]

[1]3rd year Veterinary Science, University of Liverpool; [2]3rd year Biochemistry, University of Oxford

As undergraduate students three years into our respective science degrees, I'm sure both of us like to think we know our apples from oranges, mitochondria from chloroplasts, and transcription from translation. This also means that at a time when health and disease have become dinner-time conversation and the forefront of the mainstream media, it has been hugely beneficial and empowering to understand the science around us. Unfortunately, this is not something that is always easy for people without a background in science.

"2020 has undoubtedly proven the importance of science being accessible and the issues that arise when it's not."

2020 has undoubtedly proven the importance of science being accessible and the issues that arise when it's not. At the peak of the pandemic, there were 'COVID-19' parties whereby people would gather in the hope of contracting the virus to develop immunity and people who died from injecting bleach; not to mention conspiracy theories ranging from 5G towers spreading the virus to the government filling vaccines with microchips in a bid to control the population. Science accessibility has always been important to us, mainly because we think science is amazing and everyone should have access to it; and that science can be even more interesting than what is taught in a classroom. But 2020 definitely put science accessibility into a new light.

Luckily for us, we're not the only ones who feel this way and who want to do something about it. So, back in September 2020, when we saw a new, student-run organisation had been set up that aimed to present science news in a brand new, more accessible way, we had to get involved!

Scimos (www.scimos.org) is an entirely student-led organisation (something we're very proud of!) created in May 2020 and launched in September. As of yet, we have twenty-five members, ranging from managers to writers, and we're continually growing, with numerous students expressing interest each month. The majority of us are University students from across the country, including Oxford, Durham, Newcastle and Liverpool. Currently, there are a total of 53 articles published on Scimos, spanning our Learn and News sections, covering articles on recent

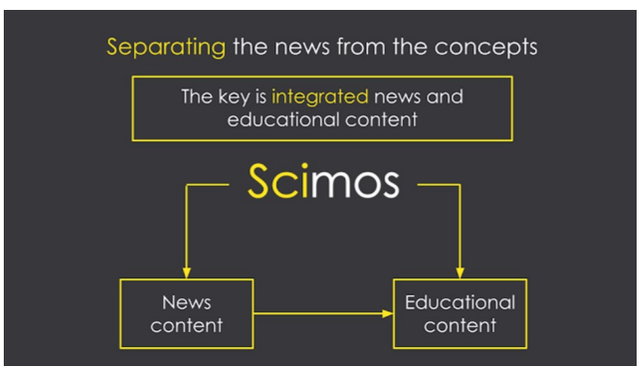


Figure 1. The underlying concept of Scimos relies on integration of news and educational content. This will allow readers to explore more about areas that either interest them or aren't generally

developments, such as the COVID-19 vaccine or the SpaceX Starship Prototype SN8 test launch, and educational content from immunology to algebra, to name a few. In January 2021, we had a total of 2300 views on our website, and we cumulatively reached 2226 people through our Instagram and Facebook.

Science can easily seem intimidating, with complex words and equations that often limit its target audience. When it comes to science articles and educational content, the majority can be very long or difficult to understand, depending on who the reader is. A lot of the time, the point the author is trying to make relies on a concept that a layman reader won't be familiar with. Meanwhile, if an explanation for a specific notion is given, for example, 'special relativity', then to the physicist, and even the scientist, this clarification is unnecessary.

"Scimos solves this problem by integrating news and educational content, a principle that is unique to our website."

Scimos solves this problem by integrating news and educational content, a principle that is unique to our website. This way, the article isn't overwhelming to those readers who aren't familiar with the topic, allowing the focus to be the news and its importance, and the article is concise enough for those with pre-existing knowledge to do the same. We aim to make science accessible to everyone, no matter their pre-existing knowledge, understanding or academic background.

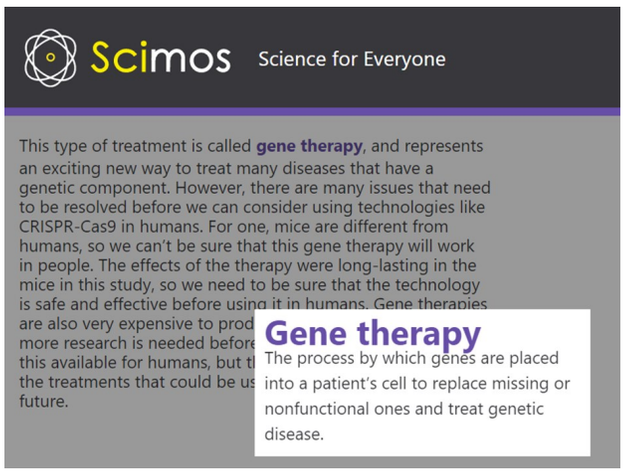


Figure 2. One of the features of Scimos is our keyword system. This allows readers to click on the words in bold for an explanation or definition. This keeps our articles concise and to the point avoiding unnecessary explanation for those who understand the term, while still providing it for those who do not.

Within Scimos, we have a News and a Learn section to optimise our content for readers of all abilities. Our News section features breakthroughs and developments in science, highlighting what these mean for society. Articles are grouped into either biology, health, tech, physics or chemistry, and by integrating science news and educational content these are written in a way that aims to be interesting, exciting and educational.

In addition to news content, the site aims to offer a comprehensive breakdown of each area of science. This is via the Learn section, which allows readers to enhance their scientific knowledge. Our Learn section is broken down into topics, subtopics and articles across biology, chemistry, physics and maths. To provide content for a varied audience, we delegate a 'Tier' to each learn article. The three tiers represent different academic levels (as opposed to different COVID alert levels!), which ensures every article is accessible and appropriate. This also allows users to choose, and therefore only view the articles that are suitable to them.

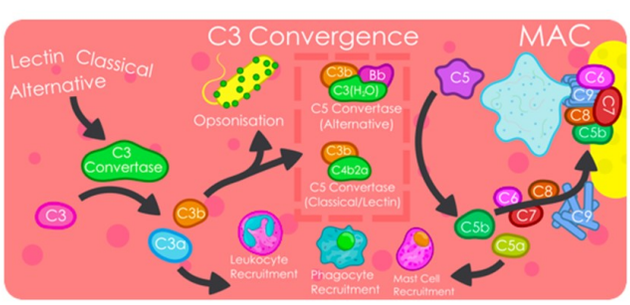


Figure 3. Many of our members also create images for the site, such as this one on the convergence of pathways in the complement system (as part of our Learn series on immunology). Using unique, eye-catching diagrams adds another dimension to our articles, supplementing our writing and helping to further explain concepts.

This means that those with an advanced level of pre-existing knowledge don't have to read introductory articles with long-winded explanations, and vice-versa. Our Tier system is still a work in progress, and should be completely implemented within the next few months.

Our website is always changing and developing, and we have some exciting updates coming soon, including changes to our News section, and an entirely new section, the Explore section.

We are continually expanding, refining and recruiting, and we are looking forward to seeing just how far we can grow in 2021! Scimos has been a fantastic opportunity for us to improve our writing skills, further our scientific understanding and management skills, and increase self-confidence, as well as serving as a great addition to our CVs.

It has also been extremely encouraging to see us already achieving some of the things we set out to do. For instance, one of the historical problems in science is diversity, with access to careers in science being heavily dominated by white males. As two women in science, something personally great to see is that almost two-thirds of followers on our social media accounts, and currently over 75% of our writers, are other women and girls!

We're continually growing and expanding our team. If Scimos sounds like a project you'd like to be involved with, please visit the "write for us" section on our website!

Research

Research from students at Liverpool

Read about a range of research being undertaken by both undergraduate and masters students.

^{1}H NMR lipidomic analysis of synovial fluid in equine osteochondrosis

Eleanor Rowland[1], James R. Anderson[2], Emily J. Clarke[2], Marie M. Phelan[3], Luis M. Rubio-Martinez[4], Mandy J. Peffers[2]

[1] Year 3 Veterinary Science, Institute of Veterinary Science, University of Liverpool, L69 3GB; [2] Institute of Life Course and Medical Science, University of Liverpool, Liverpool L7 8TX; [3] Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool L69 7BE; [4]Sussex Equine Hospital, Billingshurst Road, Ashington, West Sussex RH20 3BB.

Osteochondrosis (OC) is a significant developmental disorder seen in growing horses and affects the cartilage and bone within the joints, subsequently causing pain, lameness and reduced athletic ability. Synovial fluid is located within the joint cavity and acts to reduce friction and thereby enhance the mobility of the joint. Synovial fluid is predominately affected by the health status of the joint, with the lipid (or fat) content of synovial fluid reflecting this, implying that synovial fluid is a good source for biomarker discovery and therefore the biofluid of choice to investigate OC. Synovial fluid samples were analysed using nuclear magnetic resonance (NMR). NMR enables the analysis of the molecular structure of a sample by measuring the interaction of nuclear spins when inserted into a powerful magnetic field. It allows for efficient analysis of small volumes of synovial fluid, requires minimal sample preparation and is non-invasive and non-destructive, providing robust and reproducible results. Statistical analyses were utilised to identify any differences in the lipid content of synovial fluid obtained from the joints of horses with and without OC. No statistically significant differences were detected. Time limitations and limited access to specialised software prevented the identification of the individual lipids within the synovial fluid samples. Future studies will focus their attention on identifying these lipids. While this study was unable to identify any significant differences between the joints with and without OC, it was the first study to our knowledge to investigate the synovial fluid lipid profiles with NMR.

Abstract

Osteochondrosis is a significant developmental disorder of horses and is caused by the disruption or failure of the endochondral ossification process within epiphyseal articular cartilage or growth plates, resulting in pain, lameness and reduced athletic performance. Synovial fluid is located in close proximity to articular tissues and is predominantly affected by joint pathology. The lipid profile of synovial fluid is said to reflect the health status of the joints, therefore presenting a potential source of biomarker discovery. Nuclear magnetic resonance (NMR) allows for efficient analysis of the molecular structure of small volumes of synovial fluid with minimal sample preparation as well as being non-invasive and non-destructive, providing robust and reproducible results. ^{1}H NMR was utilised to analyse and compare the lipid profiles of synovial fluid from horses with osteochondrosis (n=five) and controls without osteochondrosis (n=four), and was shown to be a sufficient technique to investigate this biofluid. Univariant and multivariant statistical analyses found there were no significant differences between the lipid profiles of the two groups ($p>0.05$). Time limitations and limited access to specialised software prevented identification of the lipids within the synovial fluid samples. Any future studies would need to focus their attention on identifying these lipids. While this study was unable to identify any significant differences between the two groups, it is the first study to use NMR lipidomics to investigate equine synovial fluid's role in osteochondrosis.

Introduction

Osteochondrosis (OC) is a significant developmental orthopaedic disease of growing horses (Mendoza *et al.*, 2015) and is characterised by the focal disturbance of endochondral ossification within epiphyseal articular cartilage (De Grauw *et al.*, 2011, Rejnö & Strömberg, 1978) and growth plates (Ytrehus *et al.*, 2007). This disturbance can result in detached fragments (osteochondrosis dissecans, OCD), subchondral bone cysts or fissures forming on the epiphyseal growth cartilage (Rejnö & Strömberg, 1978, Ytrehus *et al.*, 2007). In addition, the blood supply to epiphyseal cartilage channels can be interrupted resulting in focal regions of chondronecrosis which forms clefts that extend through the articular cartilage into subchondral bone (McCoy *et al.*, 2013). One of the most common outcomes of OC is OCD. OCD is associated with the failure of cellular differentiation in growing cartilage, causing it to become thicker or retained, the development of fissures and subsequent cartilaginous flaps from the joint (Carlson *et al.*, 1995). These detached fragments can subsequently lead to lameness, pain and diminished athletic performance in young horses (McIlwraith, 1993, Verwilghen *et al.*, 2013). Biomechanics, exercise, nutrition, rapid growth as well as genetics (Philipsson, 1996) have been identified as the main risk factors associated with OC (Jeffcott, 1991), although how they influence endochondral ossification remains unclear (Donabédian *et al.*, 2008, McCoy *et al.*, 2013). It commonly affects the tarsocrural joint, with an incidence of up to 30-40% in some breeds (Philipsson, 1996) and has a prevalence of 23% in Thoroughbreds (Russell *et al.*, 2017).

Synovial fluid is located within articular joints, providing a pool of nutrients to neighbouring tissues as well as lubricating articular cartilage, resulting in frictionless movement of the joint (Blewis *et al.*, 2007). The synovial fluid lipid profile is associated with the joint's health status (Kosinska *et al.*, 2016), with alterations to the lipid profile resulting in reduced lubrication and altered inflammatory state of the joint (Kosinska *et al.*, 2013, Kosinska *et al.*, 2014). Elevated synovial fluid concentrations of prostaglandin E2 and leukotriene B4 were identified in horses with clinical signs of OC (Billinghurst *et al.*, 2004, De Grauw *et al.*, 2006, Donabédian *et al.*, 2008),

demonstrating that these substances have the potential for diagnostic purposes. Synovial fluid is an essential biofluid for investigating OC due to neighbouring articular tissues which are primarily affected during joint pathology, thus presenting a potential source of biomarker discovery (Anderson *et al.*, 2018).

Previously, high-resolution ^{1}H nuclear magnetic resonance (^{1}H NMR), has been utilised to analyse metabolites, lipids and small molecules in various body fluids including cerebrospinal fluid (Musteata *et al.*, 2013), plasma (Le Moyec *et al.*, 2014, Nicholson *et al.*, 1984) and synovial fluid (Anderson *et al.*, 2018, Lacitignola *et al.*, 2008). The main advantages of NMR analysis of biological fluids, compared with previously utilised techniques, are that it requires minimal sample preparation and with its non-destructive and non-invasive nature produces results which are robust and reproducible (Keun & Athersuch, 2011). To date, ^{1}H NMR has been used to analyse metabolite biomarkers within synovial fluid (Anderson *et al.*, 2018) however, this technique is yet to be used to investigate the lipid profile of synovial fluid from OC joints.

This study aimed to use ^{1}H NMR to identify lipid biomarkers in synovial fluid to enable differentiation between horses with and without OC, thereby advancing diagnostic and prognostic capabilities and enhancing equine health and welfare.

Methods

Sample cohorts and collection

Adhering to owner consent and ethical approval, synovial fluid was aspirated from the joints of horses presented to The Philip Leverhulme Equine Hospital, University of Liverpool, between 2014 and 2016. It was aspirated from the affected joints at the start of surgical arthroscopy under general anaesthetic. 500 µL of each sample was submitted for NMR lipidomic analysis. Affected joints comprised of femorotibial, glenohumeral, metacarpophalangeal, metatarsophalangeal and tarsocrural. Horses were organised into two groups: OC (n=5) and controls (n=4). The controls consisted of four horses which suffered from non-joint related disorders. OC diagnosis occurred via a combination of arthroscopy, radiography and ultrasonography. Synovial fluid was immediately deposited into uncoated 1.5 mL collection tubes and processed within an hour of collection. Centrifugation (4°C, 2450 g for 5 min) removed particulate matter from the synovial fluid and the cell-free supernatant was placed into a clean, uncoated 1.5 mL collection tube, snap-frozen with liquid nitrogen and stored at -80°C.

Sample preparation

Following thawing on ice, the synovial fluid samples underwent centrifugation (4°C, 14000 g for 10 min). The supernatant was removed and treated with 1 µg/ml hyaluronidase (bovine origin, Sigma-Aldrich) at 37°C for 1 hour and subsequently centrifuged at 2500 g for 10 min. 200 µL of the sample was combined with 250 µL deuterated chloroform and incubated on ice for 5 min. The mixture was vortexed for 30 s, centrifuged at 1200 g and 4° C for 1 min and incubated on ice for a further 1 min to allow the aqueous and lipid phase to separate. Using a glass pipette 200 µL of the lower (lipid) phase was transferred into 3 mm outer diameter clean NMR tubes.

NMR optimisation, acquisition and processing

One dimensional ^{1}H NMR spectra with Nuclear Overhauser Effect Spectroscopy (NOSEY) filter was utilised to attain signals from lipids on a 700 MHz NMR Bruker Avance III spectrometer. Spectra were obtained at 15°C, with a 4 s interscan delay, 256 transients and a 26 parts per million (ppm) spectral width. Topspin 3.6.2 and IconNMR 4.67 software with automated baseline and phasing correction as well as standard vendor processing routine were used to attain and process data.

Lipid annotation and identification

All spectra were screened to guarantee they met the recommended quality control criteria before statistical analysis. The quality control criteria included a line-width half height which is of representative residual ^{1}H chloroform peak aligned to 7.26 ppm within one standard deviation and a flat baseline. The spectra were separated into 'bins' or 'buckets' according to the lipid annotation from TameNMR (Grauslys, n.d.). Each spectrum was separated into 99 bins and the intensity of each bin divided by the bin width to counteract the intensity variance. Before statistical analysis, the bins were normalised to the median spectrum and Pareto scaled in MetaboAnalyst (MetaboAnaylst, n.d.). Residual ^{1}H chloroform ($C^{1}HCl_3$) was identified in the spectra as it is present in the deuterated chloroform ($C^{2}HCl_3$ (<1%)) and was used to dilute the synovial fluid sample. Therefore, for multivariant analysis, the bins assigned to chloroform were excluded.

Lipidomic statistical analysis

The synovial fluid spectra were divided into two groups: OC and controls. MetaboAnalyst 4.0 was used for normalisation, t-tests, principal component analysis (PCA) scores plots as well as box plots used the R package of statistical computing software (Chong *et al.*, 2019). For t-tests, $p \leq 0.05$ was considered statistically significant.

Results

NMR spectra quality control

Spectra were successfully acquired from the nine samples. Upon an initial overview of the NMR spectra, three distinct, large peaks were identified (Figure 1A). On closer inspection, these were determined to be due to contamination with deuterated chloroform at 7.26 ppm (peak 1), an artefact from water suppression at 5.0 ppm (peak 2) and water at 1.6 ppm (peak 3) (Figure 1B). As these were deemed contaminants they were subsequently omitted from further analysis. The spectra representing the 99 lipid metabolite peaks from all nine synovial fluid samples were subsequently analysed.

Data normalisation

Data were normalised to the median and Pareto scaled. Figure 2 shows the data before and after normalisation.

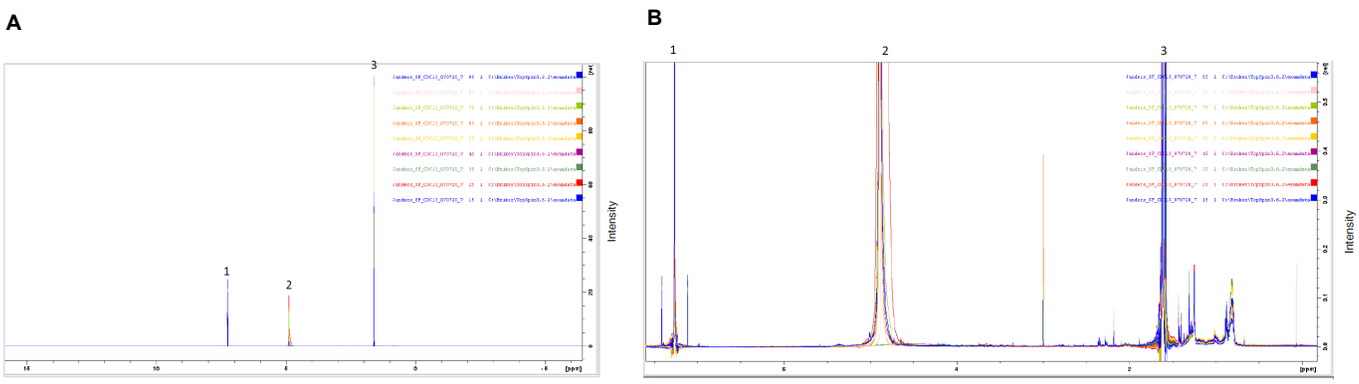


Figure 1. (A) Initial lipidomic NMR spectra of synovial fluid from four control and five OC donors with annotated contaminated peaks. Contaminant annotations include: chloroform (1) and water (2 and 3). These contaminants were omitted from analysis. All nine samples are represented in this NMR spectra with their intensity along the y-axis and parts per million (ppm) along the x-axis. **(B)** Expanded lipidomic NMR spectra of synovial fluid from all samples. This NMR spectra ranges from 1-7.5 ppm and shows 99 peaks. The contaminant chloroform at 7.26 ppm (1), artefact of water suppression at 5.0 ppm (2) and water peak at 1.6 ppm (3) can be seen in this spectra. All nine samples are represented in this NMR spectra with their intensity along the y-axis and ppm along the x-axis.

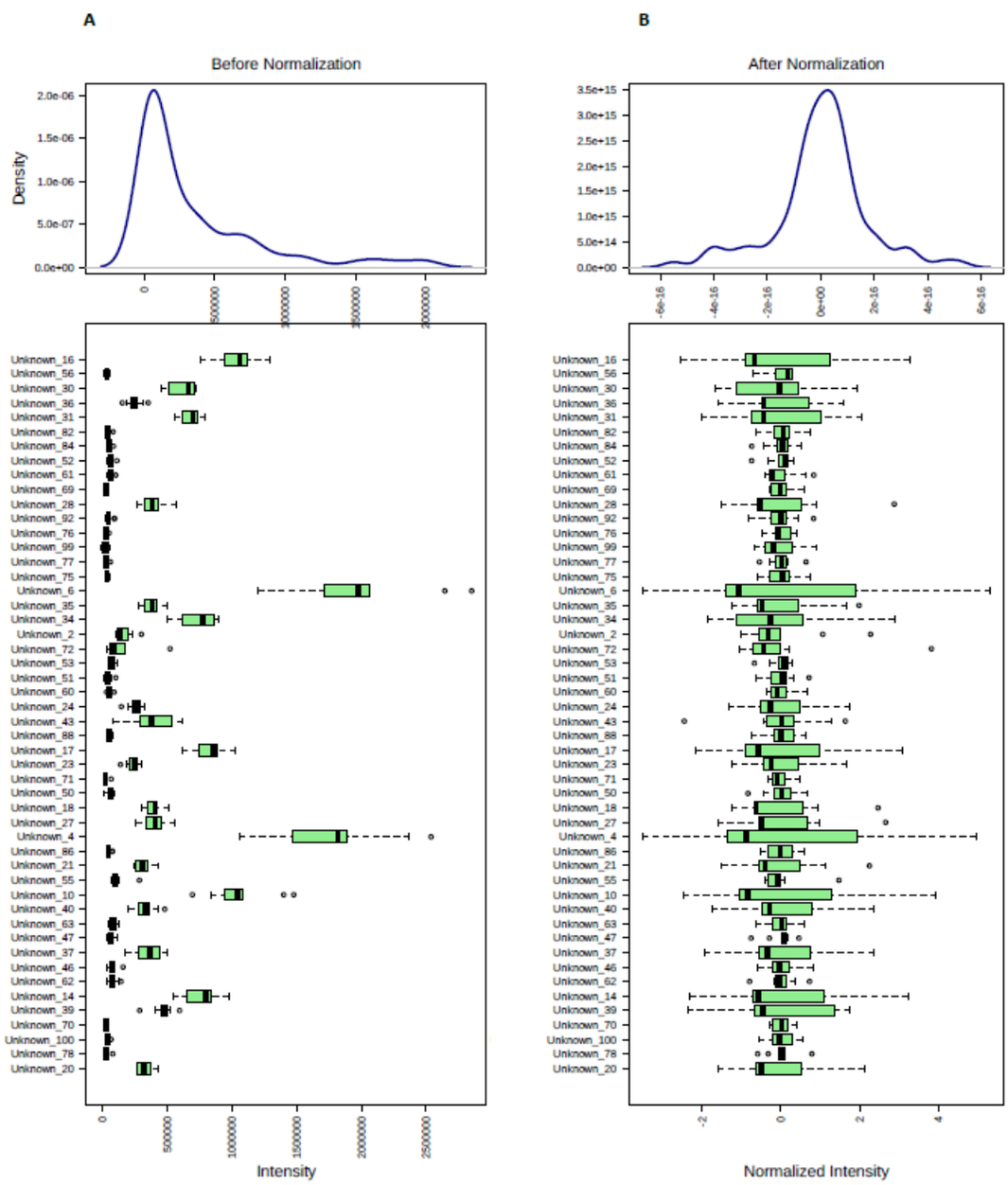


Figure 2. NMR data of the OC and control groups before and after normalisation. **(A)** Prior to normalisation. **(B)** After normalisation. The data were normalised to the median and Pareto scaled prior to statistical analysis to ensure that the data were directly comparable. The density is displayed in the graphs with the intensity of each lipid peak displayed directly below as green box pots.

Prior to normalisation, the density is skewed to the left and the lipid boxplots vary in intensity with Unknown_4 and Unknown_6 displaying the greatest intensity. Following normalisation, the density curve is near-symmetrical and the boxplot intensities are all central to 0.

Lipidomic statistical analysis

Unsupervised multivariate principal component analysis (PCA) scores were utilised to identify significant differences in the lipid profile between the synovial fluid aspirated from OC and control joints. No clear separation was observed between the different groups (Figure 3A). There was variation in the shape and size of the two clusters, with the control group being more clustered together. In the synchronised 3D plot, the coloured markers show that there is some separation between the two groups along the principal component (PC) 3 axis (Figure 3B). However, this axis accounts for 4.5% of the variance, thus does not display any statistical significance.

Data were further analysed using a loadings plot. Figure 4A represents the loading plot associated with the PCA scores described in Figure 3A and enabled comparison of each lipid peak identified in control and OC samples. The loadings plot showed that Unknown_43 (highlighted in the blue box) (p=0.71) had the most significant effect (Figure 4A). Expression of Unknown_43 was higher in the OC group although not statistically significant (Figure 4B).

Univariate t-test analysis of the spectra determined no significant differences (p>0.05) between the lipid profile of the 99 peaks from the synovial fluid obtained from the two groups.

Discussion

OC is a developmental disorder caused by a disturbance to endochondral ossification (De Grauw *et al.*, 2011, Rejnö and Strömberg, 1978) subsequently affecting the cartilage of joints. Due to its close association with articular tissue and being influenced by joint pathology, synovial fluid is an important source of biomarker discovery, thereby an essential biofluid to investigate OC (Anderson *et al.*, 2018).

The lipid profile of synovial fluid was investigated as it is related to the health status of the joint (Kosinska *et al.,* 2016), with modifications affecting the inflammatory state and lubrication of the joint (Kosinska *et al.*, 2013, Kosinska *et al.*, 2014). It is thought this is the first report of the use of NMR lipidomics to interrogate synovial fluid in OC.

No lipids were found to be statistically different between the OC and control groups. Synovial fluid collected from the control group was more clustered, suggesting that these samples shared similar lipid profiles. It also implies that there is more variation in the lipid biomarkers associated with equine OC. Similarly, Zhang *et al.,* (2020) raised the possibility of lipid biomarkers being used for disease diagnosis with Alzheimer's disease (AD) mice displaying significant changes in many lipid species and clear separation between AD and control mice.

Loadings plots are used to analyse which variables have the greatest effect on each component, in this case being OC and control horses. From the loadings plot, Unknown_43 was identified as a significant driver and appears to be associated with the OC group. Further investigations would be needed to identify this lipid and thus its role in OC.

It is imperative to identify contaminants in spectra prior to further analysis. On the NMR spectra, chloroform and water were identified as contaminants and thus omitted from further investigation. To confirm that chloroform was the peak at 7.26 ppm, the same process, as stated in the methods, should be utilised to analyse chloroform alone and determine whether this peak matches. Due to the separation of the aqueous and lipid portions of synovial fluid prior to NMR analysis, water is a probable contaminant. It would be difficult to obtain a pure sample without water peaks.

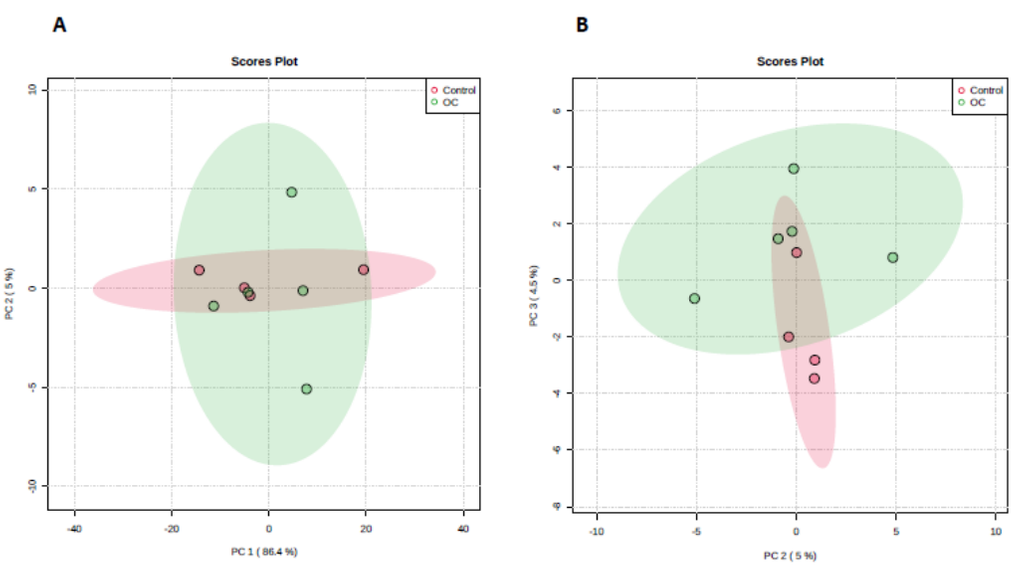


Figure 3. Principle component analysis (PCA) of lipid peaks identified within synovial fluid aspirated from OC affected horses (n=5) and controls (n=4) using ^{1}H NMR. **(A)** Equine synovial fluid lipidomic 2D PCA plot. Groups are represented as green (OC) and red (control) coloured areas. PC1 and PC2 account for 89.4% variance. Shading represents 95% of the confidence region. **(B)** Equine synovial fluid synchronised 3D plot shown in 2D orientation. Groups are represented as green (OC) ore red (control). PC2 and PC3 account for 9.5% variance.

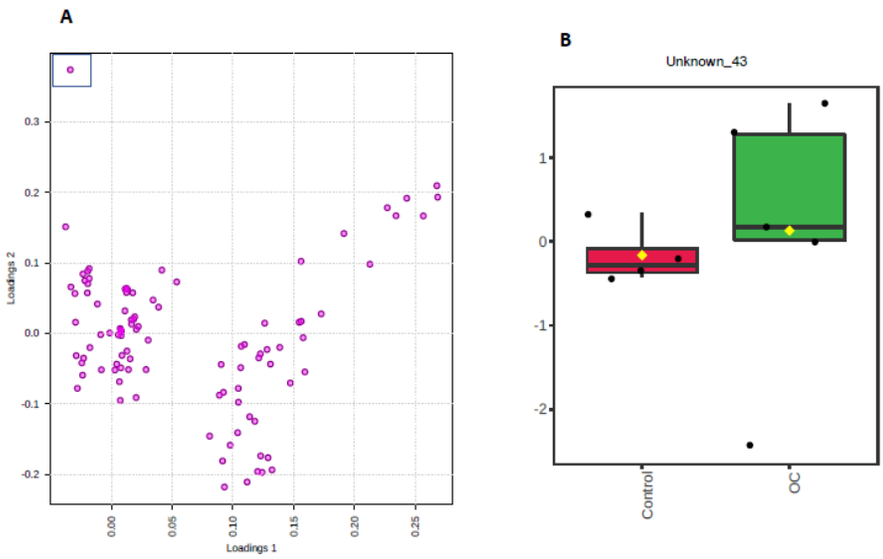


Figure 4. Loadings plots of lipid peaks identified within synovial fluid aspirated from OC affected horses (n=5) and controls (n=4) using ^{1}H NMR. **(A)** Equine synovial fluid loadings plot. The peak most different, Unknown_43, is highlighted. **(B)** Unknown_43 normalised box plot comparing the effect of Unknown_43 between control and OC groups.

Before further analysis, it is vital to normalise data so that all the samples are directly comparable (Craig *et al.*, 2006, Dieterled *et al.*, 2006). Normalising the data using the median and Pareto scaling proved to be sufficient, with the normalised data producing a near symmetrical curve. Additionally, methyl (CH_3) normalisation has also been recommended for lipidomic statistical analysis compared with other methods, such as probabilistic quotient normalisation, as they do not skew normalised data (Morgan, 2019).

This pilot study used a small sample size, nine samples, and this is a major limitation. The preliminary data could be used in a power calculation for future work to determine the number of subjects and/or samples required to detect a significant effect. A larger sample size would be beneficial to identify any statistical significance in future studies.

Due to time limitations and limited access to specialised software, lipid metabolites could not be identified. Therefore, a follow up to this study would be the identification of lipids within the synovial fluid aspirated from the two groups by comparing it with spectra of known lipids or mass spectrometry to determine the lipid landscape. Additional studies should compare and contrast the lipid profiles of synovial fluid aspirated from OC and osteoarthritic equine joints as they commonly cause pain and lameness in horses.

Conclusion

This is the first study to utilise ^{1}H NMR to study lipidomics of synovial fluid from horses with osteochondrosis. Although we were unable to discriminate between OC affected and non-affected joints, this study is a pilot for this area of research, with the potential for significant biomarker discovery within the near future.

Acknowledgements

The author would like to thank Professor Mandy Peffers and her team for the opportunity to work alongside them and their ongoing support throughout the studentship. I would also like to thank The Academy of Medical Sciences, University of Liverpool Vet School INSPIRE award for funding this project.

References

Anderson, J. R., Phelan M. M., Clegg P. D., Peffers, M. J. & Rubio-Martinez, L. M. 2018. Synovial fluid metabolites differentiate between septic and nonseptic joint pathologies. *Journal of Proteome Research*, 17, 2735-2743.

Billinghurst, R. C., Brama, P. A., Van Weeren, P. R., Knowlton, M. S. & McIlwraith, C. W. 2004. Evaluation of serum concentrations of biomarkers of skeletal metabolism and results of radiography as indicators of severity of osteochondrosis in foals. *Am J Vet Res*, 65, 143-50.

Blewis, M., Nugent-Derfus, G., Schmidt, T., Schumacher, B. & Sah, R. 2007. A model of synovial fluid lubricant composition in normal and injured joints. *Eur Cell Mater*, 13, 26-39.

Carlson, C. S., Cullins, L. D. & Meuten, D. J. 1995. Osteochondrosis of the Articular-Epiphyseal Cartilage Complex in Young Horses: Evidence for a Defect in Cartilage Canal Blood Supply. *Veterinary Pathology*, 32, 641-647.

Chong, J., Wishart, D. S. & Xia, J. 2019. Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. *Current Protocols in Bioinformatics,* 68, e86.

Craig, A., Cloarec, O., Holmes, E., Nicholson, J. K. & Lindon, J. C. 2006. Scaling and normalization effects in NMR spectroscopic metabonomic data sets. *Analytical Chemistry*, 78, 2262-2267.

De Grauw, J., Brama, P. A. J., Wiemer, P., *et al.* 2006. Cartilage-derived biomarkers and lipid mediators of inflammation in horses with osteochondritis dissecans of the distal intermediate ridge of the tibia. *American Journal of Veterinary Research,* 67.

De Grauw, J., Donabédian, M., Van De Lest, C., *et al.* 2011. Assessment of synovial fluid biomarkers in healthy foals and in foals with tarsocrural osteochondrosis. *The Veterinary Journal*, 190, 390-395.

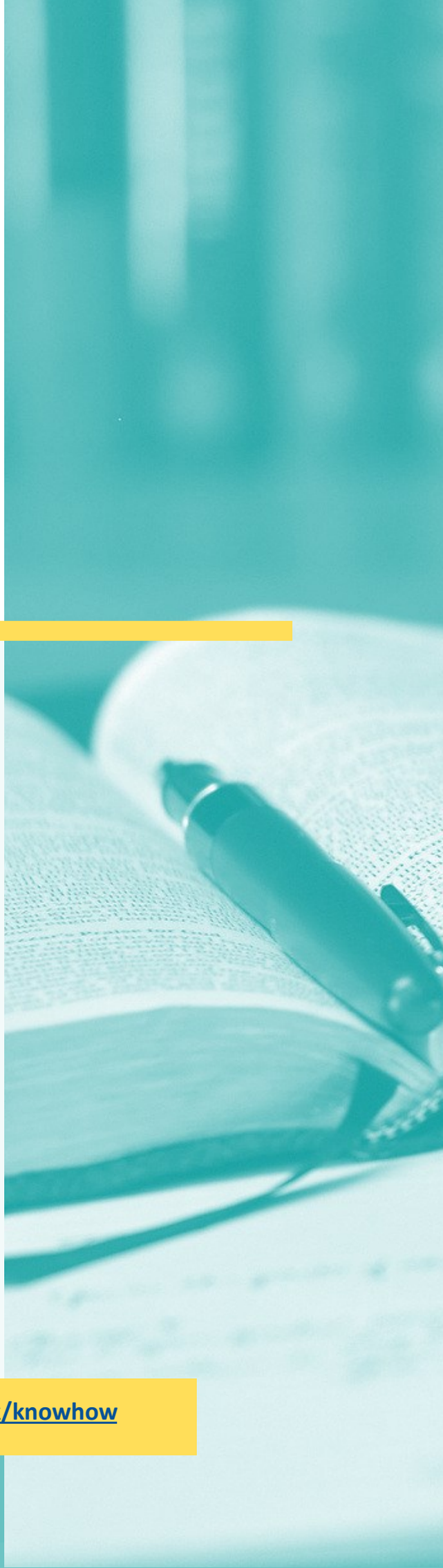
Dieterled, F., Ross, A., Schlotterbeck, G. & Senn, H. 2006. Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics. *Analytical Chemistry*, 78, 4281-4290.

Donabédian, M., Van Weeren, P. R., Perona, G., *et al.* 2008. Early changes in biomarkers of skeletal metabolism and their association to the occurrence of osteochondrosis (OC) in the horse. *Equine Veterinary Journal,* 40, 253-259.

Grauslys, A. n.d. PGB-LIV/tameNMR [Online]. GitHub. Available: https://github.com/PGB-LIV/tameNMR [Accessed 2020].
Jeffcott, L. B. 1991. Osteochondrosis in the horse--searching for the key to pathogenesis. *Equine Vet J*, 23, 331-8.
Keun, H. C. & Athersuch, T. J. 2011. *Nuclear magnetic resonance (NMR)-based metabolomics*. Metabolic Profiling. Springer.
Kosinska, M. K., Liebisch, G., Lochnit, G., *et al.* 2013. A Lipidomic Study of Phospholipid Classes and Species in Human Synovial Fluid. *Arthritis & Rheumatism,* 65, 2323-2333.
Kosinska, M. K., Liebisch, G., Lochnit, G., *et al.* 2014. Sphingolipids in human synovial fluid-a lipidomic study. *PloS one*, 9, e91769.
Kosinska, M. K., Mastbergen, S. C., Liebisch, G., *et al.* 2016. Comparative lipidomic analysis of synovial fluid in human and canine osteoarthritis. *Osteoarthritis Cartilage*, 24, 1470-8.
Lacitignola, L., Fanizzi, F. P., Francioso, E. & Crovace, A. 2008. 1H NMR investigation of normal and osteo-arthritic synovial fluid in the horse. *Vet Comp Orthop Traumatol*, 21, 85-8.
Le Moyec, L., Robert, C., Triba, M. N., *et al.* 2014. Protein catabolism and high lipid metabolism associated with long-distance exercise are revealed by plasma NMR metabolomics in endurance horses. *PloS one*, 9, e90730.
McCoy, A. M., Toth, F., Dolvik, N. I., *et al.* 2013. Articular osteochondrosis: a comparison of naturally-occurring human and animal disease. *Osteoarthritis and Cartilage*, 21, 1638-1647.
Mcilwraith, C. W. 1993. Inferences from referred clinical cases of osteochondritis dissecans. *Equine Veterinary Journal*, 25, 27-30.
Mendoza, L., Piquemal, D., Lejeune, J. P., *et al.* 2015. Age-dependent expression of osteochondrosis-related genes in equine leukocytes. *Veterinary Record Open*, 2, e000058-e000058.
Metaboanalyst. n.d. MetaboAnalyst [Online]. MetaboAnalyst Available: https://www.metaboanalyst.ca [Accessed 2020].
Morgan, R. S. 2019. Understanding the role of HACD enzymes and very long chain fatty acids in muscle development and disease. *Proc Physiol Soc,* 43, PC222.
Musteata, M., Nicolescul, A., Solcan, G. & Deleanu, C. 2013. The 1H NMR profile of healthy dog cerebrospinal fluid. PloS one, 8, e81192-e81192.
Nicholsonl, J., O'Flynn, M. P., Sadler, P., *et al.* 1984. Proton-nuclear-magnetic-resonance studies of serum, plasma and urine from fasting normal and diabetic subjects. *Biochemical Journal*, 217, 365-375.
Philipsson, J. 1996. Pathogenesis of osteochondrosis–genetic implications. WB Saunders Philadelphia, PA.
Rejnö, S. & Strömberg, B. 1978. Osteochondrosis in the horse. II. Pathology. *Acta Radiol Suppl*, 358, 153-78.
Russell, J., Matika, O., Russell, T. & Reardon, R. J. M. 2017. Heritability and prevalence of selected osteochondrosis lesions in yearling Thoroughbred horses. *Equine Veterinary Journal*, 49, 282-287.
Verwilghen, D. R., Janssens, S., Busoni, V., *et al.* 2013. Do developmental orthopaedic disorders influence future jumping performances in Warmblood stallions? *Equine Veterinary Journal*, 45, 578-581.
Ytrehus, B., Carlson, C. S. & Ekman, S. 2007. Etiology and Pathogenesis of Osteochondrosis. *Veterinary Pathology*, 44, 429-448.
Zhang, X., Liu, W., Zan, J., Wu, C. & Tan, W. 2020. Untargeted lipidomics reveals progression of early Alzheimer's disease in APP/PS1 transgenic mice. *Scientific Reports*, 10, 14509.

Tutorials and video resources

to help improve your academic skills



- Presentations
- Note taking
- Academic Integrity
- Referencing
- Academic Writing
- Statistics

Find out more here: https://libguides.liverpool.ac.uk/knowhow

Identifying genetic mutations which aid *Streptococcus pneumoniae* in intra-host transmission from the lung to the blood

Isobel Maclean[1], Daniel Neill[2] and Angharad Green[2]

[1] Advanced Biological Sciences MSc (Microbiology), School of Life Sciences, Liverpool, UK, L69 7ZB; [2] Department of Clinical Infection, Microbiology and Immunology, University of Liverpool, Liverpool, L69 7BE.

***Streptococcus pneumoniae* (pneumococcus) is a bacterial species normally found living in the throat of humans. The bacteria normally cause no harm, however they can spread through the body to the lungs and blood. Here, the bacteria can cause the development of diseases such as pneumonia and septicaemia (sepsis) respectively. The processes by which the pneumococcus spreads and causes these diseases are not understood. This study aims to identify genetic adaptations that are acquired by the bacteria during infection of the lungs and blood. Mice were infected with *S. pneumoniae*, which resulted in the development of pneumonia, and in some cases, sepsis. Bacterial samples were taken and analysed to reveal genetic mutations present within the population, and their frequency. The study found three different genes that are potentially beneficial to the pneumococcus during infection of the lungs and blood. The functions of these genes were researched and are related to bacterial metabolism. In conclusion, the proteins that these genes encode for may be potential targets for future treatments or vaccines, as they enable the bacteria to cause disease.**

Abstract

Streptococcus pneumoniae or pneumococcus is a Gram-positive bacterium which colonises the human nasopharynx, mostly asymptomatically. The pneumococcus possesses the ability to infect diverse host niches within the human body, which can result in the development of invasive diseases such as pneumonia, septicaemia, and meningitis. The incidence of fatality for these diseases is as high as 11-30% within the U.S and Europe. Experimental evolution was performed via the passage of 10 independent lineages, using a mouse pneumonia model, to identify acquisition of genetic mutations in lung and blood populations of pneumococcus. Samples of the pneumococcal population in the lung and the blood were taken at passage numbers 1, 5, 10, 15 and 20. Bioinformatic analyses show the genetic variants present within the blood and lung isolates, and these variants were compared. The results show evidence of niche specific adaptation to the blood environment. Genetic variants in *ilvD*, *pyk* and *lctO*, were present within multiple independent lineages within the blood isolates showing evidence of parallel evolution. The gene products of *pyk* and *lctO* play roles in pyruvate metabolism. *IlvD* is involved in amino acid biosynthesis and iron acquisition. Iron acquisition is a key challenge for bacterial pathogens when colonising the blood, as most iron is bound to haemoglobin. Genes *ilvD* and *pyk* were shown to have significantly higher gene expression within *S. pneumoniae* D39 when bacteria were grown in blood mimicking conditions as compared to lung mimicking conditions. In conclusion these genetic variants may be potential targets for new treatments or vaccines.

Introduction

Streptococcus pneumoniae (pneumococcus) is a Gram-positive bacterium which acts as an opportunistic pathogen within the human host. Pneumococcus causes diseases such as otitis media, and invasive diseases; pneumonia, septicaemia, and meningitis (Aprianto *et al*., 2018).

A common complication of community acquired pneumococcal pneumonia is the development of bacteraemia or septicaemia (sepsis) (Ceccato & Torres, 2018). This can often progress to septic shock, which has a high mortality rate even in treated cases. In studies in Europe and the US, case fatality rates for invasive pneumococcal disease and sepsis are reported to be as high as 11-30% (Askim *et al*., 2016; Chavanet, 2012). The mechanisms that allow pneumococcus to colonise diverse host niches are not well understood, for example the mechanisms by which pneumococcus is able to penetrate the epithelial barrier of the alveoli and enter the blood is still unclear.

The hypothesis of this study is that pneumococcal colonisation of the blood from the lung is associated with a bottleneck of just one cell, this has already been demonstrated in a previous study (Kono *et al*., 2016). Due to reduced diversity within the pneumococcal population, as pneumococcus replicates within the blood, the new environmental pressures may result in the selection of a novel, advantageous genotype. Only the genotype with the most advantageous mutations will survive. As well as the accumulation of new genetic variants, changes in gene expression patterns may also aid in colonisation of the blood. The aim of this study is to identify and determine the function of these genes in which variants are selected during the lung to blood transition. This will provide an insight into the pathogenicity of pneumococcal sepsis, potentially contributing to the development of new treatments.

Methodology

In vivo experimental evolution

Ancestor strain *S. pneumoniae* D39 (serotype 2) was grown overnight from a single colony. The following day, the bacteria were passaged to new growth medium, and permitted to grow to mid-exponential stage. Ten mice were inoculated, establishing 10 distinct pneumococcal lineages. Infection led to the development of pneumonia. After each passage, bacteria were recovered from the lungs. A small number of bacteria were swabbed onto gentamicin BAB agar, and colonies were confirmed as *S. pneumoniae* by an optochin disk. The pneumococcal population was then resuspended in solution and stored in three tubes at -80°C, to be used for analysis of the genome. One was used in re-infection of a new mouse (passage number 2). The passage process was repeated 20 times for each of the 10 lineages as shown in Figure 1. Samples of pneumococcal population from the blood in these infected mice were stored, alongside the samples kept from the lungs.

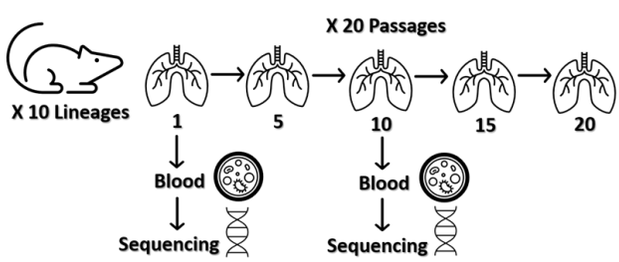


Figure 1. Experimental evolution mouse pneumonia model. Demonstration of how *S. pneumoniae* was passaged a total of 20 times via a mouse pneumonia model, for 10 separate independent lineages. Samples were taken from the lung after passage numbers 1, 5, 10, 15 and 20. If mice had developed a blood infection, a sample of the pneumococcal population from the blood was also stored. All samples were sequenced.

Data Analysis

Genetic variants that arose within the lungs were compared to those that arose within the blood. To begin the comparison, a spreadsheet was created using Microsoft Excel which included genetic variants exclusively present in lung evolved lineages/ blood evolved lineages, and the frequency of the genetic variant within the pneumococcal population.

Genes of interest were compiled into a spreadsheet and were narrowed down to a final list for lung-specific variants, and a separate list for blood-specific variants. The variants on the final list were researched using literature searches, Uniprot (The UniProt, 2018), KEGG (Kanehisa, 2019), PneumoBrowse (Slager *et al*., 2018) and PneumoExpress (Aprianto *et al*., 2018).

Aprianto *et al*., (2018) used *S. pneumoniae* D39 to quantify relative expression levels of the transcriptome in 22 different infection relevant conditions, including lung-mimicking conditions (LMC) and blood-mimicking conditions (BMC). PneumoExpress data was used to perform a one-way ANOVA Sidak's multiple comparison test, selecting LMC and BMC for gene variants *ilvD*, *lctO* and *pyk*. Statistical analyses were performed using GraphPad Prism version is 8.4.3.

Results

Table 1 was constructed from raw data. In particular, variants in *pyk*, *lctO* and *ilvD* are of interest. This is because they are all present in 3 different lineages, suggesting evidence of parallel evolution. The function of these genes, as shown in the table, include pyruvate metabolism and iron acquisition.

Figure 2A shows expression levels (TPM) of the gene "*ilvD*" in *S. pneumoniae* strain D39 in a range of infection relevant conditions, including lung mimicking conditions (LMC), blood mimicking conditions (BMC), nose mimicking conditions (NMC) and throughout the time course of an infection, measured in minutes post infection (mpi). The graphs show that expression of *ilvD* was highest for BMC, and at 30 mpi.

Figure 2B and 2C show the same graph as described above for genes "*pyk*" and "*lctO*" respectively. In Figure 2B expression levels of *pyk* were significantly higher within BMC than LMC. Overall, the highest levels of expression were seen for the infection conditions "Infection, 60 mpi". Figure 2C shows expression levels of *lctO* were relatively low and were comparable in BMC and LMC.

Lineage	Lung or Blood	Variant	Frequency in blood	Frequency in lung	Mutation	Protein	Function
2	Both	rny → I14I (ATC→ATT)	100%	100%	G ⟶ A	Ribonuclease Y	Endoribonuclease activity that initiates mRNA decay
4	Both	P0_01974 → A608S (GCA→TCA)	74.3%	30.3%	C ⟶ A	Lanthionine biosynthesis protein (LanM)	Catalytic activity
4	Both	sdhB → G14R (GGA→AGA)	72.2%	15.9%	C ⟶ T	L-serine dehydratase	Gluconeogenesis and 4 iron, 4 sulfur cluster binding
7	Both	modC → L160L (TTG→TTA)	61.5%	23.7%	C ⟶ T	Molybdenum import ATP-binding protein	Part of the ABC transporter complex ModABC involved in molybdenum import
7	Both	P0_00735 → P90A (CCA→GCA)	61.4%	18.8%	C ⟶ G	Hypothetical protein	N/A
7	Both	ettA_1 → K62N (AAG→AAT)	59.5%	16.9%	C ⟶ A	ABC transporter ATP -binding protein	ATP binding and ATPase activity
Multiple	Blood	pyk → F57L (TTC→TTA)	21.5%	N/A	C ⟶ A	Pyruvate kinase	Involved in synthesis of pyruvate (glycolysis pathway)
Multiple	Both	lctO → V326G (GTC→GGC)	10.5%	49.3% (lineage 2)	T ⟶ G	L lactate oxidase	L-lactate dehydrogenase activity - catalyses production of pyruvate
Multiple	Blood	ilvD → L278I (CTT→ATT)	9.6%	N/A	G ⟶ T	Dihydroxy-acid dehydratase	Amino acid synthesis and metal ion binding Iron-sulfur (4Fe-4S)

Table 1. Table constructed from raw data. In particular, variants in *pyk*, *lctO* and *ilvD* are of interest. This is because they are all present in 3 different lineages, suggesting evidence of parallel evolution. The function of these genes, as shown in the table, include pyruvate metabolism and iron acquisition.

P values were obtained demonstrating that the expression of *ilvD* ($p < 0.0001$) and *pyk* ($p = 0.0079$) were both significantly higher within BMC than LMC. The gene *lctO* was slightly more expressed within BMC than LMC, however the difference was not significant, $P>0.05$.

Discussion

The results of this study show evidence of niche specific adaptations within *S. pneumoniae* to the blood environment. It was found that two genes were both involved in pyruvate metabolism, *pyk* and *lctO*. Gene *ilvD* is also linked to pyruvate as it is involved in the pathway by which pyruvate is converted into valine. The results show that all three of these gene variants are present within multiple lineages within blood isolates and are all linked to or involved in pyruvate metabolism. There are many enzymes involved in pyruvate metabolism and it has been shown that the loss of these enzymes within the pyruvate node can affect pneumococcal virulence (Echlin *et al*., 2020).

The gene *pyk* encodes for the enzyme pyruvate kinase, the function of this enzyme is in the metabolic pathway glycolysis. In glycolysis, pyruvate is synthesised from D-glyceraldehyde 3-phosphate. Pyruvate kinases are present in many bacterial species, for example Gram positive bacterium *Staphylococcus aureus*, due to the unstable nature of bacterial enzymes, pyruvate kinase has not been extensively studied. A study by Zoraghi *et al*., (2010) has suggested that pyruvate kinase may be a novel target for antimicrobial treatments. The results of the study showed that within *S. aureus*, pyruvate kinase was essential to survival of the bacterium. It was also found that pyruvate kinase activity was higher during growth phase of the bacterium. These data suggest pyruvate kinase may be a potential target for antimicrobial therapies. In future studies, similar experiments should be performed using *S. pneumoniae* pyruvate kinase, to assess if the enzyme is essential to pneumococcal survival within the blood.

Gene *lctO* encodes for enzyme L-lactate oxidase which oxidises L-lactate to produce pyruvate, a key role in the generation of energy (ATP) in an aerobic environment (The UniProt, 2018). *LctO* is closely linked to *SpxB*, as they are both part of the central metabolism of pneumococcus in aerobic conditions. *SpxB* encodes for the enzyme pyruvate oxidase, this enzyme decarboxylates pyruvate, releasing ATP. In addition to this function *SpxB* the main producer of hydrogen peroxide, which inhibits the growth of neighbouring bacterial species (Redanz *et al*., 2018). *SpxB* was not identified as a gene variant within the blood isolates in my dataset. This may indicate the production of hydrogen peroxide is not beneficial to the colonisation of the blood, and that if *lctO* is under selection, it is likely to be due to another function of *lctO*, such as the generation of pyruvate.

A genetic variant in *ilvD* occurred within multiple lineages, in blood isolates only. The expression of this gene was significantly higher within BMC than LMC (Aprianto *et al*., 2018). The gene encodes for the protein dihydroxy acid dehydratase (DHAD). This enzyme catalyses the fourth

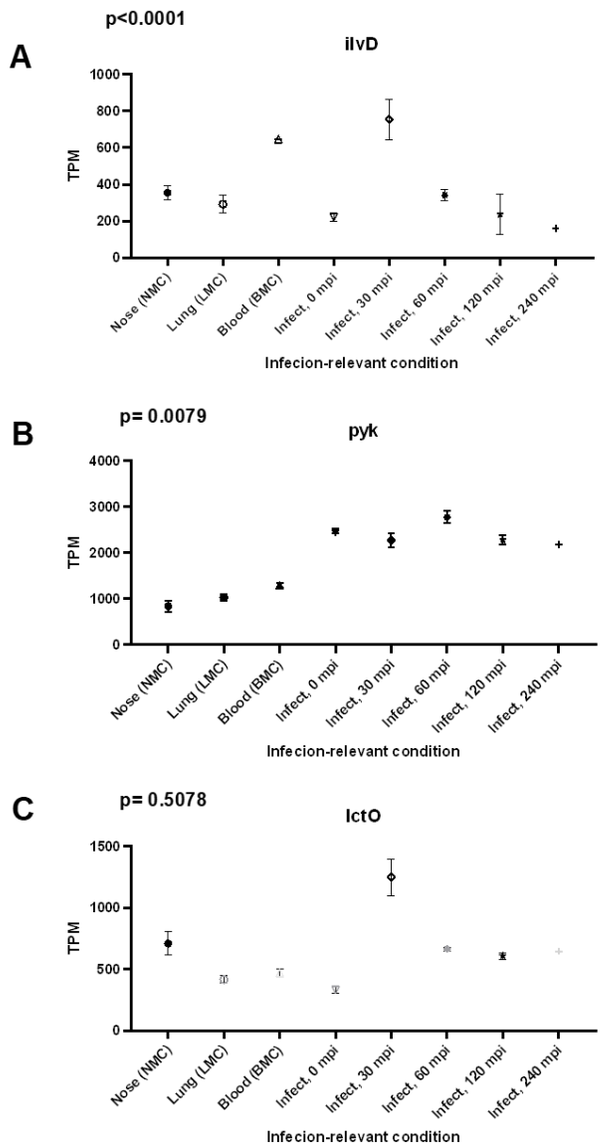


Figure 2. Expression of gene variants in *Streptococcus pneumoniae* D39 in different infection-relevant conditions. Using data provided by PneumoExpress each graph represents a different gene (titled). Graphs show the transcripts per million reads (TPM) of the genes in *S. pneumoniae* D39 for each infection relevant condition. P values gained from output of one-way ANOVA multiple comparison test in which blood (blood-mimicking conditions) were compared to lung (lung-mimicking conditions).

step in the biosynthesis of isoleucine and valine. The DHAD synthesises L-valine from pyruvate, indicating that *ilvD* may be linked to pyruvate metabolism, which is a key role of the gene products mentioned above, for genes *pyk* and *lctO*.

The molecular function of *ilvD* also includes 4 iron, 4 sulfur cluster binding and metal ion binding (UniProt, 2018). Enzymes encoded for by *ilvD* (DHAD) contain an [Fe-S] cluster as a co-factor in the active site (Rahman, *et al*., 2018). This indicates that *ilvD* is involved in the acquisition of iron. Within the blood, iron is bound to haemoglobin with a high affinity. This can create a challenge for bacteria when colonising the blood, as bacterial metabolism requires the use of free iron. *IlvD* may provide an advantage to *S. pneumoniae* in colonisation of the blood by enabling the acquisition of free iron. To confirm this theory, a mutant strain of *S. pneumoniae* without gene *ilvD* may be created using knockout methods.

Conclusion

This study has found evidence of niche specific adaptations to the blood environment, with pyruvate metabolism and iron acquisition being the function of these genes. The research focused on genes *ilvD*, *lctO* and *pyk*. These three genes occurred in multiple lineages, and at multiple passage timepoints. This shows evidence of parallel evolution, and that these genes were under selection within the blood environment. Further research is required, for example, the creation of knockout mutants of *S. pneumoniae* without *ilvD*, *lctO* and *pyk* would confirm if their function is essential in colonisation of the blood. These genes and their gene products may be potential targets for treatments in pneumococcal sepsis.

References

Aprianto, R., Slager, J., Holsappel, S & Veening, J.-W. 2018. High-resolution analysis of the pneumococcal transcriptome under a wide range of infection-relevant conditions. *Nucleic Acids Research*, 46 (19), 9990-10006.

Askim, Å., Mehl, A., Paulsen, J., *et al*. 2016. Epidemiology and outcome of sepsis in adult patients with Streptococcus pneumoniae infection in a Norwegian county 1993-2011: an observational study. *BMC Infectious Diseases*, 16 223-223.

Ceccato, A. & Torres, A. (2018) Sepsis and community-acquired pneumonia. *Annals of Research Hospitals*, 2(7).

Chavanet, P. (2012). Pneumococcus infections: Is the burden still as heavy? *Médecine et Maladies Infectieuses*, 42(4), 149-153.

Echlin, H., Frank, M., Rock, C. & Rosch, J. W. 2020. Role of the pyruvate metabolic network on carbohydrate metabolism and virulence in Streptococcus pneumoniae. *Molecular Microbiology*, 114(4), 356-552.

Kanehisa, M. 2019. Toward understanding the origin and evolution of cellular organisms. *Protein Sci,* 28(11),1947-1951.

Kono, M., Zafar, M. A., Zuniga, *et al*. 2016. Single Cell Bottlenecks in the Pathogenesis of *Streptococcus pneumoniae*. *PLOS Pathogens*, 12 (10), e1005887.

Rahman, M. M., Andberg, M., Koivula, A., Rouvinen, J. & Hakulinen, N. 2018. The crystal structure of D-xylonate dehydratase reveals functional features of enzymes from the Ilv/ED dehydratase family. *Scientific Reports*, 8(1), 865.

Redanz, S., Cheng, X., Giacaman, R. A., *et al*. 2018. Live and let die: Hydrogen peroxide production by the commensal flora and its role in maintaining a symbiotic microbiome. *Molecular Oral Microbiology*, 33 (5), 337-352.

Slager, J., Aprianto, R & Veening, J. W. 2018. Deep genome annotation of the opportunistic human pathogen Streptococcus pneumoniae D39. Nucleic Acids Research. 46(19), 9971-9989.

The UniProt. 2018. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Research*, 47(D1), D506-D515.

Zoraghi, R., See, R. H., Gong, H., *et al.* 2010. Functional Analysis, Overexpression, and Kinetic Characterization of Pyruvate Kinase from Methicillin-Resistant *Staphylococcus aureus*. *Biochemistry*, 49(35), 7733-7747.

Insights

What are your peers thinking about?

In-depth reviews of some really interesting topics, learn about something new!

Chelation therapy: an alternative medicine or breakthrough cardiovascular treatment?

Joseph Carr

3rd year Biochemistry BSc

Human blood vessels contain a diverse group of enzymes needed to speed up the rate of chemical reactions in our cells known as matrix metalloproteinases (MMPs). These require certain metals to operate, for example zinc and calcium. What is interesting about MMPs is that when they become uncontrolled, they contribute to a range of diseases including atherosclerosis and myocardial infarction (heart attack). This is because MMPs can cause the blood vessels to remodel themselves, fatty plaques in the vessel can become detached and perhaps spread to the brain or heart. To avoid this cells have a mechanism in place to stop MMPs going awry, namely, tissue inhibitors of metalloproteinases (TIMPs). TIMPs are small proteins that turn off MMPs. In the diseases mentioned, there is an imbalance in MMPs being active and TIMPs turning them off. This commentary looks at novel strategies that scientists have developed to prevent this imbalance such as chelation therapy, MMP inhibitors and compounds that cause our cells to make more TIMPs. Finally, this commentary will highlight the direction of future research into therapies and the likelihood of their use.

Metalloproteinases and MMPs: what are they and how are they regulated?

The use of enzymes in biological systems that utilise the characteristics of certain metals, commonly zinc, cobalt and manganese, to exert a catalytic effect are well understood (Schitler, 2019). Perhaps the most interesting class of these enzymes are the metalloproteinases which are used to break the peptide bond in protein chains. For example, pre-sequence protease (PreP) is used in degrading some mitochondrial proteins and the β-amyloid protein (Johnson *et al.*, 2006). Hence, it is unsurprising that a significant reduction in expression of PreP is seen in Alzheimer's disease, where β-amyloid deposits form pathologically significant plaques in the brain. PreP, also referred to as metalloproteinase-1, requires a Zn^{2+} ion to function and is, therefore, considered a metalloproteinase.

On a chemical level, the metal component of a metalloproteinase coordinates with three amino acid residues and a fourth coordination occurs with a water molecule. The water molecule binds fleetingly and is replaced by a target substrate amide bond (Hernick & Fierke, 2010). The importance of the metal is to polarise the carbonyl group of the target peptide which then allows the hydrolysis reaction to proceed.

The most significant subcategory of metalloproteinases is the matrix metalloproteinases (MMP) although adamalysins, serralysins and astacins also exist. MMPs contain zinc, but also depend on the presence of calcium (Mutluay-Tezvergil *et al.*, 2010). Their main role is to degrade extracellular membrane proteins, necessary for such processes as apoptosis, proliferation, migration and defence. Broadly speaking, there are six categories of MMP: collagenases, matrilysins, stromelysins membrane-type metalloproteinases, gelatinases and, finally, zinc- and calcium-dependent endopeptidases.

There are three mechanisms of action elucidated for MMPs: a base-catalysis reaction (Browner *et al.*, 1995), the Matthew acid-base reaction (Kester & Matthews, 1977) (proposing further use for water and the Zn^{2+} ion) and the Manzetti mechanism (Manzetti *et al.*, 2003). The latter showed Matthew's acid-base reaction was unlikely, and that histidine plays a more important role than zinc and water. Nevertheless, these hydrolysing mechanisms target the extracellular matrix, in particular, collagen and elastin fibres such as those found in the tunica media, adventitia and intima layers of the arterial wall (Xu & Shi, 2014). They are usually secreted from cells in an inactive zymogenic form.

A key regulator of MMPs are the TIMPs family of inhibitors, of which there are four (TIMP-1, TIMP-2, TIMP-3 and TIMP-4). TIMPs are biologically conserved endogenous proteins present in both primitive and higher eukaryotes (Murphy, 2011). Because of their essential role indicated by their prevalence in nature, it is unsurprising that any imbalance between MMP and TIMP activity can develop into a significant clinical pathology, e.g. cardiovascular disease.

Cardiovascular disease and MMPs

Recently, the pathological significance of certain MMPs has been observed in cardiovascular disease, as well as cancer metastasis, osteoarthritis and cirrhosis, as shown in Table 1. In this, MMPs seem to encourage and accelerate atherosclerotic lesion formation in the intima layer of vascular walls (see Figure 1). MMPs degrade various extracellular proteins to allow vascular smooth muscle cell (VSMC) migration to the aforementioned intima layer (Ravn & Falk, 1999).

MMPs become pathologically significant during vascular plaque remodelling. In this, a pre-existing plaque undergoes a change. This involves the growth and breakdown of collagen fibres in the arterial wall's intima layer, the latter is performed by MMPs. Thus, active atherosclerotic plaques are associated with far greater

MMP Biomarker	Pathology	Reference
Over-expression of TIMP-1, excess inhibition of MMP	Hepatic Cirrhosis	(Roeb, 2018)
MMP-2, 3 and 9 in synovial joint	Rheumatoid arthritis and Osteoarthritis	(Burrage *et al.*, 2006)
MMP-2, 9, 14 break-down of periostin	Myocardial Fibrosis and Heart Failure	(DeLeon-Pennell *et al.*, 2018)
Over-expression of MMP-2	Breast cancer metastasis	(Figueira *et al.*, 2009)
Over-expression of MMP-3, 7 and 9	Atherosclerosis and plaque instability	(Olejarz *et al.*, 2020)

Table 1. Various pathologies associated with digression from normal MMP/TIMP expression and regulation.

levels of MMPs, especially MMP-3 and MMP-9. Pro-remodelling factors are secreted by local macrophages which activate MMPs causing matrix degradation. Disequilibrium between active MMP and inactive TIMP increases the risk of plaque rupture and eventual cardiovascular events. (Liu *et al.*, 2006)

During active plaque remodelling the fibrous cap (composed of VSMCs) becomes weaker, leading to a potentially dangerous instability and eventual rupture. This can have downstream consequences, such as myocardial infarction (MI), stroke and other ischaemic conditions associated with microvascular plaque deposition. Furthermore, a genetic polymorphism within the promoter region of the genes coding MMP enzymes seems to dictate the susceptibility for cardiovascular disease, in particular coronary artery disease (Shalia *et al.*, 2010). As a result of this, the polymorphic promoter can act as a genetic biomarker for pathological significant cardiovascular disease.

MMPs and TIMPs as therapeutic targets for cardiovascular disease

Because MMPs depend on metal to function, removing this metal with a chelating agent could provide therapeutic potential. One such chelating agent is ethylene-diaminetetraacetic acid (EDTA), commonly used in the treatment of periodontal disease (Liu *et al.*, 2016).

To best appreciate the nature of EDTA as a chelation therapy, one must understand the complex chemistry of chelates. That being, a chelate is a molecule with the ability to remove metal from a source, in this case, the MMP enzyme. This is due to complex thermodynamics making the higher dentate ligand (EDTA) more capable of forming a metal complex than the original source of the metal (MMP) (see Figure 2). This is the so-called chelate effect (Vallet *et al.*, 2003).

The first reported use of EDTA to treat cardiovascular disease was reported by Clarke *et al.* in 1956, against angina pectoris and symptomatic coronary pathologies. This study demonstrated that angina was diminished in the majority of cases.

What does the future hold for MMP targeting?

EDTA and chelation therapy has not been approved for the treatment of MI and other cardiovascular diseases, although a large-scale study is currently being undertaken. TACT2 is a randomized, double blind controlled trial investigating EDTA and high-dose oral vitamins and minerals as a way to prevent recurrent cardiac events in diabetic patients with a prior MI and follows a decade long study which identified a significant effect of EDTA infusions on diabetic patients post-MI (Escolar *et al.*, 2014).

The current study, TACT2, aims to reach fruition by 2023 (Clinicaltrials.gov, NCT02733185). In particular, TACT2 aims to determine if chelation-based therapy increases the time to the first occurrence of adverse cardiovascular outcomes including hospitalization for unstable angina.

Besides EDTA it is also logical to assume that because TIMPs are endogenous inhibitors of MMPs the upregulation of TIMPs could serve as a therapeutic target. In fact, deficiency in certain TIMP subtypes, e.g. TIMP3, has been shown to precipitate pathological remodelling of the heart, cardiac fibrosis, abdominal aortic aneurysm and atherosclerosis, amongst other cardiovascular diseases (Fan & Kassiri, 2020).

Studies of TIMP infusion therapy in mice and rats has shown promising results. Studies have shown that reducing MMP activity in post-MI mice by administration of injections

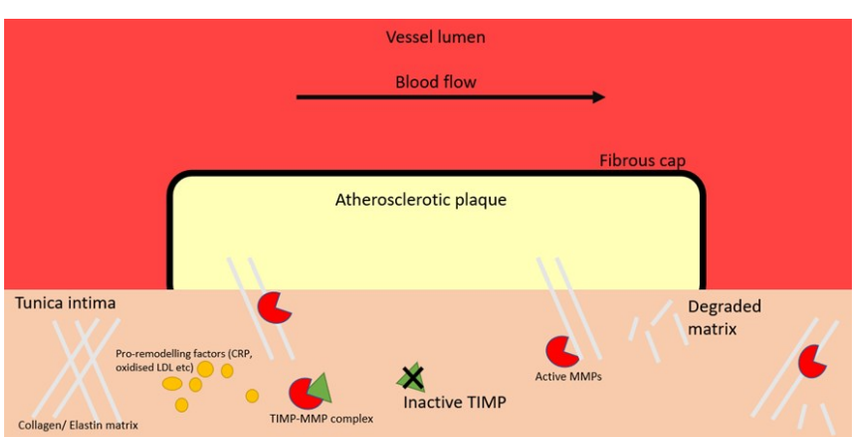


Figure 1. Vascular remodelling of the collagen/elastin matrix in the tunica intima .

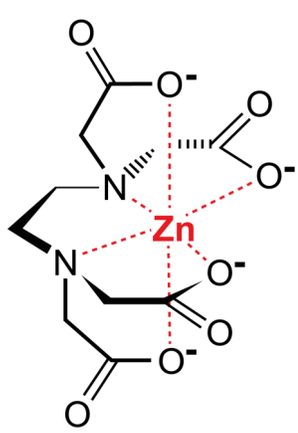


Figure 2. Molecular structure of the EDTA-zinc complex formed as a product of an MMP chelation reaction.

containing adenovirus' overexpressing TIMP3 into the peri-infarct area resulted in improved cardiac function after a single week (Takawale *et al.*, 2017). It is because of this that further research is being undertaken into the pharmacological implications for TIMPs as treatments for cardiovascular disease.

Conclusion

This commentary does not have the scope available to discuss the complete breadth of the effects of MMPs on the cardiovascular system. Greater information is available on a range of conditions due to excess MMP activity and metal deposition in the body, e.g. Ca^{2+} in the brain forming acervuli and MMP activity in tumour migration and osteoporosis (Vigh *et al.*, 1998).

Furthermore, when the TACT2 results are published, further information on the effects of EDTA chelation in post-MI diabetic patients will be available. We may even see enhanced use of chelation therapy, perhaps offered by the NHS, for cardiovascular pathology in the future. However, this still would depend on regulatory approval and cost-effectiveness.

Overall, the aim to use chelation therapy to inactivate metalloenzymes and treat cardiovascular disease remains controversial due to a lack of substantial, long-term effectiveness data. The fact that chelating agents remove metal from the body is well accepted within the scientific community, although its transition from alternative to conventional cardiovascular therapy is currently met with caution (Sultan *et al.*, 2017). Despite the use of chelation therapy within the NHS to treat heavy metal toxicity and thalassemia (Fulgenzi *et al.*, 2015), redeployment within a cardiovascular scenario is yet to be demonstrated as efficacious. Despite the vast understanding of metal biochemistry, there remain many unanswered questions-especially involving MMPs and how significant they really are to human pathology.

References

Browner, M. F., Smith, W. W. & Castelhano, A. L. 1995. Matrilysin-inhibitor complexes: common themes among metalloproteases. *Biochemistry*, 34(20), 6602-10.

Burrage, P. S., Mix, K. S. & Brinckerhoff, C. E. 2006. Matrix metalloproteinases: role in arthritis. *Frontiers in Bioscience*, 1(11), 529-43.

Clarke, C. N., Clarke, N. E. & Mosher, R. E. 1956. Treatment of angina pectoris with disodium ethylene diamine tetraacetic acid. *American Journal of Medical Sciences*, 232(6), 654-666.

ClinicalTrials.gov [Online] National Library of Medicine (US). 2016 April 11. Identifier NCT02733185, Trial to Assess Chelation Therapy 2 (TACT2). Available from: https://clinicaltrials.gov/ct2/show/NCT02733185.

DeLeon-Pennell, K. Y., Meschiari, C. A., Jung, M., *et al.* 2018. Matrix Metalloproteinases in Myocardial Infarction and Heart Failure. *Progress in Molecular Biology and Translational Science*, 147, 75-100.

Escolar, E., Lamas, G.A., Mark, D.B., *et al.* 2014. The effect of an EDTA-based chelation regimen on patients with diabetes mellitus and prior myocardial infarction in the Trial to Assess Chelation Therapy (TACT). *Circulation: Cardiovascular Quality and Outcomes*, 7(1), 15-24.

Fan, D. & Kassiri, Z. 2020. Biology of Tissue Inhibitor of Metalloproteinase 3 (TIMP3), and Its Therapeutic Implications in Cardiovascular Pathology. *Frontiers in Physiology*, 11(61), DOI: 10.3389/fphys.2020.00661.

Figueira, R. C. S., Gomes, L.R, Neto, J.S, *et al.* 2009. Correlation between MMPs and their inhibitors in breast cancer tumor tissue specimens and in cell lines with different metastatic potential. *BMC Cancer*, 9(20), DOI: 10.1186/1471-2407-9-20.

Fulgenzi, A., De Giuseppe, R., Bamonti, F., *et al.* 2015. Efficacy of chelation therapy to remove aluminium intoxication. *Journal of Inorganic Biochemistry*, 152, 214-8.

Hernick, M. & Fierke, C. 2010. Mechanisms of Metal-Dependent Hydrolases in Metabolism. *Comprehensive Natural Products II*, 8, 547-581.

Johnson, K. A., Bhushan, S., Ståhl, A., *et al.* 2006. The closed structure of presequence protease PreP forms a unique 10,000 Angstroms3 chamber for proteolysis. *Europe PMC*, 25(9), 1977-1986.

Kester, W. R. & Matthews, B. W. 1977. Crystallographic study of the binding of dipeptide inhibitors to thermolysin: implications for the mechanism of catalysis. *Biochemistry*, 16(11), 2506-2516.

Liu, P., Sun, M. & Sader, S. 2006. Matrix metalloproteinases in cardiovascular disease. *Canadian Journal of Cardiology*, 22, 25-30.

Liu, X., Mao, M. & Ma, T. 2016. The effect of EDTA root conditioning on periodontal surgery outcome: A meta-analysis. *Quintessence International*, 47(10), 833-841.

Manzetti, S., Herrington, A. C., van der Spoel, D. *et al.* 2003. Modeling of enzyme–substrate complexes for the metalloproteases MMP-3, ADAM-9 and ADAM-10. *Journal of Computer-Aided Molecular Design*, 17, 551-565.

Murphy, G. 2011. Tissue inhibitors of metalloproteinases. *Genome Biology*, 12(233), DOI: 10.1186/gb-2011-12-11-233.

Mutluay-Tezvergil, A., Agee, K.A., Hoshika, T., *et al.* 2010. The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. *Dental Materials*, 26(11), 1059-1067.

Olejarz, W., Lacheta, D., Kubiak-Tomaszewska, G. 2020. Matrix Metalloproteinases as Biomarkers of Atherosclerotic Plaque Instability. *International Journal of Molecular Sciences*, 21(11), 3946, DOI: 10.3390/ijms21113946.

Ravn, H. B. & Falk, E. 1999. Histopathology of plaque rupture. *Cardiology Clinics,* 17(2), 263-270.

Roeb, E. 2018. Matrix metalloproteinases and liver fibrosis (translational aspects. *Matrix Biology*, 68-69, 463-473.

Schitler, D. 2019. Finding the right match. *Nature Reviews Chemistry*, 3 (130), DOI: 10.1038/s41570-019-0083-5.

Shalia, K. K., Shah V.K., Mashru, M.R., *et al.* 2010. Matrix metalloproteinase-3 (MMP-3) -1612 5A/6A promoter polymorphism in coronary artery disease in Indian population. Indian *Journal of Clinical Biochemistry*, 25(2), 133–140.

Sultan, S., Murarka, S., Jahangir, A., *et al.* 2017. Chelation therapy in cardiovascular disease: an update. *Expert Review of Clinical Pharmacology,* 10(8), 843-854.

Takawale, A., Zhang, P., Patel, V.B., *et al.* 2017. Tissue Inhibitor of Matrix Metalloproteinase-1 Promotes Myocardial Fibrosis by Mediating CD63-Integrin β1 Interaction. *Hypertension*, 69(6), 1092-1103.

Vallet, V., Wahlgren, U. & Grenthe, I. 2003. Chelate Effect and Thermodynamics of Metal Complex Formation in Solution: A Quantum Chemical Study. *Journal of the American Chemical Society*, 125(48), 14941-14950.

Vígh, B., Szél, A., Debreceni, K., *et al.* 1998. Comparative histology of pineal calcification. *Histology and Histopathology*, 13, 851-870.

Xu, J. & Shi, G. P. 2014. Vascular wall extracellular matrix proteins and vascular diseases. *Biochimica et Biophysica Acta*, 1842(11), 2106-2119.

A summary of Avian Influenza Virus in Antarctic penguins

Katie Micklewright

1st year MBiolSci (Biological Sciences)

Penguins on the Antarctic Peninsula are a vital part of polar ecology and the marine food chain, making them useful biological indicators and worthy of protection. Despite this, little is known about the viral threats facing the eight penguin species on the continent. Avian influenza viruses (AIVs) are of particular interest due to their global distribution and prevalence in migratory birds. Investigation into the possible AIV pathways into Antarctica is necessary to understand and predict future viral outbreaks.

Whilst research into AIVs in Antarctic penguins is limited due to the cost and difficulty of polar research a number of studies have provided pioneering insights. Prior to 2013, the only evidence of AIV in penguins came from serological studies, in which the presence of antibodies for the H1, H3, H7 and H9 influenza A subtypes were described in multiple species of penguins, including Adélie penguins (*Pygoscelis adeliae*) and chinstrap penguins (*Pygoscelis antarcticus*) (Morgan & Westbury, 1981; Baumeister *et al.*, 2004). In 2013, the RNA of four highly homologous H11N2 influenza A viruses were isolated from Adélie penguins in Admiralty Bay, King George Island and Rada Covadonga (Hurt *et al.*, 2014). In 2015, H5N5 influenza virus RNA was isolated from chinstrap and gentoo penguins (*Pygoscelis papua*) on Aitcho Island (Barriga *et al.*, 2016), and one chinstrap penguin on King George Island (Hurt *et al.*, 2016). Figure 1 displays the locations of each viral discovery.

Phylogenetic analysis of each of the three strains demonstrated a strong evolutionary relationship with North American AIVs. For the H11N2 virus, Hurt *et al.,* (2014) suggest a possible oral-faecal transmission route into penguin colonies via south polar skuas (*Stercorarius maccormicki*) and southern giant petrels (*Macronectes giganteus*), due to their notoriety for carrying H11 viruses, their close integration with penguin colonies, and their Antarctic migration coinciding with penguin nesting season. Barriga *et al.,* (2016) specify the Pacific-American flyway as a potential route for the H5N5 virus, theorising that shorebirds like the ruddy turnstone (*Arenaria interpres*) could act as vectors for AIVs. This is supported by the H5N5 neuraminidase segment clustering with a Eurasian clade and ruddy turnstones have previously been found to carry AIVs with Eurasian N5 segments (Kraus *et al.*, 2010). Additionally, there is a small amount of evidence for transmission via South America. One of the H5N5 viruses clustered with a strain found in Chilean kelp gulls (*Larus dominicanus*) in 2008, a species found on the Antarctic Peninsula, with two out of eight gene segments also showing South American linkage (Barriga *et al.*, 2016). However, Hurt *et al.,* (2014) notes that H11 viruses are unreported in South American wild birds; this implies that they are not direct vectors, or that they represent one of multiple transmission routes. In summary, the virus strains found thus far implicate North American shorebirds and seabirds as a viral entryway to Antarctica, with potential input from South American gulls. However, until more penguin AIVs are analysed, routes of entry to Antarctica are uncertain.

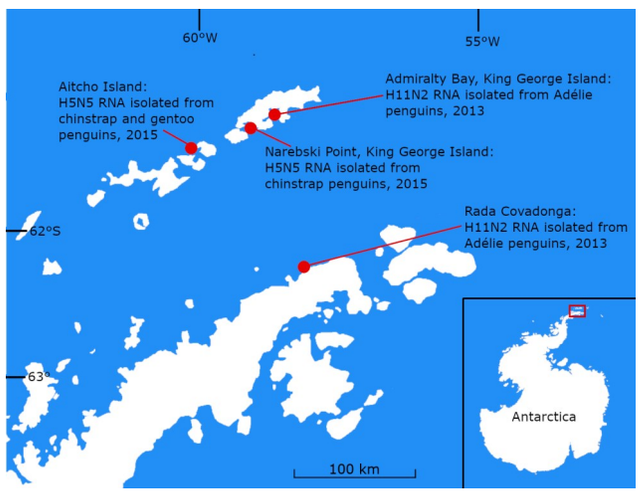


Figure 1. A map showing the four locations where viral RNA was discovered on the Antarctic Peninsula. Labels include the year of discovery, subtype of virus, and species of penguin that each virus was isolated from. The corresponding research for each location is as follows: Hurt *et al.,* (2014) for Rada Covadonga and Admiralty Bay, King George Island; Barriga *et al.,* (2016) for Aitcho Island; Hurt *et al.,* (2016) for Narebski Point, King George Island. Drawn using GNU Image Manipulation Program software (GIMP) 2.10.

At present, there is little evidence to suggest that the presence of AIVs in Antarctic penguins causes significant harm or mortality. Morgan and Westbury (1981) surveyed locations and linked an increase in Adélie chick death to a high serological AIV presence, and Barriga *et al.,* (2016) noted that the H5N5 virus samples were taken from juvenile chinstrap penguins that were “weak, depressed, and possibly ill”. These accounts are based on small samples and therefore are inconclusive, but they do demonstrate a need to thoroughly explore both the effect of AIVs on penguin species, and the likelihood of a more pathogenic virus infecting the continent. Out of the eight Antarctic penguin species, the International Union for Conservation of Nature (IUCN) list two as vulnerable, two as near-threatened, and the remaining four in least-concern category (IUCN, 2020). Despite this being a relatively mild assessment, the prognosis for penguins

may be far more serious due to their behaviour and the global threats facing their habitat. Each of these species congregate in dense breeding colonies, increasing the likelihood of airborne or faecal transmission between individuals. Consequently, if a deadly AIV strain appeared in a colony, the spread could quickly decrease population numbers. In addition, climate change is predicted to alter the populations of certain Antarctic penguin species; whilst some species appear to initially favour warmer temperatures, there is evidence to suggest that this is limited by a decline in krill quantity (Trivelpiecea *et al.*, 2011). Furthermore, species that rely on sea ice for their habitat such as emperor penguins (*Aptenodytes forsteri*) and king penguins (*Aptenodytes patagonicus*) may see population decreases as global temperatures rise (Cristofari *et al.*, 2018; Barbraud & Weimerskirch, 2001). When taking these pressures into account, the vulnerability of Antarctic penguins to future disease may be significantly greater than anticipated.

To prevent a potentially devastating virus reaching Antarctic penguin colonies, it is vital to monitor AIVs and other viruses in migratory seabirds. If a highly virulent AIV strain is detected, a quick response could intercept its spread and safeguard vulnerable avian species. There should be a particular focus on avian viruses found in North and South America, as the current evidence implicates transmission via American flyways. It would also be beneficial to conduct longitudinal studies to evaluate the immune response to AIVs in Antarctic penguins, allowing a better assessment of the risk posed by viral threats. As Antarctic wildlife responds to climate change, protecting penguins from preventable disease will provide a greater understanding of Antarctic ecology whilst allowing them to remain as biological indicators for an unpredictable future.

References

Barbraud, C. & Weimerskirch, H. 2001. Emperor penguins and climate change. *Nature*, 411(6834), 1353-1359.

Barriga, G. P., Boric-Bargetto, D., San Martin, M. C., *et al.* 2016. Avian Influenza Virus H5 Strain with North American and Eurasian Lineage Genes in an Antarctic Penguin. *Emerg. Infect. Dis.*, 22(12), 2221–2223.

Baumeister, E., Leotta, G., Pontoriero, A., *et al.* 2004. Serological evidences of influenza A virus infection in Antarctica migratory birds. *Int.Congr.*, 1263, 737-740.

Cristofari, R., Liu, X., Bonadonna, F., *et al.* 2018. Climate-driven range shifts of the king penguin in a fragmented ecosystem. *Nat. Clim. Change*, 8, 245 –251.

Hurt, A., Chuan Fang Su, Y., Aban, M., *et al.* 2016. Evidence for the Introduction, Reassortment, and Persistence of Diverse Influenza A Viruses in Antarctica. *J. Virol.*, 90(21), 9674-9682.

Hurt, A., Vijaykrishna, D., Butler, J., *et al.* 2014. Detection of Evolutionarily Distinct Avian Influenza A Viruses in Antarctica. *mBio*, 5(3), e01098-14 DOI: 10.1128/mBio.01098-14.

IUCN. 2020. *The IUCN Red List of Threatened Species*. Available at: https://www.iucnredlist.org (Accessed: 26 Dec 2020).

Kraus, S., Stallknecht, D. E., Negovetich, N. J., *et al.* 2010. Coincident ruddy turnstone migration and horseshoe crab spawning creates an ecological 'hot spot' for influenza viruses. *Proc. Biol. Sci.*, 277(1699), 3373–3379.

Morgan, I. R. & Westbury, H. A. 1981. Virological Studies of Adélie Penguins (*Pygoscelis adeliae*) in Antarctica. *Avian Dis.*, 25(4), 1019-1026.

Trivelpiecea, W. Z., Hinkea, J. T., Millera, A. K., *et al.* 2011. Variability in krill biomass links harvesting and climate warming to penguin population changes in Antarctica. *Proc. Natl. Acad. Sci. U.S.A.*, 108(18), 7625-7628.

Microbial Dysbiosis in Crohn's Disease:

Causes and Solutions

Scott Harris

1st year Biochemistry BSc

Introduction

Crohn's Disease (CD), along with Ulcerative Colitis (UC), are the two inflammatory bowel diseases (IBDs); illnesses that involve chronic inflammation and pain within the gastrointestinal (GI) tract (Coates *et al.,* 2020). Other symptoms of IBD include: diarrhoea, blood in stool, fatigue, lack of appetite and weight loss (NHS, 2020). Unlike colitis, CD can be found in patches anywhere along the tract, however the ileocolonic region (where the small and large intestine are joined) tends to be the most commonly affected (Day *et al.,* 2012). It was estimated in 2014 by the National Institute for Health and Care Excellence that 157 in 100,000 people have CD (NICE, 2014), but many of those with the condition report stigma and negative psychological effects as a direct result (Gamwell *et al.,* 2020). It is therefore important for awareness for CD to be raised, and its status as a relevant issue recognised. It is known that CD is caused by and/or influences the gut microbiome - the communities of bacteria and small amounts of other microorganisms of the intestines (Daniel *et al.,* 2007). In a healthy individual, the majority of these bacteria are commensal (non-harmful), and many provide benefits, like the production of vitamins and the prevention of too many pathogenic bacteria from growing. The gut microbiome of CD patients is characterised by a lack of diversity and an imbalance, or dysbiosis, of bacteria that perform different functions (Daniel *et al.,* 2007). This is at least partially responsible for inflammation; pro-inflammatory bacteria outweigh anti-inflammatory bacteria (Leylabadlo *et al.,* 2020). Both current treatments for CD – anti-inflammatory treatments and immunosuppressants - only work in a small portion of patients (Hart *et al.,* 2020; Schoepfer *et al.,* 2014; Feagan *et al.,* 2013; Feagan *et al.,* 2016; Rutgeerts *et al.,* 2005; Syal, Kashani & Shih, 2018), with little understanding of why. Research into the reasons why people get CD and possible alternative therapies are therefore essential.

Although CD is rarely fatal, it can cause a severe decrease in quality of life to many who suffer with it. Recurring abdominal pain is one of the most common symptoms of the disease, being found in around 70% of patients (Bielefeldt, Davis & Binion, 2009; Coates *et al.,* 2020). Anti-inflammatory treatments, specifically the usage of the 5-Aminosalicylate (5-ASA) drug group, are one of the most commonly used to treat CD, however there is often disagreement on their efficacy, with a large discrepancy between cases (Hart *et al.,* 2020; Schoepfer *et al.,* 2014). Recently, immunosuppressants have been approved by the US Food and Drug Administration (FDA) for CD treatment, but these are also seen to be ineffective in many patients – between 1/3 and 1/2, depending on the specific drug (Feagan *et al.,* 2013; Feagan *et al.,* 2016; Rutgeerts *et al.,* 2005; Syal, Kashani & Shih, 2018). There is potential for other treatments with more decisive evidence to be developed; this includes the discovery of nutraceuticals that have similar effects (Ortiz *et al.,* 2020), and faecal microbiota transplants. These have already been used to great success within other gastrointestinal diseases, specifically *Clostridium difficile* infections (Costello *et al.,* 2015), but their usage within CD is still in its experimental phase, with the lack of understanding of its pathogenesis causing paradoxical issues, such as the increase of gut microbiota diversity with no apparent effect on the patient's pain levels.

Dysbiosis of the Gut Microbiome

There is a great amount of taxonomic variance within the gut microbiome. *Escherichia coli* can be found in most individuals through culture growth, and the phyla Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Verrucomicrobia are also present in the majority of cases, though the latter three in smaller numbers. However, the possibility of finding a more specific core set of bacteria than this, such as universal genera or species, is very unlikely, and even the few species that have been grown in great enough numbers to justify such a claim can be present in amounts as low as 0.5% of the total flora (Lozupone *et al.,* 2012). The diversity in patients' microbiomes is the basic premise of bacterial dysbiosis – the abnormal make-up of bacterial colonies, leading to possible over and/or under-presence of certain organisms that leads to disease. Given the huge amount of variation within microbiomes, even being able to characterise a 'normal' and 'abnormal' state seems impossibly complex; therefore, metagenomic approaches have become increasingly popular within this field of research. It is important to know what exact type of bacteria one has in their GI tract much less than simply what they are actually doing, both within the bacterial communities and towards the host system. Functional searching to assess gene profiles can give light to ideas on the main effect of different 'types' of bacteria usually, but not necessarily, related to their taxonomy (aerobic/anaerobic living style, metabolic intake and output, etc.) (Lozupone *et al.,* 2012).

Of course, it is important to note that not all variance is necessarily bad; many factors affect an individuals' microbiome composition. This can include: diet and geographical location (De Filippo *et al.,* 2010); genetics, especially mutations/allelic variation of proteins which

regulate innate immunity to commensal bacteria (Zaruhi *et al.,* 2008); and age. Infants generally possess the most variability between individuals, while taxonomic diversity is seen to increase during pregnancy. There is also the tendency for Bacteroidetes species to dominate the microbiota with old age (Kostic, Howitt & Garrett, 2013; Lozupone *et al.,* 2012) (Figure 1). One of the most notable presentations of CD is the greatly decreased amount of both diversity and overall count of bacteria within the gut (Dicksved *et al.,* 2008; Daniel *et al.,* 2007; Peterson *et al*., 2008). It is well known that the gut microbiome is a multifaceted, key part of human health. Species create short-chain fatty acids (SCFAs), such as butyrate and acetate, which are used as fuel by the colonic epithelial cells (Huda-Faujan *et al.,* 2010). A well-balanced gut microbiome is also shown to stimulate the immune system, leading to a lower rate of autoimmune disease (Nishida *et al.,* 2017). In particular, patients with CD are likely to have a lowered amount of bacteria from the phylum Firmicutes, including the key bacterium *Faecalibacterium prausnitzii*.

F. prausnitzii is known to be a key bacterium species for a healthy gut microbiome for two principal reasons. The first is that it is one of the above mentioned SCFA-producing bacteria, specifically a butyrate-producing bacteria (BPB). This molecules' abundance has been shown to affect multiple areas of the human body, such as the intestinal cell life cycle and the halting of the development of cancer, though the exact mechanisms are not yet determined in most cases (Leylabadlo *et al.,* 2020). Low levels of available butyrate have also been associated with increased levels of pathogenic bacteria, such as *E. coli* (Nishida *et al.,* 2017). The second key aspect of *F. prausnitzii* is its anti-inflammatory and immune system-related effect (Miquel *et al.,* 2013; Stein & Shaker, 2015). In particular, *F. prausnitzii* is known to stimulate the production of a great amount of the primary anti-inflammatory cytokine IL-10 compared to other bacterial strains, while it produces among the lowest amount of the pro-inflammatory cytokine IL-12. The ratio of IL-10:IL-12 stimulation is the metric generally used for determining the anti-inflammatory/inflammatory effect of a species; it seems from this data that *F. prausnitzii* is one of the most potent (Sokol *et al.,* 2008). An additional change in the composition of a CD patient microbiome is the increase in bacteria associated with mucolysis, the breaking down of the gut mucus. Ruminococcus species are known to usually be the most abundant varieties. They are shown to specifically break down the MUC2 protein within mucus, while other species are able to enzymatically catalyse the degradation of the oligosaccharides which surround it (Hoskins & Boulding, 1981; Png *et al.,* 2010).

The Possibility of Faecal Microbiota Transplantation as a Therapy for Crohn's Disease

Faecal Microbiota Transplantation (FMT) is the introduction of faecal matter from a healthy individual into a patient, with the goal of rebalancing the levels of bacteria within the gut. The treatment is currently given as either enemas or colonoscopies, and there have been no disadvantages for the growing use of frozen faecal matter, with the advantage that it can be stored for much longer periods of time (Costello *et al.,* 2015; Ramai *et al.,* 2019). There is also growing support for the use of oral capsules, as their administration is much less invasive and prevents any risk of gut perforation (Ramai *et al.,* 2019). The capsules contain lyophilised faecal powder mixed with a cryoprotectant, such as glycerol, thickly encapsulated to prevent dissolution by stomach acid (Tian *et al.,* 2015). Historically, it was preferred that the donated sample was taken from healthy close relatives, however this has not been shown to be an important factor in its efficacy (Ramai *et al.,* 2019), potentially allowing the treatment to be conducted on a larger amount of patients. FMT is believed to be a potential treatment candidate for CD (and IBDs in general), as it has been repeatedly shown that the treatment restores balance within the gut microbiome. FMT grants the individual a higher amount of colony resistance—the presence of commensal bacteria that are able to prevent the spread of pathogenic bacteria (Ademe 2020; Ooijevaar *et al.,* 2019). It also increases the number of SCFA bacteria which are known to be specifically lacking in IBD patients, as well as the restoration of a healthy bile composition. Primary bile, mainly made up of cholic and taurocholic acid, is the substance secreted by the patient's liver, while bacteria within the gut deconjugate and dehydroxylate these molecules to create

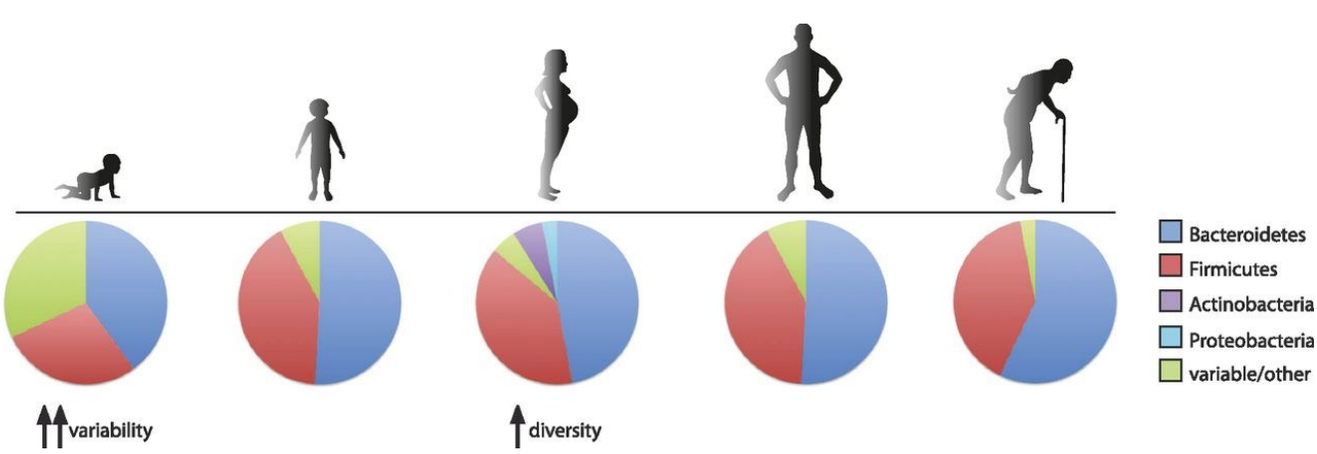


Figure 1. The relative amounts of certain bacterial phyla in healthy individuals at different life stages. There is increased variability between infant individuals, and diversity in pregnant individuals increases, including the growth in proportion of otherwise less common phyla. Adapted from Kostic, Howitt & Garrett, 2013.

secondary bile (Weingarden *et al.,* 2014). Secondary bile is the main constituent of bile within healthy individuals and patients post-FMT, and can prevent the spread of pathogenic bacteria which are known to use primary bile as a germination source, most notable *Clostridium difficile* (Khoruts & Sadowsky, 2016). FMT is most currently used for *C. difficile* infections, where it has been demonstrated to be quite a success (Weingarden *et al.,* 2014), and increasing the therapy's scope to other gastrointestinal diseases has been the focus of great amounts of research. Targeted disorders include CD and UC, but also Irritable Bowel Syndrome (IBS) and issues following low amounts of commensal bacteria after long antibiotic treatments. However, use of FMT for IBD is controversial, with a great variety in its efficacy between patients. It is unknown why the treatment works well in some patients but poorly in others (Angelberger *et al.,* 2013; Rossen *et al.,* 2015). It is usually shown that there is a positive change in the gut microbiome of patients, and often some improvement is seen, but it is not very often a long term change; the best time for patients in terms of their illness is usually 1 month post-FMT (Collins & DeWitt, 2020).

There is a strong possibility that the conflicting conclusions behind studies taken on this subject are at least partly due to the fact that all so far have been of a small-scale with limited numbers of patients; a set of 'well-designed randomised controlled trials' are needed to grant more decisive results (Anderson, Edney & Whelan, 2012). However, it does reinforce the fact that CD, and IBDs in general, are still poorly understood, complex diseases, of which gut dysbiosis is only one part (Caldeira *et al.,* 2020; Khan *et al.,* 2019). It may also be related that CD patients have been found to have a different dysbiosis to other GI diseases, including UC, IBS, etc; there are unique features of the imbalances in each, but CD has been proven to have the most distinct pattern (Bernstein & Forbes, 2017). This could lead to the conclusion that current FMT treatments may not work for CD as often as *C. difficile* infections because the samples do not contain enough of the correct type of bacteria for the disease to be able to go into a state of remission. There has been a list published that details 'Microbes of Interest' specifically within CD and UC (Bernstein & Forbes, 2017), and synthetically altered FMT samples could be generated to create a more personalised approach.

References

Ademe, M. 2020. Benefits of fecal microbiota transplantation: A comprehensive review. *Journal of Infection in Developing Countries*, 14 (10), 1074-1080.

Anderson, J. L., Edney, R. J. & Whelan, K. 2012. Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. *Alimentary Pharmacology & Therapeutics*, 36(6), 503-516.

Angelberger, S., Reinisch, W., Makristathis, A. *et al.* 2013. Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. *The American journal of gastroenterology*, 108(10), 1620-1630.

Bernstein, C. N. & Forbes, J. D. 2017. Gut Microbiome in Inflammatory Bowel Disease and Other Chronic Immune-Mediated Inflammatory Diseases. *Inflamm. Intest. Dis.*, 2(2), 116-123.

Bielefeldt, K., Davis, B. & Binion, D. G. 2009. Pain and Inflammatory Bowel Disease, *Inflamm. Intest. Dis.*, 15(5), 778-788.

Caldeira, L. d. F., Borba, H. H., Tonin, F. S. *et al.* 2020. Fecal microbiota transplantation in inflammatory bowel disease patients: A systematic review and meta-analysis. *PLoS ONE*, 15(9), 1-17.

Coates, M. D., Johri, A., Gorrepati, V. S., *et al.* 2020. Abdominal pain in quiescent inflammatory bowel disease. *International Journal of Colorectal Disease,* 36(1), 93-102.

Collins, M. & DeWitt, M. 2020. Fecal microbiota transplantation in the treatment of Crohn disease. *JAAPA: Journal of the American Academy of Physician Assistants*, 33(9), 34-37.

Costello, S. P., Conlon, M. A., Vuaran, M. S., Roberts-Thomson, I. C. & Andrews, J. M. 2015. Faecal microbiota transplant for recurrent *Clostridium difficile* infection using long-term frozen stool is effective: clinical efficacy and bacterial viability data. *Alimentary pharmacology & therapeutics*, 42(8), 1011-1018.

Day, A. S., Ledder, O., Lemberg, D. A. & Leach, S. T. 2012. Crohn's and colitis in children and adolescents. *World Journal of Gastroenterology*, 18(41), 5862-5869.

De Filippo, C., Cavalieri, D., Di Paola, M., *et al.* 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 107(33), 14691-14696.

Dicksved, J., Halfvarson, J., Rosenquist, M., *et al.* 2008. Molecular analysis of the gut microbiota of identical twins with Crohn's disease. *The ISME journal*, 2(7), 716-727.

Feagan, B. G., Rutgeerts, P., Sands, B. E., *et al.* 2013. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *New Engl. J. Med.,* 369(8), 699-710.

Feagan, B. G., Sandborn, W. J., Gasink, C., *et al.* 2016. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. *New England Journal of Medicine*, 375(20), 1946-1960.

Frank, D. N., St. Amand Allison, L., Feldman Robert, A., *et al.* 2007. Molecular-Phylogenetic Characterization of Microbial Community Imbalances in Human Inflammatory Bowel Diseases. *Proceedings of the National Academy of Sciences of the United States of America*, 104 (34), 13780-13785.

Gamwell, K. L., Roberts, C. M., Espeleta, H. C., *et al.* 2020. Perceived stigma, illness uncertainty, and depressive symptoms in youth with inflammatory bowel disease: The moderating effect of mindfulness. *Psychology, Health & Medicine*, 25(9), 1037-1048.

Hart, A., Ng, S. C., Watkins, J., *et al.* 2020. The use of 5-aminosalicylates in Crohn's disease: a retrospective study using the UK Clinical Practice Research Datalink. *Annals of Gastroenterology*, 33(5), 500-507.

Hoskins, L. C. & Boulding, E. T. 1981. Mucin degradation in human colon ecosystems. Evidence for the existence and role of bacterial subpopulations producing glycosidases as extracellular enzymes. *Journal of Clinical Investigation*, 67(1), 163-172.

Huda-Faujan, N., Abdulamir, A. S., Fatimah, A. B., *et al.* 2010. The Impact of the Level of the Intestinal Short Chain Fatty Acids in Inflammatory Bowel Disease Patients Versus Healthy Subjects, The *Open Biochemistry Journal,* 4, 53-8.

Khan, I., Ullah, N., Zha, L., *et al.* 2019. Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? *IBD Treatment Targeting the Gut Microbiome*, 8(3), 126.

Khoruts, A. & Sadowsky, M. J. 2016. Understanding the mechanisms of faecal microbiota transplantation. *Nature reviews. Gastroenterology & hepatology*, 13(9), 508-516.

Kostic, A. D., Howitt, M. R. & Garrett, W. S. 2013. Exploring host-microbiota interactions in animal models and humans. *Genes Dev.,* 27(7), 701-18.

Leylabadlo, H. E., Ghotaslou, R., Feizabadi, M. M., *et al.* 2020. The critical role of *Faecalibacterium prausnitzii* in human health: An overview. *Microbial Pathogenesis*, 149, 104344.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R. 2012. Diversity, stability and resilience of the human gut microbiota. *Nature*, 489, 220-230.

Miquel, S., Martín, R., Rossi, O., *et al.* 2013. *Faecalibacterium prausnitzii* and human intestinal health. *Current Opinion in Microbiology*, 16(3), 255-261.

Nishida, A., Inoue, R., Inatomi, O., *et al.* 2017. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clinical Journal of Gastroenterology*, 11(1), 1-10.

Ooijevaar, R. E., Terveer, E. M., Kuijper, E. J., Verspaget, H. W. & Keller, J. J. 2019. Clinical application and potential of fecal microbiota transplantation. *Annu. Rev. Med.,* 70, 335-351.

Ortiz, T., Arguelles-Arias, F., Illanes, M., *et al.* 2020. Polyphenolic Maqui Extract as a Potential Nutraceutical to Treat TNBS-Induced Crohn's Disease by the Regulation of Antioxidant and Anti-Inflammatory Pathways. *Nutrients,* 12(6), 1752.

Peterson, D. A., Frank, D. N., Pace, N. R. & Gordon, J. I. 2008. Metagenomic Approaches for Defining the Pathogenesis of Inflammatory Bowel Diseases. *Cell Host & Microbe*, 3(6), 417-427.

Png, C. W., Lindén, S. K., McGuckin, M. A., *et al.* 2010. Mucolytic bacteria with increased prevalence in IBD mucosa augment *in vitro* utilization of

mucin by other bacteria. *American Journal of Gastroenterology*, 105,(11), 2420-2428.
Ramai, D., Zakhia, K., Ofosu, A., Ofori, E. & Reddy, M. 2019. Fecal microbiota transplantation: donor relation, fresh or frozen, delivery methods, cost-effectiveness. *Annals of Gastroenterology*, 32(1), 30-38.
Rossen, N. G., Fuentes, S., van der Spek, M. J., *et al.* 2015. Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis. *Gastroenterology*, 149(1), 110-118.
Rutgeerts, P., Sandborn, W. J., Feagan, B. G., *et al.* 2005. Infliximab for induction and maintenance therapy for ulcerative colitis. *New England Journal of Medicine*, 353(23), 2462-2476.
Schoepfer, A. M., Bortolotti, M., Pittet, V., *et al.* 2014. The gap between scientific evidence and clinical practice: 5-aminosalicylates are frequently used for the treatment of Crohn's disease. *Alimentary pharmacology & therapeutics*, 40(8), 930-937.
Sokol, H., Pigneur, B., Watterlot, L., *et al.* 2008. *Faecalibacterium prausnitzii* Is an Anti-Inflammatory Commensal Bacterium Identified by Gut Microbiota Analysis of Crohn Disease Patient. *Proceedings of the National Academy of Sciences of the United States of America*, 105(43), 16731-16736.
Stein, D. J. & Shaker, R. 2015. Inflammatory Bowel Disease. A Point of Care Clinical Guide, Springer International Publishing.
Syal, G., Kashani, A. & Shih, D. Q. 2018. Fecal Microbiota Transplantation in Inflammatory Bowel Disease: A Primer for Internists. *The American Journal of Medicine*, 131(9), 1017-1024.
Tian, H., Ding, C., Gong, J., *et al.* 2015. Freeze-dried, capsulized fecal microbiota transplantation for relapsing *Clostridium difficile* infection. *Journal of Clinical Gastroenterology*, 49(6), 537-538.
Weingarden, A. R., Sadowsky, M. J., Chen, C., *et al.* 2014. Microbiota transplantation restores normal fecal bile acid composition in recurrent *Clostridium difficile* infection. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 306(4), G310-G319.
Zaruhi, A. K., Zhanna, A. K., Gayane, P. M., *et al.* 2008. Predominant role of host genetics in controlling the composition of gut microbiota. *PLoS ONE*, 3(8), e3064-e3064.

Insider Imprint

Follow us on social media

Like and follow our pages to see our latest news and receive updates about writing and art opportunities!

twitter.com/insiderimprint

facebook.com/insiderimprint

instagram.com/insiderimprint

Guardian of the Genome:

An insight into the response dynamics of tumour suppressor protein 53 following DNA damage and hyperproliferative signals

Amy Steele

4th year MBiolSci (Pharmacology)

Tumour suppressor protein 53 (p53), also canonically referred to as the guardian of the genome, is a crucial cellular stress sensor involved in coordinating responses to cellular insults such as hypoxia, ribonucleotide depletion, telomere attrition, oncogene activation, oxidative damage and hyperproliferation, amongst other factors such as UV light and γ-irradiation. The types of response to cellular damage include activation of cell cycle arrest, DNA repair pathways, senescence, and in the most severe cases of stress, apoptosis. Loss of p53 is highly associated with cancer development due to the absence of the vital genome protection that p53 is renowned for, thus an enhanced understanding of p53 function is vital to facilitate cancer detection, prognosis and treatment. However, the exact mechanisms of cellular protection are yet to be fully elucidated. Within this article, the most well-defined mechanisms regarding p53 regulation and function are discussed.

Brief introduction to p53

Cancer is defined by a population of cells proliferating and behaving in an uncontrolled manner (Feroz and Sheikh, 2020). Throughout time, cells have evolved many mechanisms to challenge stress in order to protect the genome (Feroz & Sheikh, 2020). Without these mechanisms, mutations and chromosomal abnormalities associated with oncogene activation or tumour suppressor repression, transmitted to daughter cells during mitosis, may initiate development of cancer (Feroz & Sheikh, 2020). Located on chromosome 17 the tumour suppressor 53 (TP53) gene, encoding transcription factor p53, is a highly studied gene involved in orchestrating many responses to genomic stress, resulting in its canonical name: “the guardian of the genome” (Feroz & Sheikh, 2020). In fact, the significance of p53 within prevention of tumorigenesis is undeniable, given that p53 activity is altered significantly in over 50% of all sporadic human cancers, either by mutation at the TP53 locus or by oncogenic events that reduce the functionality of wild type p53, for example upregulation of p53 repressors and downregulation of p53 activators (Sullivan *et al.*, 2018). This makes p53 the most frequently mutated protein in human cancer. Similarly, people with Li-Fraumeni syndrome, characterised by inheritance of a mutant TP53 allele, have an increased predisposition to cancer (Bieging, Mello & Attardi, 2014). Clearly, inactivation of p53 is pivotal to carcinogenesis. Here, we discuss the most well-defined mechanisms by which p53 is known to protect the cell.

Regulation of p53

The maintenance of genomic integrity is regulated by distinct signalling mechanisms (Giaccia & Kastan, 1998). Activation of p53 is initiated following a host of diverse cellular insults, such as oncogene activation, telomere attrition, ribonucleotide depletion, hyper-proliferation, oxidative damage, in addition to environmental factors, including γ-irradiation, UV light and chemotherapeutics (Giaccia & Kastan, 1998). Under normal physiological conditions, ideally parading a scarcity of cellular stressors, p53 levels are minimal as a result of the critical p53 regulator, mouse double minute 2 homolog (MDM2) (Chen, 2012). This protein negatively regulates p53 activity and keeps p53 levels low in the absence of DNA damage signals by antagonising the N-terminal trans-activation domain of p53, a region critical for activation of p53 (Kruse & Gu, 2009). Therefore, MDM2 represses p53 transactivation function. Moreover, MDM2 exhibits ubiquitin E3 ligase activity through binding and poly-ubiquitinating lysine residues in the p53 C-terminus in preparation for proteasome-mediated degradation of p53 (Zhang & Xiong, 2001). This process involves covalently attaching a repeating chain of the small regulatory protein called ubiquitin to a lysine residue on the substrate protein, which ultimately stabilises the substrate protein and marks it as a target for degradation by the 26S proteosome (Callis, 2014). Interestingly, MDM2 is also a p53 responsive gene, meaning that upon transcription of p53, MDM2 transcription is simultaneously activated resulting in increased MDM2 protein levels (Aubrey *et al.*, 2018). This generates a negative feedback loop responsible for maintaining low p53 levels, vulnerable to disruption only when cellular stress signals are high (Aubrey *et al.*, 2018).

Perhaps the most extensively defined signalling pathways leading to p53 activation are the ataxia telangiectasia mutated (ATM)-dependent response to acute DNA damage and the ADP-ribosylation factor (ARF)-dependent response to hyperproliferative signals (Feroz & Sheikh, 2020). Regarding response to DNA damage, specifically DNA double-strand breaks, ATM and ataxia telangiectasia

and Rad3 related (ATR) protein kinases are recruited for phosphorylation and thus activation of checkpoint kinases CHK1 and CHK2 (Feroz & Sheikh, 2020). These kinases phosphorylate p53 on serine 20 whilst ATM and ATR phosphorylate p53 on serine 15 (Sakaguchi *et al.*, 1998). Each of these post-translational modifications disrupt MDM2 binding to p53, preventing negative regulation and allowing p53 to perform its various DNA damage response activities (Chen *et al.*, 2005). Similarly, hyperproliferative signals induce the ARF-dependent response (Cheng & Chen, 2010). Here, unregulated cell division results in amplified liberation of the E2F transcription factor, which plays a role in stimulating ARF transcription, an important tumour suppressor (Cheng & Chen, 2010). ARF forms stable complexes with MDM2, sequestering MDM2 within nucleoli and inhibiting MDM2 E3 ubiquitin ligase activity, dissociation of the MDM2-p53 complex again permitting the transcriptional endeavours of p53 (Kruse & Gu, 2009).

Role in tumour suppression

Stabilisation of p53 by ATM, ATR, and ARF in response to cellular stress allows induction of manifold downstream transcriptional targets, encoding genes that ultimately share the same goal: preserving genomic integrity (Bieging, Mello & Attardi, 2014). A broad range of target genes are activated by p53, mainly implicated in cell cycle arrest, DNA repair, senescence and apoptosis (Bieging, Mello & Attardi, 2014). An overview of the role of p53 in tumour suppression is detailed in Figure 1.

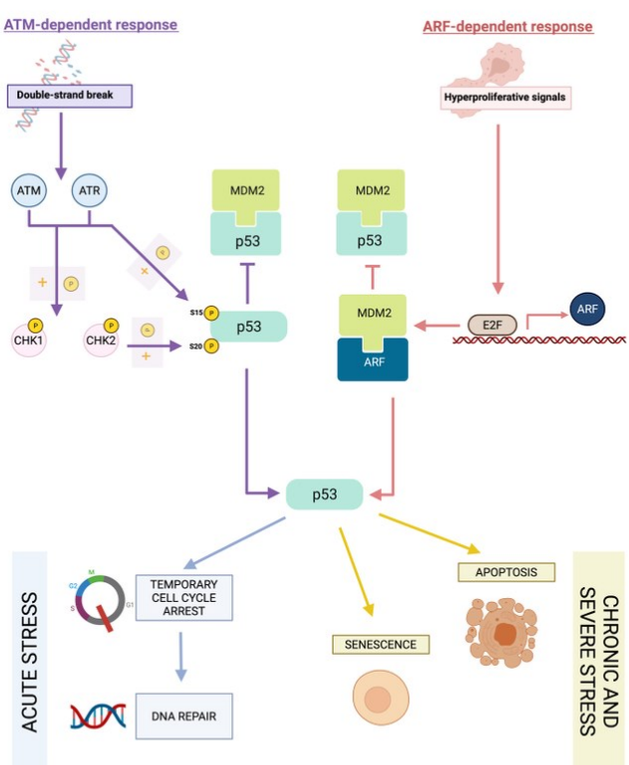


Figure 1. A schematic overview of the activation of p53 by the ATM-dependent and ARF-dependent pathways following cellular stresses such as DNA damage and hyperproliferative signals, respectively, involving uncoupling of p53 from its negative regulator, MDM2. This leads to p53 functional responses, those being temporary cell cycle arrest and DNA repair regarding transient cellular stresses, and senescence or apoptosis regarding prolonged and severe cellular stresses. Created with BioRender.com

To achieve cell cycle arrest, p53 prompts transcriptional activation of CDKN1A, encoding p21, a protein responsible for binding to cyclin E/cyclin-dependent kinase 2 (CDK2) and cyclin D/cyclin-dependent kinase 4 (CDK4) complexes (Hafner *et al.*, 2019). In normal circumstances, these complexes would phosphorylate retinoblastoma protein (pRb) complexed with E2 transcriptional factor (E2F), subsequently triggering conformational changes that liberate E2F from the complex in order to initiate expression of genes important for DNA replication and G1/S transition in the cell cycle (Burke *et al.*, 2010). Yet, p21 binds these cyclin/CDK complexes, preventing phosphorylation of pRb protein, arresting the cell cycle at the G1/S regulation point (Slebos *et al.*, 1994). Similarly, p53 stabilisation obstructs cells at the G2/M phase by repressing 14-3-3s promoters, which usually encode proteins that sequester cell division cycle 25C (CDC25C) within the cytoplasm, a process needed to activate cyclin/CDK complexes (Hermeking *et al.*, 1997). This temporary cell cycle arrest provides essential cell-cycle checkpoints which grant the cell with enough time to repair possible genomic lesions before the cell begins cycling again and DNA replication begins (Chen, 2016). This enhances survival of damaged cells and prevents propagation of DNA aberrations to progeny cells from which malignancies might arise (Chen, 2016). To take advantage of the quiescent cellular states implemented by p53, specialised DNA repair machineries within the cell pursue damage removal (Bieging, Mello & Attardi, 2014). Examples of such repair pathways include nucleotide excision repair (NER), base excision repair (BER) and non-homologous end-joining (NHEJ) (Bieging, Mello & Attardi, 2014). Often, p53 also directly plays a direct role in these pathways, both through modulation by transcriptional activation of target genes and participation in the pathway itself (Bieging, Mello & Attardi, 2014).

Despite the best efforts of the cellular machinery to repair damaged DNA, sometimes it is simply not possible to counteract the more severe and prolonged cellular stressors (Amaral *et al.*, 2010). In these circumstances, to prevent further proliferation and propagation of genetic defects possessing the potential to generate neoplasia, p53 may induce permanent cell cycle arrest, termed senescence, as opposed to the temporary cell cycle arrest induced by transient stimuli (Mijit *et al.*, 2020). Senescence is defined as irreversible cell cycle arrest by which the cell remains functional but further replication is inhibited (Kruiswijk, Labuschagne & Vousden, 2015). p53 achieves this by sustained transcriptional activation of p21 (Qian & Chen, 2013). Moreover, should the severity and duration of stress become extreme, p53 will even induce cell death by apoptosis via transcriptional activation of pro-apoptotic B-cell lymphoma 2 (BCL-2) family proteins such as BCL-2 homologous antagonist killer (BAK1), phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1) and p53 upregulated modulator of apoptosis (PUMA) (Hafner *et al.*, 2019). Evidently, p53 plays a central role in evaluating the fate of cells; as such, p53 has even been referred to as “a lifeguard with a licence to kill” (Kruiswijk, Labuschagne & Vousden, 2015).

Conclusion and future considerations for p53 research

In conclusion, p53 is a highly relevant tumour suppressor which under normal circumstances is coupled with its negative regulator MDM2 (Kruiswijk, Labuschagne & Vousden, 2015). In the presence of various stress stimuli, p53 is released from the MDM2-p53 complex by either the ATM-dependent response or the ARF-dependent response to permit coordination of an adaptive gene expression programme resulting in either growth arrest or cell death, depending on the transience of the stress stimuli (Kruiswijk, Labuschagne & Vousden, 2015). Only the most extensively defined mechanisms have been described in this article. Despite several decades of research, the comprehensive role of p53 in tumour suppression is currently unclear and has yet to be fully dissected, owing to its complex dynamics and multifaceted functions. It is even likely that additional functions of p53 are yet to be discovered. For example, it was recently discovered that p53 may also modulate invasion and tumour-stromal cell cross talk within the tumour microenvironment (Bieging, Mello & Attardi, 2014). Moreover, current knowledge of p53 activity is founded on experimental data obtained from mouse models or cell culture studies which often do not consider important variables such as age, sex and ethnicity (Sullivan *et al.*, 2018). With novel technologies, addressing these concerns can be made possible through isolation of various cell lineages *ex-vivo* to determine potential differences in p53 chromatin binding, activation and regulation (Sullivan *et al.*, 2018). Likewise, the question of how exactly p53 tumour suppressor function can be heightened for cancer treatment has yet to be fully answered. Currently, many MDM2 inhibitors, which mechanistically act by preventing the negative regulation of p53, are deemed ineffective as monotherapies and induce significant haematological toxicity following long term treatment regimens (Tisato *et al.*, 2017). Nevertheless, it has been suggested that therapeutic combinatorial approaches may prove effective through modifying the function of vital p53 cofactors or target genes, with hopes that synergistic activity will reduce toxicity and strengthen the tumour suppressive activity of MDM2 inhibitors (Sullivan *et al.*, 2018). Therefore, future directions for p53 research include systematic biochemical and cytological studies to decipher the specific details of p53-mediated tumour suppression and answer the many open questions still existing within p53 research. Not only will this refine comprehension of p53 function, but enhanced understanding of the components involved in p53-mediated tumour suppression will also facilitate cancer detection and prognosis, in addition to increased flexibility during identification of potential therapeutic targets for cancer treatment.

References

Amaral, J. D., Xavier, J. M., Steer, C. J. & Rodrigues, C. M. 2010. The role of p53 in apoptosis. *Discov. Med.,* 9(45), 145-52.

Aubrey, B. J., Kelly, G. L., Janic, A., Herold, M. J. & Strasser, A. 2018. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? *Cell Death & Differentiation*, 25(1), 104-113.

Bieging, K. T., Mello, S. S. & Attardi, L. D. 2014. Unravelling mechanisms of p53-mediated tumour suppression. *Nature reviews: Cancer*, 14(5), 359-370.

Burke, J. R., Deshong, A. J., Pelton, J. G. & Rubin, S. M. 2010. Phosphorylation-induced conformational changes in the retinoblastoma protein inhibit E2F transactivation domain binding. *The Journal of Biological Chemistry*, 285(21), 16286-16293.

Callis, J. 2014. *The ubiquitination machinery of the ubiquitin system*. The Arabidopsis Book, 12, pp. e0174-e0174.

Chen, J. 2012. The Roles of MDM2 and MDMX Phosphorylation in Stress Signaling to p53. *Genes & Cancer*, 3(3-4), 274-282.

Chen, J. 2016. The Cell-Cycle Arrest and Apoptotic Functions of p53 in Tumor Initiation and Progression. *Cold Spring Harb Perspect Med*, 6(3), a026104.

Chen, L., Gilkes, D. M., Pan, Y., Lane, W. S. & Chen, J. 2005. ATM and Chk2-dependent phosphorylation of MDMX contribute to p53 activation after DNA damage. *The EMBO Journal*, 24(19), 3411-3422.

Cheng, Q. & Chen, J. 2010. Mechanism of p53 stabilization by ATM after DNA damage. *Cell Cycle*, 9(3), 472-478.

Feroz, W. & Sheikh, A. M. A. 2020. Exploring the multiple roles of guardian of the genome: P53. *Egyptian Journal of Medical Human Genetics*, 21(1), 49.

Giaccia, A. J. & Kastan, M. B. 1998. The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev*, 12(19), 2973-83.

Hafner, A., Bulyk, M. L., Jambhekar, A. & Lahav, G. 2019. The multiple mechanisms that regulate p53 activity and cell fate. *Nature Reviews Molecular Cell Biology*, 20(4), 199-210.

Hermeking, H., Lengauer, C., Polyak, K., He, T. C., Zhang, L., *et al.* 1997. 14-3-3sigma is a p53-regulated inhibitor of G2/M progression. *Mol Cell*, 1 (1), 3-11.

Kruiswijk, F., Labuschagne, C. F. & Vousden, K. H. 2015. p53 in survival, death and metabolic health: a lifeguard with a licence to kill. *Nature Reviews Molecular Cell Biology*, 16(7), 393-405.

Kruse, J.-P. & Gu, W. 2009. Modes of p53 Regulation. *Cell*, 137(4), 609-622.

Mijit, M., Caracciolo, V., Melillo, A., Amicarelli, F. & Giordano, A. 2020. Role of p53 in the Regulation of Cellular Senescence. *Biomolecules,* 10(3), 420.

Qian, Y. & Chen, X. 2013. Senescence regulation by the p53 protein family. *Methods in Molecular Biology,* 965, 37-61.

Sakaguchi, K., Herrera, J. E., Saito, S. I., Miki, T., Bustin, M., *et al*. 1998. DNA damage activates p53 through a phosphorylation–acetylation cascade. *Genes & Development*, 12(18), 2831-2841.

Slebos, R. J., Lee, M. H., Plunkett, B. S., Kessis, T. D., Williams, B. O., *et al.* 1994. p53-dependent G1 arrest involves pRB-related proteins and is disrupted by the human papillomavirus 16 E7 oncoprotein. *Proc. Natl. Acad. Sci. U.S.A.*, 91(12), 5320-4.

Sullivan, K. D., Galbraith, M. D., Andrysik, Z. & Espinosa, J. M. 2018. Mechanisms of transcriptional regulation by p53. *Cell Death Differ.*, 25(1), 133-143.

Tisato, V., Voltan, R., Gonelli, A., Secchiero, P. & Zauli, G. 2017. MDM2/X inhibitors under clinical evaluation: perspectives for the management of hematological malignancies and pediatric cancer. Journal *of Hematology & Oncology*, 10(1), 133.

Zhang, Y. & Xiong, Y. 2001. Control of p53 ubiquitination and nuclear export by MDM2 and ARF. *Cell Growth Differ.*, 12(4), 175-86.

CRISPR/Cas9 as an *in-vivo* gene editing technology for human genetic disease

Alice Ibbotson

3rd year Genetics BSc

CRISPR/Cas9 is a gene editing technology derived from a bacterial defence mechanism to fight viral infection. It uses a small RNA as a guide to bring the endonuclease Cas9 to a specific location within the genome, where once it has matched the target DNA sequence, Cas9 induces a double strand break (DSB) within the target and the sequence can be altered. This DSB triggers homology directed repair mechanisms (HDR) to correct the mutation using a donor template. This can be used to correct mutations which are responsible for genetic disease. Many studies have been performed to look at the finer details of this mechanism and to improve on the accuracy in an effort to reduce off-target effects. Various methods to get CRISPR/Cas9 inside cells have been devised and these range from physical to viral vector-based delivery methods. The emergence of such a promising gene editing technology has encountered multiple ethical issues with a lot of attention being put on the protest against inheritable germline mutations and the impact that gene editing will have on the process of evolution. It is safe to say that much more work is required to improve precision and reduce off-target effects before CRISPR/Cas9 is used as an *in-vivo* therapeutic for genetic disease in humans, but when it has been approved for this type of use, it will revolutionise the treatment of genetic diseases, improving the lives of many people.

Mechanism of CRISPR/Cas9

Genetic disorders are caused by mutations in an individual's DNA. These mutations can be at the level of the DNA sequence in the form of single nucleotide polymorphisms (SNPs) or at the level of the chromosome. This review will focus on single gene mutations which cause genetic disease in humans and how they can potentially be corrected with gene editing technology using the CRISPR/Cas9 system. This system is derived from the natural CRISPR/Cas system used in bacterial cells to protect themselves from viral infection (Doudna & Charpentier, 2014). CRISPR is an acronym for clustered regularly interspaced short palindromic repeats and Cas9 is a CRISPR-associated endonuclease (Hsu, Lander & Zhang, 2014). The process makes use of a small RNA molecule, around 20 nucleotides in length, called a single guide RNA (sgRNA) to direct the Cas9 protein to the target sequence in the DNA, this interaction is shown in Figure 1. The sgRNA binds to the target DNA sequence through Watson-Crick base pairing where Cas9 can create a double strand break (DSB) in a precise location (Doudna & Charpentier, 2014; Hsu, Lander & Zhang, 2014). The presence of DSBs triggers the action of DNA repair mechanisms using either error-free homology-directed repair (HDR) or the error-prone process of non-homologous end joining (NHEJ) (Hsu & Zhang, 2014; Chu *et al*., 2015). This can be applied to genetic disease by artificially constructing a sgRNA that is complementary to a disease-causing mutation. Using CRISPR/Cas9, the mutation can be corrected with HDR to incorporate a new DNA sequence which contains the wild-type allele, usually from a donor template (Chu *et al*., 2015). The idea of using CRISPR/Cas9 technology in humans to treat genetic diseases has caused many debates concerning the ethical issues. At present, applications of the CRISPR/Cas9 system have only gone as far as using human cells *in-vitro* with a small number being used *in-vivo*. This article will review the literature surrounding the progression to *in-vivo* use of CRISPR/Cas9 in humans, how scientists intend to make it safer and the ethical issues that must be tackled.

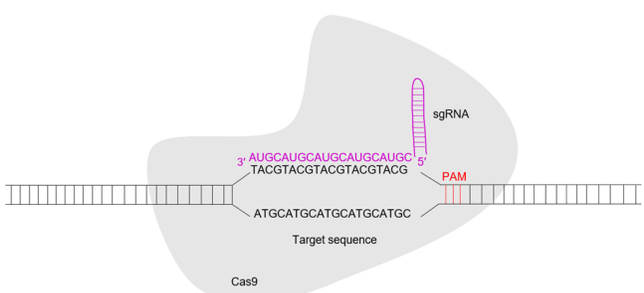


Figure 1. CRISPR/Cas9 System. A simplified diagram of the interaction between the sgRNA, in purple, with the target DNA sequence, in black, and the protospacer adjacent motif, in red, present after the 5′ region of the sgRNA. The Cas9 protein is shown in grey and facilitates this interaction and the DSB which ultimately occurs when there is a match. Adapted from Zhang *et al*., 2015.

How can we get CRISPR/Cas9 into human cells?

There are different methods for delivery of CRISPR/Cas9 into the host cells. The individual components of the process can also be delivered in a range of ways. Both the Cas9 sequence and the sgRNA can be integrated as part of a DNA vector which holds the genetic information for them to be transcribed and, in the case of Cas9, translated. They can also both be delivered as RNA with Cas9 mRNA and sgRNA, or alternatively, in a ribonucleoprotein complex, with Cas9 being delivered as a protein together with the sgRNA (Liu *et al*., 2017; Lino *et al*., 2018). All three options have advantages and disadvantages, and this allows them all to have their specific applications. For instance, using a DNA vector is

more stable than using mRNA but the DNA vector exists longer in the cells than mRNA, potentially allowing for more negative, off-target effects (Liu *et al*., 2017). Arguably, the most efficient method of delivering CRISPR/Cas9 is through the physical process of microinjection (Yang *et al*., 2013). Other delivery methods more suited to *in-vivo* applications include viral vectors, such as adeno-associated virus vectors (AAV), adenovirus or lentivirus vectors, and non-viral vectors, such as lipid nanoparticles (Lino *et al*., 2018). The advantages and disadvantages of each of these methods are outlined in Table 1.

Delivery method	Advantages	Disadvantages
Adeno-associated virus vector	Low toxicity in host. Many serotypes for wide application.	Can integrate into host genome.
Lentivirus vector	Efficient at infecting cells	Can integrate into host genome. Cannot control quantity of
Adenovirus vector	Does not integrate into host genome. Many serotypes for wide application.	High immunogenicity. Cannot control quantity of
Microinjection	Extremely efficient. Reliable to get a known quantity into	Targets one cell at a time. Limited *in-vivo* application.
Lipid nanoparticles	Protects DNA/RNA from degradation. Easy entry to the cell.	Easily broken down by endosome. No direct delivery to the nucleus.

Table 1. A brief description of the advantages and disadvantages of the different delivery methods to introduce CRISPR/Cas9 into host cells. Information was obtained and adapted from (Liu *et al*., 2017; Lino *et al*., 2018).

When using a gene editing technology such as CRISPR/Cas9 to treat genetic disease in humans, it is important to consider methods of targeting the correct cells, especially when the disease is not systemic. For example, junctional epidermolysis bullosa (JEB) is a genetic skin disease that mainly occurs due to mutations in the laminin 332 protein which plays a vital role in adhesion of the epidermis and the dermis through the basement membrane (Benati *et al*., 2018). To treat this disease, the laminin mutations specifically in defective skin cells must be edited, and not the cells in the rest of the body. This is proving to be a considerable obstacle in the process of applying CRISPR/Cas9 systems to targeted cell types both *in-vitro* and *in-vivo*. One promising way to target cells is by adding selective components into the vector construct which cause it to be expressed only in the correct conditions, particularly in the presence of a molecule or mutation which only occurs in the affected cells and not any others. This was demonstrated in the treatment of a fusion oncogene in a human Ewing sarcoma cell line where the CRISPR/Cas9 DSBs are only induced when a detrimental chromosome rearrangement has occurred, bringing two genes into close proximity to form a fusion oncogene (Martinez-Lage *et al*., 2020). This allows for CRISPR/Cas9 targeted gene editing in specific cell types which contain the mutation of interest while remaining inactive in the wild-type cells. This could be applied to the example of JEB where the DNA vector construct only becomes active in keratinocytes that contain a skin-specific promoter.

How can off-target effects be minimised?

One of the main concerns with using CRISPR/Cas9 is the inaccuracy of the sgRNA to bind only to the target sequence and not anywhere outside this region to other similar sequences with high homology. These are known as off-target effects and have been investigated extensively. An additional component of the CRISPR/Cas9 system as yet unmentioned is a short nucleotide sequence which comes after the sgRNA target and is known as a protospacer adjacent motif (PAM) (Zhang *et al*., 2015) which is shown in Figure 1. A well documented Cas9 protein is that of SpCas9, derived from bacterial species Streptococcus pyogenes and the PAM sequence for this variant is NGG, but this sequence varies between each species of Cas9 (Hsu, Lander & Zhang, 2014). It has been proposed that the Cas9 protein first recognises the PAM sequence and then identifies whether or not the preceding sequence matches the sgRNA (Szczelkun *et al*., 2014), indicating that the PAM plays a very important role in CRISPR/Cas9 binding to the correct target sequence. Sequence mis-matches between the sgRNA and the target DNA are better tolerated when they are closer to the PAM, which means any deviations from the 3′ end of the sgRNA could lead to a DSB being made in the wrong place in the genome (Zhang *et al*., 2015).

One method which has been used to reduce the off-target effects of CRISPR/Cas9 is to use a Cas9 protein which induces a single stranded break (SSB) rather than a DSB at the target sequence. These are known as nickase enzymes and would require two to work at the same time, generating a SSB on both the forward and the reverse strands containing the target sequence (Lino *et al*., 2018). Using co-operative nickase forms of Cas9 would still generate the DSB needed for HR to correct the mutation, but any off-target binding, and strand breakage would only cause a SSB which is much less detrimental, and easier to resolve, than off-target DSBs (Lino *et al*., 2018). Using genome databases, it is possible to identify off-target sequences of a sgRNA and predict where they may occur (Zhang *et al*., 2015). In this case, it may be possible one day to determine the consequences of such off-target effects and evaluate the impact they may have on an individual. It is plausible that some off-target effects may be much less drastic than the symptoms of a genetic disease such as severe JEB, and therefore this would possibly alleviate the disease.

Ethical Concerns

The advancement of gene editing technologies has been met with many concerns about the moralities and practicalities surrounding application in humans. One of the major worries is that a vector used to infect the cells with the CRISPR/Cas9 components will incorporate into the host genome and cause adverse effects, including the activation of oncogenes. This is a reasonable concern and viral vectors, such as AAV can have these kind of side effects but it has also been found that adenovirus vectors

do not merge into the host genome (Lino *et al*., 2018) which is one way around this problem. Another major ethical concern is that of progression to producing germline modifications that become inheritable and the view that this is interfering with the process of evolution. For this, it should be noted that there are strict regulations in place in many countries, including the UK, against the use of genetically modified embryos or gametes for any reproductive purposes (Reyes & Lanner, 2017).

Conclusion

In conclusion, CRISPR/Cas9 is a revolutionary gene editing technique with the potential to change the course of treatment for many genetic diseases. Before CRISPR/Cas9 can be introduced to human trials on a wider scale there are improvements that must be made to increase the safety and efficiency of the process. Off-target effects are still too frequent and unpredictable to make this a feasible *in-vivo* technique to treat human genetic disease. Here, some of the methods scientists have developed in an effort to make CRISPR/Cas9 a more effective and precise method of gene editing have been outlined. While already being used in laboratories all over the world for *in-vitro* experiments, once improved, the process of CRISPR/Cas9 is likely to become much more common as part of *in-vivo* gene editing used for therapeutics of genetic disease. If this happens, scientists will have to stick to strict regulations in order to make sure the applications remain controlled without any disastrous consequences.

References

Benati, D., Miselli, F., Cocchiarella, F., *et al.* 2018. CRISPR/Cas9-Mediated *In Situ* Correction of LAMB3 Gene in Keratinocytes Derived from a Junctional Epidermolysis Bullosa Patient. *Molecular Therapy*, 26(11), 2592-2603.

Chu, V. T., Weber, T., Wefers, B., *et al.* 2015. Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells. *Nature Biotechnology*, 33, 543-548.

Doudna, J. & Charpentier, E. 2014. The new frontier of genome engineering with CRISPR-Cas9. *Science,* 346(6213), 1077.

Hsu, P., Lander, E. S., & Zhang, F. 2014. Development and applications of CRISPR-Cas9 for genome engineering. *Cell,* 157(6), 1262-1278.

Lino, C., Harper, J. C., Carney, J. P. & Timlin, J. A. 2018. Delivering CRISPR: a review of the challenges and approaches. *Drug Delivery*, 25 (1), 1234-1257.

Liu, C., Zhang, L., Liu, H. & Cheng, K. 2017. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. *Journal of Controlled Release*, 266, 17-26.

Martinez-Lage, M., Torres_Ruiz, R., Puig-Serra, P., *et al.* 2020. *In vivo* CRISPR/Cas9 targeting of fusion oncogenes for selective elimination of cancer cells. *Nature Communications*, 11.

Plaza Reyes, A. & Lanner, F. 2017. Towards a CRISPR view of early human development: applications, limitations and ethical concerns of genome editing in human embryos. *Development*, 144(1), 3-7.

Szczelkun, M. D., Tikhomirova, M. S., Sinkunas, T. *et al.* 2014. Direct observation of R-loop formation by single RNA-guided Cas9 and Cascade effector complexes. *PNAS*, 111(27), 9798-9803.

Yang, H., Want, H., Shivalila, C. S., *et al.* 2013. One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. *Cell*, 154(6), 1370-1379.

Zhang, X.-H., Tee, L. Y., Wang, X.-G., Huang, Q.-S. & Yang, S.-H. 2015. Off -target Effects in CRISPR/Cas9-mediated Genome Engineering. *Molecular Therapy Nucleic Acids*, 4(11), e254.

Foetal Alcohol Spectrum Disorders and their Prevalence in the North West

Daniel Byrne

2nd year Biochemistry BSc

Foetal Alcohol Spectrum Disorders (FASDs) are a range of disorders associated with the consumption of alcohol during pregnancy and its impact on a foetus (National Organisation on Foetal Alcohol Syndrome UK, 2009). The prevalence of these disorders is difficult to measure due to the ambiguity of the disorders that are associated with FASDs. This spectrum of disorders may well affect large proportions of the population as only since 2016, UK government guidelines on drinking during pregnancy have encouraged women to abstain completely from alcohol where previously advice limited consumption to 1-2 units up to twice a week (Schölin *et al.,* 2019). The broad nature of symptoms of the disease and lack of diagnosis mechanism mean that FASDs remain a huge problem in society.

What are FASDs?

FASDs are a variety of disorders associated with the consumption of alcohol during pregnancy. It has been identified by the British Medical Association as the most common, non-genetic cause of learning disability in the UK (British Medical Association, 2007), causing significant concern over its preventability through education. Alcohol is normally processed in the body by two enzymes, alcohol dehydrogenase and cytochrome P450 2E1 (CYP2E1) (Heit *et al.,* 2013). In an adult, the enzymes would process the metabolism of alcohol, converting it to acetaldehyde for removal from the body. CYP2E1 is not present in a foetus' liver until 19, 23 and 24 weeks gestation, meaning there is a build-up of alcohol within the foetus due to a lack of action from the enzymes (Burd *et al.,* 2012). The presence of alcohol within a foetus can be detrimental, acting as an oxidative stress inducer and bringing about the destruction of cell membranes and mitochondria, whilst also interfering with cell signalling involved in development of the foetus (Gupta *et al.,* 2016). Not only this, the presence of alcohol can also alter expression of certain genes through DNA methylation and histone tail modification, and these changes to DNA can be carried for generations (Sarkar, 2016). Changes to DNA and cell death during development can be detrimental to a cell, and the random nature of DNA methylation and this cell destruction brought about by alcohol suggests why the symptoms of the disorder are so broad. Figure 1 demonstrates the changes to cellular mechanisms brought about by alcohol exposure. The effects of the aforementioned consequences of alcohol presence during development include physical defects, neural problems and behavioural issues- these include, but are not limited to, deformities of joints, intellectual disability and poor social skills respectively (Mayo Clinic, 2018).

Diagnosis and Treatment

The symptoms of FASDs are broad and lend themselves to improper diagnosis. Symptoms such as intellectual disability and poor social skills are something that cannot be assessed until the child has developed sufficiently to display such symptoms. Diagnosis of FAS at birth therefore requires three facial abnormalities, documentation of growth deficits and documentation of central nervous system abnormalities (National Organisation on Foetal Alcohol Syndrome, 2020). We are yet to discover a universal marker for FASDs, whether that be a genetic loci, or a biomarker produced only in individuals with the disorder. Despite the information we have in regards to how alcohol brings about cell death and genetic changes, we have very little understanding in regards to how to identify those with the disorder, and also how to treat them. Further study into biomarkers that are present solely in FASD individuals or understanding how alterations to physiological pathways may cause changes to similar genetic loci may bring about further investigation into treatment as a result. Investigations have been conducted into the administration of antioxidants *in vitro*, however a lack of action was seen *in vivo* due to the global and random nature of damage, meaning there was a lack of antioxidant present at the site of damage (Ehrhart *et al.,* 2018). The gaps in our understanding of how alcohol interferes with complex physiological pathways such as methylation throughout the body prevents us from finding a diagnosis method or treatment of the disease as a result.

FASD and Liverpool

The study of FASDs by region is difficult as it requires study into drinking habits and socio-economic status to provide context. Alcohol abuse remains a huge problem in Liverpool, with the city being top of the list in regards to female hospital admissions for alcohol-related abuse (BBC, 2011). We can look at a series of studies conducted by the Office for National Statistics (ONS) to provide context for the raised hospital admissions in relation to the rest of the country. A study that identified the degree of alcohol consumption in regions of the UK showed that the North West had the highest alcohol consumption in a week and heaviest drinking day, when 7100 individuals were asked a series of questions about their drinking habits (number of units of alcohol consumed) outside of the South East (John, 2017). Another study conducted into socio-economic status of participants and their drinking status found that economically inactive women (unemployed and not seeking work or enrolled as

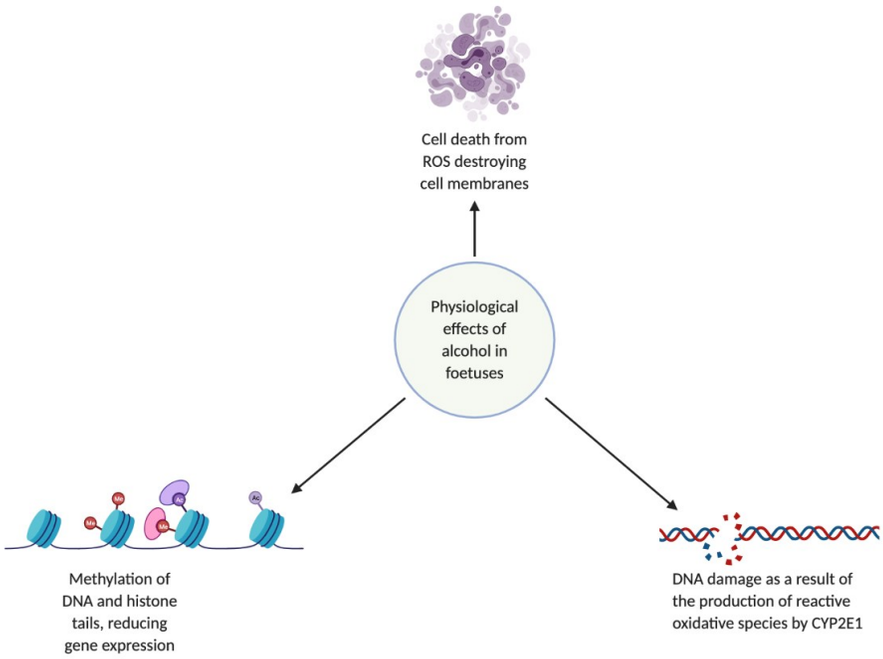


Figure 1. The effects of alcohol on cellular mechanisms in a foetus. The lack of breakdown of alcohol in the foetus allows the breakdown of DNA through the oxidative nature of the molecule. This also brings about the degradation of the cell membrane, thus resulting in apoptosis. Alcohol presence also encourages methylation, which prevents key genes from being transcribed and so bringing about the side effects commonly seen with FASDs. Created with BioRender.com

students) were the most likely to exceed 9 units of alcohol on their heaviest drinking day, and most likely to drink on 5 or more days in a week (Watson, 2018). The North West had the highest inactivity rate during the period of the study, at 23.1% (Watson, 2018). A global study into the prevalence of alcohol consumption during pregnancy places the UK second in the world, with 41% of pregnant women consuming some alcohol during their pregnancy (Popova *et al.,* 2017). These data suggest that the North West could well be one of the worst affected areas in the UK in regards to FASDs, however we cannot be sure of such a conclusion due to the lack of diagnosis methods for the disorder. Knowing why 41% of mothers in the UK consume alcohol during pregnancy despite the risk to their foetus is something we cannot for certain answer. A briefing from the National Institute for Health and Care Excellence (NICE), looking to better our treatment of FASDs in the UK, sought to better educate all women of child-bearing age, as they felt that guidance was ambiguous and misleading (NICE, 2019). Ensuring that mothers understand that any alcohol can have serious effects on their foetus is vital in ensuring that whenever we can quantitatively identify the prevalence of FASDs, Liverpool is not top of the global list.

References

British Broadcasting Corporation. 2011. *Liverpool and Blackpool top alcohol problem lists*. Available: https://www.bbc.co.uk/news/uk-england-14652900. (Accessed 21st Dec 2020).

British Medical Association. 2007. *Fetal alcohol spectrum disorders: a guide for healthcare professionals*. Available: http://bmaopac.hosted.exlibrisgroup.com/exlibris/aleph/a23_1/apache_media/3UQ4QIHNR25DH7623BMFDY45UIK7LH.pdf. (accessed 23rd Dec 2020).

Burd, L., Blair, J. & Dropps, K. 2012. Prenatal alcohol exposure, blood alcohol concentrations and alcohol elimination rates for the mother, fetus and newborn. *Journal of Perinatology*, 32, 652-659.

Ehrhart, F., Roozen, S., Verbeek, J., *et al.* 2019. Review and gap analysis: molecular pathways leading to fetal alcohol spectrum disorders. *Molecular Psychiatry*, 24, 10-17.

Gupta, K., Gupta, V. K. & Shirasaka, T. 2016. An Update on Fetal Alcohol Syndrome-Pathogenesis, Risks, and Treatment. *Alcoholism, Clinical and Experimental Research*, 40(8), 1594-1602.

Heit, C., Dong, H., Chen, Y., *et al.* 2013. The role of CYP2E1 in alcohol metabolism and sensitivity in the central nervous system. *Subcellular Biochemistry*, 67, 235-247.

John, E. 2017. *Adult drinking habits in Great Britain*. Available: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/drugusealcoholandsmoking/datasets/adultdrinkinghabits. (Accessed 23rd Dec 2020).

Mayo Clinic Staff. 2018. *Fetal alcohol syndrome*. Available: https://www.mayoclinic.org/diseases-conditions/fetal-alcohol-syndrome/symptoms-causes/syc-20352901. (Accessed 21st Dec 2020).

National Institute for Health Care and Excellence. 2019. *Health and Social Care Directorate*. Available: https://www.nice.org.uk/guidance/gid-qs10139/documents/briefing-paper. (Accessed 28th Jan 2021).

National Organization on Fetal Alcohol Syndrome. 2020. *Recognizing FASD*. Available: https://nofas.org/recognizing-fasd/. (Accessed 28th Dec 2020).

Popova, S., Lange, S., Probst, C., Gmel, G. & Rehm, J. 2017. Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: a systematic review and meta-analysis. *The Lancet Global Health*, 5(3), 290-299.

Sarkar, D. K. 2016. Male germline transmits fetal alcohol epigenetic marks for multiple generations: a review. *Addiction Biology*, 21(1), 23-34.

Schölin, L., Watson, J., Dyson, J. & Smith, L. 2019. *Alcohol Guidelines for Pregnant Women*. Available: https://www.ias.org.uk/uploads/pdf/IAS%20reports/rp37092019.pdf. (Accessed 19th Apr 2021).

Watson, B. 2018. *Regional labour market statistics in the UK: April 2018*. Available: https://www.ons.gov.uk/employmentandlabourmarket/peopleinwork/employmentandemployeetypes/bulletins/regionallabourmarket/april2018. (Accessed 23rd Dec 2020).

Is LAMB3 a Therapeutic Target for Squamous-Cell Carcinoma?

Ziyu Fang

3rd year Genetics BSc

Squamous-cell carcinoma (SCC) is the most common cancer capable of metastasis with high morbidity and mortality, and can cause head and neck, lung, gastric and papillary thyroid cancers, among others. However, existing therapies show limited effects for patients. Laminin subunit beta-3 (LAMB3), which encodes one of the three subunits of Laminin-332, plays a crucial role in tumorigenesis and progression of several types of cancer. This review aims to examine the evidence for a role of LAMB3 in SCC as well as its potential to be a therapeutic target for SCC, and then puts forward several possible anticancer therapies using LAMB3 as target. It was reported that LAMB3 is upregulated in tumour tissues, which is associated with lymph metastasis. LAMB3 could promote cell metastasis ability by regulating EMT-related proteins, MMPs and PI3K/Akt signalling pathway, and thus contributing to tumour progression. LAMB3 could also promote cell proliferation by altering the cell cycle distribution and affecting apoptosis in some cases but there also exist some cases where LAMB3 had no effect on cell proliferation, which requires further investigation. Therefore, LAMB3 is a promising diagnostic marker as well as a potential therapeutic target for SCC. Regulating LAMB3 by targeting at relative regulatory miRNAs, inducing epigenetic modification such as DNA or histone methylation, and CRISPR/dCas9 editing tool would all be possible anticancer therapies. Henceforth, targeting LAMB3 could provide improved therapies for SCC patients with a better clinical response.

Introduction

Squamous-cell carcinoma (SCC) is a highly prevalent invasive malignant neoplasm with high morbidity and mortality. SCC, which is recognized as the most common cancer capable of metastasis, could arise in various tissues and give rise to a wide range of cancers (Marinkovich, 2007). SCC tumours can often invade adjacent tissues and metastasize to distant sites such as lymph nodes (Tran *et al.,* 2008). So far, chemotherapy for SCC has shown limited effects for patients and the mortality rate of SCC is still high despite existing therapies (Marinkovich, 2007). Hence, searching for an efficient therapeutic target for treating SCC is necessary and beneficial.

Laminins, which are crucial components of basement membrane zones, are large extracellular glycoproteins associated with a variety of biological processes including cell migration, adhesion, proliferation, and interactions with other extracellular matrix components (Patarroyo *et al.,* 2002; Jung *et al.,* 2018). Laminin-332 (formerly termed laminin-5), one of the laminin isoforms, consists of three subunits, α3, β3 and γ2, which are coded by LAMA3, LAMB3 and LAMC2 genes respectively (Benati *et al.,* 2018; Fortugno *et al.,* 2020) (Figure 1). Laminin subunit beta-3 (LAMB3) plays a crucial role in tumorigenesis and progression of several types of cancer such as head and neck squamous cell carcinoma (HNSCC), pancreatic ductal adenocarcinoma (PDAC), papillary thyroid cancer (PTC), gastric cancer and lung cancer (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018; Huang *et al.,* 2020; Wang *et al.,* 2017; Kwon *et al.,* 2011; Wang *et al.,* 2013). This review aims to examine the evidence for a role of LAMB3 in SCC to determine if it holds potential as a therapeutic target.

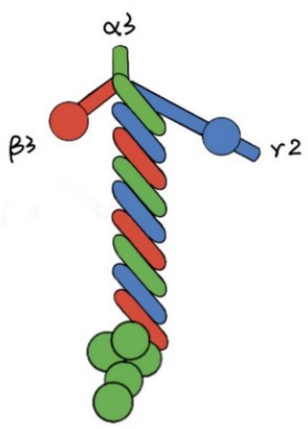


Figure 1. Laminin-332 structure with the β3 subunits in red.

LAMB3 is Positively Correlated with Tumorigenesis

Many studies performed experiments to determine the LAMB3 expression in SCC and they all showed the higher LAMB3 expression in tumour tissues than that in unaffected tissues at both mRNA and protein levels (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018; Huang *et al.,* 2020; Kwon *et al.,* 2011; Wang *et al.,* 2013). Expression levels of LAMB3 can be used to differentiate tumour tissues and unaffected ones (Wang *et al.,* 2017) and thus it could be recognized as a promising biomarker for SCC. Through analysing the association of LAMB3 expression and clinicopathological features, higher LAMB3 expression is associated with increasing risk of lymph nodes metastasis (Liu *et al.,* 2019; Wang *et al.,* 2017; Wang *et al.,* 2013). Lymph nodes metastasis is highly correlated to the poor prognosis including local recurrence and cancer-specific mortality preoperatively (Guo *et al.,* 2020; Xing *et al.,* 2020). This poor prognosis, which is also correlated with the overexpression of LAMB3, involves many types of cancer such as NHSCC, PDAC and PTC (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018).

Taken together, LAMB3 could be a prognostic biomarker for certain cancer types. Therefore, this promoted the question about whether LAMB3 contributes to tumour progression.

Controversy on the Effect LAMB3 Has on Cell Proliferation

Previously it was found that LAMB3 could promote cell proliferation in PDAC, PTC and gastric cancer cells (Kwon *et al.,* 2011; Huang *et al.,* 2020; Zhang *et al.,* 2019; Wang *et al.,* 2017). In these cases, among cells with higher expression of LAMB3 compared with unaffected cells, a larger proportion of them would be in G2/M phase while a smaller proportion would be in G1 phase. LAMB3 overexpression could lead to the upregulation of both cyclin D and BCL-2, and downregulation of p53, while its knockdown can induce the opposite effect. Cyclin D could promote the progression of cell cycle, BCL-2 plays a role in inhibiting apoptosis, and p53 could induce cell cycle arrest and promote apoptosis (Zhang *et al.,* 2019). Hence, LAMB3 can promote cell proliferation by altering the cell cycle distribution and reducing the number of early apoptotic cells (Zhang *et al.,* 2019; Huang *et al.,* 2020). However, this is not universally the case, there have been some reports where LAMB3 had no significant effect on cell proliferation in HNSCC and PTC (Liu *et al.,* 2018; Jung *et al.,* 2018). Especially, two previous studies from 2017 and 2018 respectively held contrary conclusions on the effect that LAMB3 has on cell proliferation in PTC (Wang *et al.,* 2017; Jung *et al.,* 2018). Thus, LAMB3 may promote cell proliferation in some tumour subtypes but not in other subtypes. However, no certain conclusion can be drawn on it at present and more relative studies are required.

LAMB3 Contributes to Tumour Progression in Multiple Ways

It is commonly accepted that in several cancer types LAMB3 could promote cell invasion and migration, which are two key steps of metastasis (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018; Wang *et al.,* 2017; Kwon *et al.,* 2011; Wang *et al.,* 2013). Metastasis is a crucial step in tumour progression and could give rise to related death and treatment failure (Bray *et al.,* 2018). One way that LAMB3 contributes to tumour progression is by regulating epithelial-to-mesenchymal transition (EMT)-related proteins expression (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018; Fukazawa *et al.,* 2015). EMT is associated with cell invasion in tumour progression (Jung *et al.,* 2018). During the EMT, epithelial cells lose cell-cell adhesion and then are transited to mesenchymal cells, which allows the tumour cells to invade adjacent tissues and metastasize to distant sites (Fukazawa *et al.,* 2015). Knockdown of LAMB3 has been shown to induce the upregulation of E-cadherin and downregulation of N-cadherin, vimentin and Slug (Zhang *et al.,* 2019; Liu *et al.,* 2018), where E-cadherin, N-cadherin, Slug and vimentin are well-known EMT-related proteins (Jung *et al.,* 2018). The opposite effect is observed when LAMB3 is upregulated, indicating the role of LAMB3 in regulating EMT-related proteins expression.

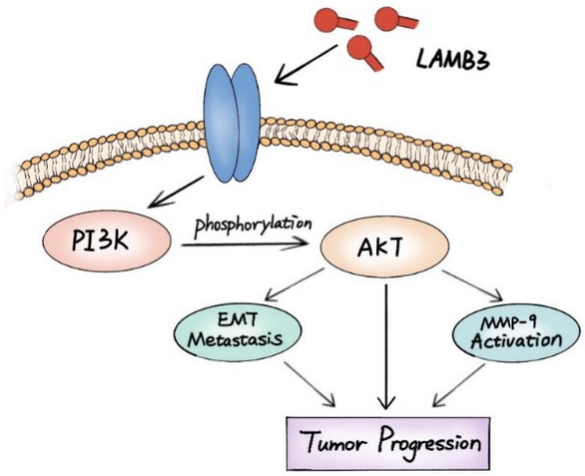


Figure 2. Mechanisms underlying the PI3K/Akt signalling pathway.

Regulating the matrix metalloproteinases (MMP) could also contribute to tumour progression by LAMB3 (Zhang *et al.,* 2019; Jung *et al.,* 2018; Kwon *et al.,* 2011; Wang *et al.,* 2013; Udayakumar *et al.,* 2003; Remy *et al.,* 2006). In order to invade the adjacent tissues and migrate to distant sites, the cancer cells have to degrade the basement membrane (BM) which acts like a barrier against cell metastasis. Normally this degradation of BM is processed via the MMP family including MT1-MMP (MMP-14), MMP-7, MMP-2 and MMP-9 (Remy *et al.,* 2006). MT1-MMP (MMP-14), which is important for the initiation of BM breakdown, is also found to activate MMP-2, which is another metastasis-related protein. LAMB3 chain is a target for MT1-MMP to cleave, thus promoting cell metastasis (Udayakumar *et al.,* 2003). Likewise, LAMB3 chain acts as a specific ligand for MMP-7, facilitating the cleavage processed by MMP-7 and enhancing cell metastasis. MMP-7, which is mainly expressed in cells of carcinomas, plays an important role in BM degradation and is associated with distant and lymph node metastasis (Remy *et al.,* 2006). In 2018, a study on PTC reported that pro-MMP-9 mRNA expression and pro-MMP-9 secretion were significantly inhibited by LAMB3 knockdown where MMP-9 is integral to cell metastasis (Jung *et al.,* 2018). Taken together, LAMB3 contributes to tumour progression by associating with MMPs.

Moreover, LAMB3 could also regulate the PI3K/Akt signalling pathway to promote tumour progression (Zhang *et al.,* 2019; Jung *et al.,* 2018; Huang *et al.,* 2020; Wang *et al.,* 2017). This pathway involves phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB/Akt), having a fundamental role in tumour progression. The activation of PI3K can mediate the phosphorylation of Akt, regulating cell invasion and migration (Xu *et al.,* 2018), The knockdown of LAMB3 could inhibit the transcription and activation of PI3K, leading to significant reduction in Akt phosphorylation, and thus giving rise to the suppression of cell invasion and migration (Zhang *et al.,* 2019). Hence, LAMB3 promotes the tumour progression by activating the PI3K-mediated Akt phosphorylation. This activation of the pathway also induces EMT and activates MMP-9, contributing to cell metastasis (Jung *et al.,* 2018) (Figure 2).

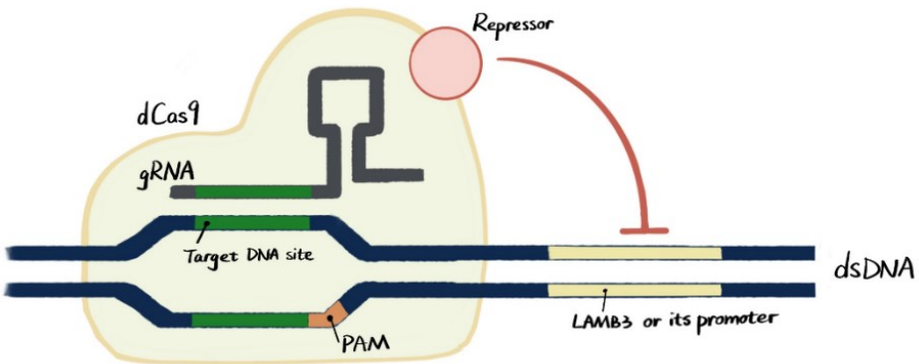


Figure 3. The mechanism of CRISPR/dCas9 gene editing tool.

Is LAMB3 a Potential Therapeutic Target for SCC?

LAMB3 was reported to be a potential diagnostic marker (Wang *et al.,* 2017), and several studies had mentioned about regulating LAMB3 as a novel therapeutic strategy (Huang *et al.,* 2020; Kwon *et al.,* 2011; Benati *et al.,* 2018), representing its potential to be a therapeutic target for SCC. One possible way is regulating the expression of LAMB3 by targeting the relative regulatory miRNAs. As most mammalian mRNAs can be conserved targets of miRNAs, finding the miRNA that could regulate LAMB3 wound be a possible approach (Friedman *et al.,* 2009). Huang *et al.,* (2020) found miR-24-3p that is an upstream miRNA of LAMB3 and could directly target the 3'UTR of the mRNA of LAMB3. And it was further shown that the miR-24-3p/LAMB3 axis could be a target for an anticancer therapeutic strategy (Huang *et al.,* 2020). Likewise, there may exist other relative miRNA could target and regulate the LAMB3 expression.

Histone methylation and DNA methylation could be another way to regulate the expression of LAMB3 given that epigenetic modification is a crucial mechanism for regulating gene expression. Kwon *et al.,* (2011) reported a strong correlation between LAMB3 expression and DNA methylation as well as histone methylation. Both promoter demethylaion and active H3K4me3 mark results in upregulation of LAMB3 (Kwon *et al.,* 2011). This previous study showed the possibility of regulating the LAMB3 expression by epigenetic mechanism. Thus, inducing the hypermethylation of LAMB3 gene promoter could be a possible way to downregulate LAMB3 expression.

LAMB3 could also be regulated by CRISPR system. CRISPR systems, which have been applied successfully in mammalian cells, can not only conduct the genome engineering with Cas9 nuclease, but also localize the target gene with dead Cas9 (dCas9) nuclease (Xiong *et al.,* 2020; Moses *et al.,* 2020). The dCas9 can be fused with the repressor domain and directed to the target DNA site guided by gRNA, thus repressing the gene expression (Moses *et al.,* 2020) (Figure 3). Likewise, dCas9 may be fused with protein that is associated with epigenetic modification, regulating LAMB3 expression via epigenetic modification such as histone methylation.

Conclusion

LAMB3 is upregulated in tumour tissues, which is associated with lymph metastasis. However, its role in cell proliferation requires further investigation as it could alter the cell cycle distribution and reduce the number of early apoptotic cells in some cases but not in other cases. LAMB3 could also promote cell invasion and migration ability and thus contribute to tumour progression by regulating EMT-related proteins, MMPs and PI3K/Akt signaling pathway. Therefore, LAMB3 is a promising diagnostic marker as well as a potential therapeutic target for SCC. Regulating LAMB3 by targeting at relative regulatory miRNAs, inducing epigenetic modification such as DNA or histone methylation, and CRISPR/dCas9 editing tool would all be possible anticancer therapies. Hence, LAMB3 is a potential therapeutic target for SCC and targeting it could provide improved therapies for SCC patients with a better clinical response.

References

Benati, D., Miselli, F., Cocchiarella, F., *et al.* 2018. CRISPR/Cas9-Mediated In Situ Correction of LAMB3 Gene in Keratinocytes Derived from a Junctional Epidermolysis Bullosa Patient. *Molecular Therapy*, 26(11), 2592-2603.

Bray, F., Ferlay, J., Soerjomataram, I., *et al.* 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.,* 68(6), 394-424.

Fortugno, P., Condorelli, A. G., Dellambra, E., *et al.* 2020. Multiple Skin Squamous Cell Carcinomas in Junctional Epidermolysis Bullosa Due to Altered Laminin-332 Function. *International Journal of Molecular Sciences,* 21(4), 1-12.

Friedman, R. C., Farh, K. K. H., Burge, C. B. & Bartel, D. P. 2009. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research,* 19(1), 92-105.

Fukazawa, S., Shinto, E., Tsuda, H., *et al.* 2015. Laminin β3 expression as a prognostic factor and a predictive marker of chemoresistance in colorectal cancer. *Japanese Journal of Clinical Oncology,* 45(6), 533-540.

Guo, K., Feng, Y., Yuan, L., *et al.* 2020. Risk factors and predictors of lymph nodes metastasis and distant metastasis in newly diagnosed T1 colorectal cancer. *Cancer Medicine*, 9(14), 5095-5113.

Huang, W., Gu, J., Tao, T., *et al.* 2020. MiR-24-3p Inhibits the Progression of Pancreatic Ductal Adenocarcinoma Through LAMB3 Downregulation. *Frontiers in Oncology*, 9(1499), 1-12.

Jung, S.-N., Lim, H. S., Liu, L., *et al.* 2018. LAMB3 mediates metastatic tumor behavior in papillary thyroid cancer by regulating c-MET/Akt signals. *Scientific Reports*, 8(1), 1-10.

Kwon, O. H., Park, J. L., Kim, M., *et al.* 2011. Aberrant up-regulation of LAMB3 and LAMC2 by promoter demethylation in gastric cancer.

Biochemical and Biophysical Research Communications, 406(4), 539-545.

Liu, L., Jung, S.-N., Oh, C., *et al.* 2019. LAMB3 is associated with disease progression and cisplatin cytotoxic sensitivity in head and neck squamous cell carcinoma. *European Journal of Surgical Oncology*, 45 (3), 359-365.

Marinkovich, M. P. 2007. Tumour microenvironment: laminin 332 in squamous-cell carcinoma. *Nature Reviews Cancer*, 7(5), 370-380.

Moses, C., Hodgetts, S. I., Ben-Ary, G., *et al.* 2020. Transcriptional repression of PTEN in neural cells using CRISPR/dCas9 epigenetic editing. *Scientific Reports*, 10(1), 1-16.

Patarroyo, M., Tryggvason, K. & Virtanen, I. 2002. Laminin isoforms in tumor invasion, angiogenesis and metastasis. *Seminars in Cancer Biology*, 12(3), 197-207.

Remy, L., Trespeuch, C., Bachy, S., Scoazec, J. Y. & Rousselle, P. 2006. Matrilysin 1 Influences Colon Carcinoma Cell Migration by Cleavage of the Laminin-5 beta3 Chain. *Cancer Research*, 66(23), 11228-11237.

Tran, M., Rousselle, P., Nokelainen, P., *et al.* 2008. Targeting a Tumor-Specific Laminin Domain Critical for Human Carcinogenesis. *Cancer Research,* 68(8), 2885-2894.

Udayakumar, T. S., Chen, M. L., Bair, E. L., *et al.* 2003. Membrane Type-1-Matrix Metalloproteinase Expressed by Prostate Carcinoma Cells Cleaves Human Laminin-5 beta3 Chain and Induces Cell Migration. *Cancer Research*, 63(9), 2292-2299.

Wang, X.-M., Li, J., Yan, M.-X., *et al.* 2013. Integrative Analyses Identify Osteopontin, LAMB3 and ITGB1 as Critical Pro-Metastatic Genes for Lung Cancer. *PLoS ONE*, 8(2), 1-11.

Wang, Y., Jin, Y., Bhandari, A., *et al.* 2017. Upregulated LAMB3 increases proliferation and metastasis in thyroid cancer. *OncoTargets and Therapy*, 11, 37-46.

Xing, Z., Qiu, Y., Yang, Q., *et al.* 2020. Thyroid cancer neck lymph nodes metastasis: Meta-analysis of US and CT diagnosis. *European Journal of Radiology*, 129, 1-9.

Xiong, Z. Q., Wei, Y. Y., Kong, L. H., *et al.* 2020. Short communication: An inducible CRISPR/dCas9 gene repression system in *Lactococcus lactis. Journal of Dairy Science*, 103(1), 161-165.

Xu, J., Gong, L., Qian, Z., Song, G. & Liu, J. 2018. ERBB4 promotes the proliferation of gastric cancer cells via the PI3K/Akt signaling pathway. *Oncology Reports*, 39(6), 2892-2898.

Zhang, H., Pan, Y.-Z., Cheung, M., *et al.* 2019. LAMB3 mediates apoptotic, proliferative, invasive, and metastatic behaviors in pancreatic cancer by regulating the PI3K/Akt signaling pathway. *Cell Death and Disease*, 10 (3), 230.

The COVID Crossroads:

How a Needle in Your Arm Will Put a Glass in Your Hand

Riley McMahon

3rd year Biochemistry BSc

Introduction

No event in recent memory has devastated the global community so completely as the COVID-19 pandemic. What began in December 2019 as an unexplained outbreak of pneumonia in Wuhan City, China, spread and before it was able to be stopped ravaged almost every facet of our society, leading to the death of 1.9 million before its second Christmas (WHO, 2021). It can be comforting in a way to characterise the disease as an evil force, an invisible killer which targets especially the elderly and the infirm. This mystification however, only serves to grant the virus undue power, which must be treated through education.

The COVID-19 disease is caused by a virus called SARS-CoV-2, shown in Figure 1. The most important component of the virus is its RNA. Much like the DNA within you, the RNA in a virus acts as a genetic blueprint, containing all the information necessary for function, divided into sections called genes. Each gene contains the information for a different characteristic, in humans, eye colour and height for example and in SARS-CoV-2 one crucial gene will instruct the cell to create spike proteins.

Spike proteins are the second component of vital importance. They extend out from the spherical surface of the viruses and each one is tipped with a region called Receptor Binding Domain (RBD) which matches a specific region on human cells called ACE2, like a key to a lock (Krammer, 2020(a)).

The combination of these two parts are what make infection by SARS-CoV-2 such a deadly prospect. The virus is incredibly small, 100,000 of them laid side by side would only span a centimetre. They can, as a result be inhaled unknowingly and once inside the body, latch onto healthy human cells using the spike protein and its RBD. Through this bond, the virus can insert its RNA into the human cell, attacking it and forcing it to act as a factory, producing more copies of the virus.

The Solution

Being the product of roughly six million years of evolution, the human body has defences in place for situations like this, namely antibodies. Antibodies are small proteins created by the body in order to defend against invaders (Krammer, 2020(b)). When created, they are specific to the invader and as such can intercept and bind to them before they reach the human cells, as shown in Figure 2.

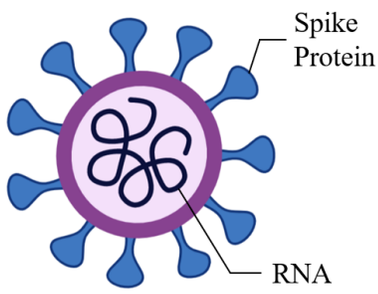


Figure 1. A Model of the SARS-Cov-2 Virus.

There is a problem however, the first time the body is exposed to an invader, the process to create these antibodies is time consuming and by the time the body has completed it, it may be too late to fight off the infection. However, after this initial exposure, the body retains memory cells which will recognize the same invader in future, meaning the specific antibodies can be created immediately. This is why vaccination is so crucial and why the recent breakthroughs hold so much promise for the future. At its core, vaccination exposes the body to harmful elements, such as the spike protein, and gives the body time to develop these memory cells, which in turn will quickly release specific antibodies in the event of an infection (Birney, 2020).

Approximately 140 vaccines are currently in development, which fall under four different methods (Callaway, 2020). This is the crossroads in the scientific community at the moment, as to the most effective way of navigating the crisis.

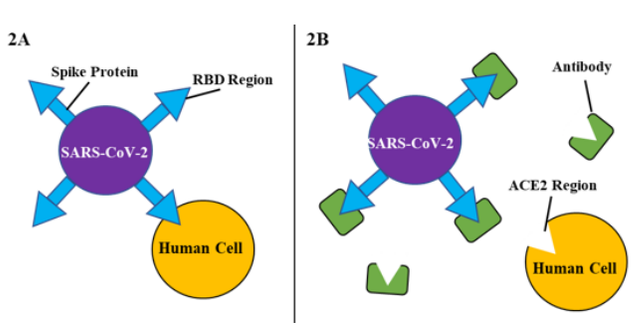


Figure 2. A model to show the impact and function of antibodies. In 2A, an example of a person without antibodies, the RBD can interact with ACE2 unobstructed, leading to infection. In 2B, the antibodies present in a person are able to bind to the RBD, which protects the human cell.

Virus Vaccines

This is the oldest method of vaccine production, used throughout history. For the elimination of polio and measles as death sentences, you have virus vaccines to thank. It is the simplest of the methods, less popular than its more modern counterparts but highly effective nonetheless. To create this vaccine, a virus in its natural state is taken, grown in favourable conditions and concentrated to produce a large amount of pure virus. It is then treated, either with heat or chemicals such as formaldehyde in order to harm the genetic information within the virus (Krammer, 2020(a)). Once treated, the surface is unchanged but the RNA has been damaged, making it unable to enter and infect human cells. It can now be delivered to a patient, who will develop antibodies without becoming infected.

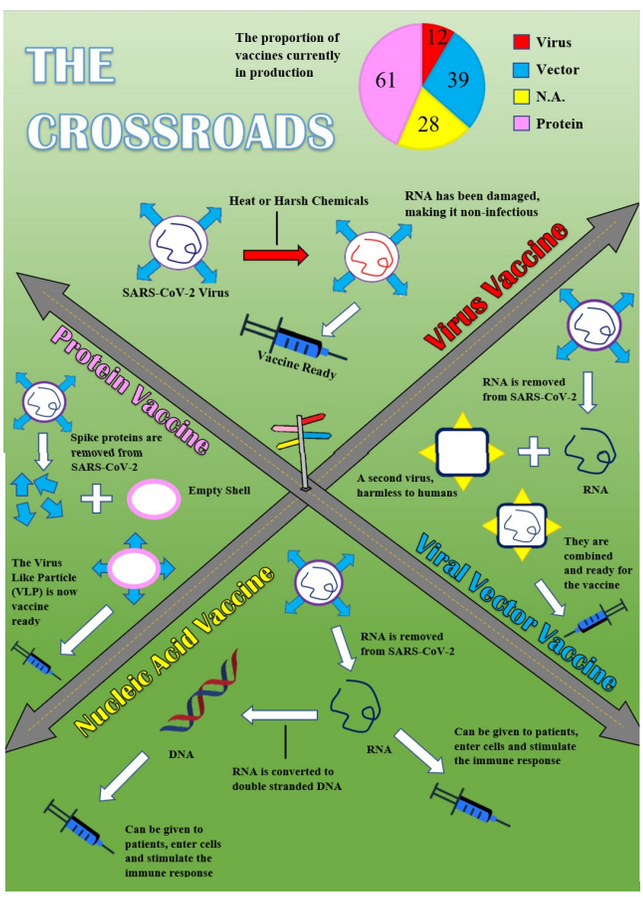


Figure 3. An infographic summarising the four most popular methods of SARS-CoV-2 vaccine production. Pie chart adapted from Callaway, 2020.

Viral Vector Vaccines

The second method available to researchers is the viral vector, which is less complex than it initially sounds. In this method, scientists take the gene in the viral RNA responsible for creating the spike proteins, and insert it into a different species of virus, which does not cause disease in humans. This second virus acts as a way of transporting this gene into human cells, which will read the gene and create a spike protein on its surface (Callaway, 2020). This will trigger the immune response and allow the specific antibodies to develop, whilst being harmless to the patient. One potential disadvantage is that a patient may be immune to this second virus and in that case, the treatment would be ineffective.

Nucleic Acid Vaccines

Nucleic acid is the NA in DNA and RNA, which codes for proteins and instructs the cell. This method is similar to the previous, opting to make human cells present the spike protein. It does this by taking a strand of RNA, with a gene which codes for the spike protein and implanting it into a patient. Alternatively, it can be converted into double stranded DNA before being given to the patient, before using a process called electroporation, which shocks the cell and creates holes the DNA can enter through (Khanh *et al.,* 2020). Once inside, cell machinery will read the gene and create the spike protein it codes for, presenting it on the surface and allowing antibodies to develop.

Protein based

It's a well-worn phrase that the simplest explanation is most often correct, whilst this may not be true in all cases, it is supported by the fourth and final method. The problem at its core is that we need to expose the human body to the spike protein in order for antibodies to develop and this fourth method does just that. Researchers take several of these spike proteins and attach them to an artificial empty shell, containing no genetic information (Krammer, 2020 (a)). This will result in a Virus-Like Particle (VLP). When given to a patient, the immune response will create antibodies and there is no risk of infection as the VLP contains no genetic material at all. A disadvantage of this method is it would require several treatments for long term immunity.

Conclusion

In conclusion, these are the four main vaccines currently being tested by researchers and each one represents a different potential route out of the COVID crisis, as shown in Figure 3. Whilst they use contrasting methods, they serve the same goal, returning us to normality, hopefully sooner rather than later.

References

Birney, E. 2020. SARS-CoV-2 vaccine tweetorial. Available from: https://twitter.com/ewanbirney/status/1327179483137404929 [Accessed 10th January 2021]

Callaway, E. 2020. The race for coronavirus vaccines: a graphical guide. *Nature,* 580, 576-577.

Khanh, T., Paris, C., Khan, K., *et al.* 2020. Nucleic Acid-Based Technologies Targeting Coronaviruses. *Trends in Biochemical Sciences*, 46(5), 351-365.

Krammer, F. 2020(a). SARS-CoV-2 vaccines in development. *Nature,* 586, 516-527.

Krammer, F. 2020(b). *SARS-CoV-2 vaccine tweetorial*. Available from: https://threader.app/thread/1310372301314101250 [Accessed 10th January 2021]

World Health Organisation, 2020. *WHO Coronavirus Disease (COVID-19) Dashboard*. Available from: https://covid19.who.int/ [Accessed 10th January 2021]


Insider Imprint
developing the student community
UNIVERSITY OF LIVERPOOL
Submit your
article
for publication
For undergraduates and master students. Find out more at www.insiderimprint.com

X Chromosome Inactivation: The Great Escape

An Insight into the Effects of 'Escapee' Genes on the Sexual Dimorphism of the Human Viral Response

Abigail Clark

3rd year Genetics BSc

Upon the entry of a pathogen into the human body, the immune response is our first line of defence. This protection mechanism has multiple ways in which to protect the body depending on the type of pathogen. When viruses enter the body, infected cells can 'warn' neighbouring cells of the viral intruder, allowing them to respond better should they become infected themselves. Although this response would be expected to be consistent between sexes, viruses appear to present differently in men and women. It is believed that a contributing factor to this is the number of X chromosomes present in the individual: females carry two X chromosomes, while males carry only one. For genetic balance to be achieved between sexes, female cells inactivate one X in each of their cells. However, not all the genes on the inactive X are silenced- some manage to escape and are subsequently expressed from both X chromosomes. Some of these 'escapees' contribute to the body's viral response and exhibit a strong female bias. One of these genes, known as Toll-like receptor 7 (*TLR7*), produces a 'viral detector' that induces the body's response upon contact with a virus. This research is of great value during the current COVID-19 pandemic. Due to their greater production of *TLR7*, it has been found that females induce a stronger viral response, allowing them better control of the disease. It has also been suggested that early induction of this response suppresses inflammation of the lungs, thereby reducing the risk of severe breathing difficulties in women. Further research into this phenomenon could help scientists identify a more personalised approach to treating not only COVID-19, but multiple viruses and other infectious diseases.

Introduction

The immune system is an intricate defence mechanism against potential infection consisting of white blood cells and multiple regulatory proteins. Interferon (IFN) responses are essential in controlling the replication of viruses, with mechanisms to 'warn' surrounding cells of the invading pathogen, however, like many of the body's complex processes, this response is regulated by cascades of reactions allowing for the ultimate release of these warning signals.

Despite the tight control of this response, many diseases, including that caused by the SARS-CoV-2 virus (Takahashi *et al.,* 2020), present themselves with clear sex biases, either being more prevalent or inducing greater severity in one sex over the other. In some cases, this may be due to lifestyle, concentrations of sex hormones, or the presence of one or two X chromosomes, as seen in the case of *TLR7*.

It is known that there exist multiple genes along the X chromosome that escape the dosage compensation process of X Chromosome Inactivation (XCI), but it has recently come to light that genes regulating responses like the IFN defence are also affected by this phenomenon. Study into this process could help researchers understand the effects of XCI escape observed within certain diseases, and use this to tailor their treatments.

X Chromosome inactivation and escape

XCI is a mammalian dosage-compensation mechanism whereby gene dosage balance of the X chromosome is achieved between the sexes, via the repression of one of the female X chromosomes.

The master regulator of this process is the long non-coding RNA, *Xist*, which becomes upregulated from the X inactivation centre of one of the X chromosomes in a random fashion (Loda & Heard, 2019). As *Xist* encompasses the DNA, it recruits epigenetic modifiers and chromatin re-modellers to ultimately form a tightly organised constitutive heterochromatic structure, known as a "Barr Body" (Figure 1). This entity is characterised by the depletion of RNA polymerase II, and multiple repressive epigenetic modifications including DNA hypermethylation, and histone modifications associated with gene silencing (Garieri *et al.,* 2018).

Despite the condensation of the inactive X (Xi), biallelic expression has been observed in approximately 15-25% of X-linked genes in females, suggesting that some of these genes escape the silencing process (Katsir & Linial, 2019). It has been shown that while many of the genes on the Xi are relocated to an area devoid of RNA polymerase II and other transcription factors, genetic 'escapees' remain on the periphery of this silenced compartment (Loda & Heard, 2019), continuing to be expressed from the otherwise inactive chromosome.

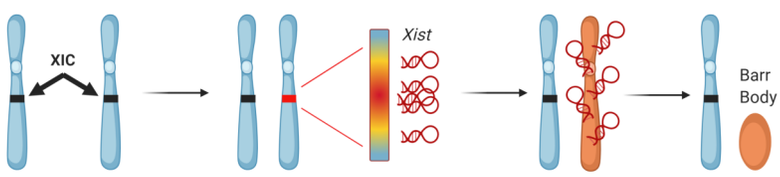


Figure 1. Formation of the Barr Body. The X Inactivation Centre (XIC) is randomly activated (Delaval *et al.,* 2007) on one chromosome. *Xist* is upregulated and gradually spreads over the chromosome, recruiting epigenetic modifiers to condense the DNA into the Barr Body.

Although this pattern of escape would be expected to be consistent, recent studies have shown that a degree of heterogeneity exists not only between individuals, but between tissues and cells of an individual. Perhaps the most surprising phenomenon is illustrated by Garrieri *et al.,* (2018) whereby allele-specific expression was investigated among fibroblasts taken from 5 female individuals. This team identified 60 escape genes, each exhibiting a varying expression profile between cells from the same individual. Different genes presented different levels of escape, with some being predominantly expressed from the active X (Xa) and others showing biallelic expression in >70% of the fibroblast sample. Interindividual inactivation profiles were also explored, revealing that the escape of the *DDX3X* gene (associated with cancer sex-bias) was highly variable among these women, suggesting that this could play a role in varying predispositions to cancer between individuals (Garieri *et al.,* 2018).

Sex biases arising from XCI escape

In line with having two X chromosomes rather than one, genes that escape XCI demonstrate a strong female bias. A 2017 study investigated the male-female differences brought about by XCI escape using RNA-seq data. Tukiainen *et al.,* (2017) found that, of 186 X chromosomal genes investigated, ~23% indicated incomplete XCI with 57% of these demonstrating variability of Xi expression between tissues, including a subset of genes appearing biallelic in just one of the 29 tissues assayed. Both *KAL1* and *CLIC2* illustrated biallelic expression specifically in the lungs and skin respectively, suggesting male-female biases in expression can arise directly from tissue-specific XCI escape, potentially influencing varying disease phenotypes between the sexes.

Conversely, genes within pseudo-autosomal region 1 (PAR1) were shown to have a consistent male bias (Tukiainen *et al.,* 2017). PARs are homologous regions along both the X and Y chromosomes, allowing for recombination between them during meiosis. It was previously believed that genes within PARs were not subject to XCI, however, Tukiainen *et al.,* (2017) demonstrated that, on average, PAR1 expression from Xi reached ~80% of Xa expression, indicating a spread of XCI beyond nonPARs, and presenting an incomplete balance of this region leading to subsequent male bias of these genes. The team ensured that these effects were from the direct influences of XCI, and not from any further regulation of the Y chromosome; there were no detections of up- or down- regulation of the PAR1 region of the Y.

Figure 2. Male-Female bias of genes on the X chromosome. The blue region represents male bias, while red represents female bias.

The rigorous investigation of 82 reported 'escapee' genes across 29 tissues illustrates an overwhelming female bias of multiple genes along the Xp arm, as well as a significant male bias of the PAR1 domain (Tukiainen *et al.,* 2017) (Figure 2).

The *TLR7* gene

Multiple genes linked with the immune response are located on the X chromosome. From the information presented above, it is hardly surprising that a number of infectious diseases present with different phenotypes between men and women.

Following the systematic analysis performed by Tukiainen *et al.,* (2017) a study was released describing the XCI escape of the *TLR7* gene, located along the Xp arm (Souyris *et al.,* 2018). *TLR7* forms part of the innate immune system capable of detecting single-stranded RNA, thus playing an essential role in antiviral responses via the induction of the type I interferon (IFN I) response and production of other inflammatory cytokines (Petes *et al.,* 2017). It was established that *TLR7* escapes XCI in primary immune cells including B cells, monocytes, and plasmacytoid dendritic cells (pDCs), presenting biallelic expression in a mosaic fashion (Souyris *et al.,* 2018). Further studies were performed to analyse the effect this had on immune responses between the sexes: it was found that the number of X chromosomes present was directly associated with pDC activation and the *TLR7*-mediated IFN response, with females indicating a significantly higher IFNα response from pDCs than males (Webb *et al.,* 2019). This higher production of interferons along with an enhanced ability to detect viral loads could potentially provide a critical immune advantage for women in the event of infection.

What could this mean for COVID-19?

Throughout the SARS-CoV-2 pandemic, it has become increasingly apparent that COVID-19 induces greater disease severity among men than women (Takahashi *et al.,* 2020). Although this is a novel virus, its sexual dimorphism is being studied continuously to enhance our understanding of how this disease behaves within the population.

Recent papers have described sex differences in the IFN response amongst pDCs, specifically analysing the effects of *TLR7* XCI escape. Cells exhibiting biallelic expression of *TLR7* have been shown to induce significantly higher levels of IFNα and IFNβ (Hagen *et al.,* 2020). This cascade of events has proved to be essential in the control of SARS-CoV-2: loss of function mutations within *TLR7* were reported in four young men with no pre-existing or underlying health conditions, and yet the progression of the disease was reported as severe, with three requiring mechanical ventilation and one succumbing to the disease (Van Der Made *et al.,* 2020). Studies investigating the related virus, MERS-CoV, identified *TLR7* as a critical player in the production of IFNs after the infection of lung epithelial cells, as well as finding that early IFN induction suppresses proinflammatory cytokine and chemokine

production in the lungs (Channappanavar *et al.,* 2019) thereby reducing the severe inflammatory phenotype observed in coronavirus patients. Following this premise, XX pDCs exhibiting biallelic *TLR7* expression may partially explain the seemingly better control of COVID-19 infection observed in women.

Further enhancing this evidence, a paper analysing the sexual dimorphism of COVID-19 found that, although viral load was equal between sexes, men exhibited higher levels of the proinflammatory cytokines, IL-8 and IL-18 (Takahashi *et al.,* 2020). The evidence from Channappanavar *et al.,* (2019) is therefore consistent with the data presented here: the higher concentration of IL-8/18 renders males more susceptible to severe inflammatory responses in the lungs than females.

The future of COVID-19 treatment

The extent of immune effects caused by XCI escape remains to be explored, but expanding on the knowledge of particular genes like *TLR7* may help scientists to further understand the clear sex bias presented by the SARS-CoV-2 virus, potentially providing a different approach to how this virus should be treated. With female genes escaping XCI in a heterogenous fashion between individuals, there may be an advantage in using a more personalised approach to treatment by examining the expression levels of particular genes. The study of sexual dimorphism observed in disease has the potential to enhance treatment not only for COVID-19, but for multiple infectious diseases, and any future pandemics yet to come.

References

Channappanavar, R., Fehr, A. R., Zheng, J., *et al.* 2019. IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. *The Journal of Clinical Investigation*, 129, 3625-3639.

Delaval, K., Govin, J., Cerqueira, F., *et al.* 2007. Differential histone modifications mark mouse imprinting control regions during spermatogenesis. *The EMBO Journal*, 26, 720-729.

Garieri, M., Stamoulis, G., Blanc, X., *et al.* 2018. Extensive cellular heterogeneity of X inactivation revealed by single-cell allele-specific expression in human fibroblasts. *Proceedings of the National Academy of Sciences*, 115, 13015.

Hagen, S. H., Henseling, F., Hennesen, J., *et al.* 2020. Heterogeneous Escape from X Chromosome Inactivation Results in Sex Differences in Type I IFN Responses at the Single Human pDC Level. *Cell Reports*, 33, 108485.

Katsir, W. K. & Linial, M. 2019. Human genes escaping X-inactivation revealed by single cell expression data. *BMC Genomics*, 20, 201.

Loda, A. & Heard, E. 2019. Xist RNA in action: Past, present, and future. *PLOS Genetics*, 15, e1008333.

Petes, C., Odoardi, N. & Gee, K. 2017. The Toll for Trafficking: Toll-Like Receptor 7 Delivery to the Endosome. *Frontiers in Immunology*, 8, 1075.

Souyris, M., Cenac, C., Azar, P., *et al.* 2018. TLR7 escapes X chromosome inactivation in immune cells. Science Immunology, 3, 8855.

Takahashi, T., Ellingson, M. K., Wong, P., *et al.* 2020. Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature*, 588, 315-320.

Tukiainen, T., Villani, A.-C., Yen, A., *et al.* 2017. Landscape of X chromosome inactivation across human tissues. *Nature*, 550, 244-248.

Van Der Made, C. I., Simons, A., Schuurs-Hoeijmakers, J., *et al.* 2020. Presence of Genetic Variants Among Young Men With Severe COVID-19. *JAMA*, 324, 663-673.

Webb, K., Peckham, H., Radziszewska, A., *et al.* 2019. Sex and Pubertal Differences in the Type 1 Interferon Pathway Associate With Both X Chromosome Number and Serum Sex Hormone Concentration. *Frontiers in Immunology*, 9, 3167-3167.

Diagnosing the biggest parasitic killers:

A review of available diagnostic tools for malaria and visceral leishmaniasis

Josephine Shepherd

3rd year Tropical Disease Biology BSc

The international effort to control malaria has received decades of global funding and resources, and for this reason rapid diagnostic testing has been rolled out to many communities with great success. Historically, malaria diagnosis has relied heavily on microscopic analysis of blood smear slides - but in the last decade there has been a huge rise in the implementation of rapid diagnostic tests (RDTs). Due to their excellent convenience and affordability, RDTs have enhanced access to accurate diagnosis. On the other hand, visceral leishmaniasis is classified as a neglected tropical disease and affects the poorest people in remote areas, which makes diagnosing the disease even more challenging. Whilst microscopy is incredibly accurate, acquiring the tissues required for biopsy is far more invasive than taking the blood sample required for the diagnosis of malaria. Serological testing, whilst not appropriate for malaria diagnosis, maintains high sensitivity and specificity and can be easily deployed to rural health outposts. Discussed herein are new diagnostics which present an incredible opportunity for the advancement of the control of these diseases and represent a major step towards elimination goals. Indeed, successful control of both diseases will require an integrated approach that considers multiple molecular and serological or antigenic techniques.

Introduction

Diagnosis is the first step in controlling the spread of disease. Diseases such as malaria and visceral leishmaniasis affect people across the world and cause immense suffering and disability if not properly diagnosed (as seen in Box 1 and 2). Combined, these diseases account for almost 415,000 deaths recorded in 2019, which re-enforces the need for new technology to diagnose and treat patients quickly and accurately (WHO, 2020, DNDi, 2021).

To this end, the World Health Organisation has identified seven criteria to aid the development of new tools to diagnose disease: The acronym ASSURED stands for Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users. For new tools to be effective in real-word settings, they must adhere to these criteria. There are many complex biological and sociological differences between malaria and visceral leishmaniasis infections, which means the technology used to diagnose the diseases must be different. Rapid diagnostic tests (RDTs) are very effective in diagnosing malaria cases, and are also affordable and accessible to poor, rural communities. However, to diagnose visceral leishmaniasis it will be more practical to use specialised technology, such as serological testing. For both diseases, microscopy is a very accurate and useful tool, but it will be difficult to find the resources to implement it on a large scale. A brief schematic of this review can be seen in Figure 1.

Specific and sensitive testing limits unnecessary administration of frontline treatments, which subsequently prevents the expansion of drug resistance. Prompt diagnosis of disease also contributes to the rapid administration of treatment to patients, thus reducing clinical manifestation and further transmission. Diagnosis of malaria has historically relied on parasite identification

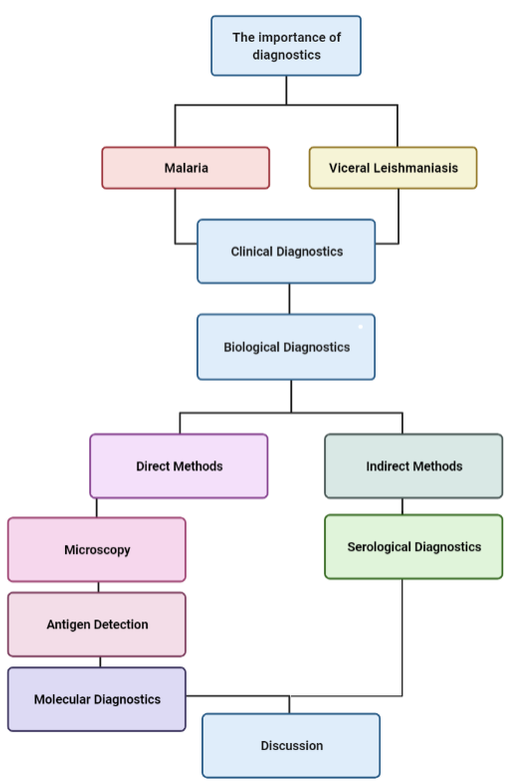


Figure 1. A brief schematic of the this review.

by microscopy, but research into new diagnostic tools for malaria have expanded the field into molecular diagnostics as well as antigen detection.

Visceral leishmaniasis, a neglected tropical disease (NTD), is identified as requiring innovative and intensified disease management (IDM) i.e., diagnosis, treatment and follow up is difficult and costly to manage, and the people affected are in rural areas where diagnosis is difficult to

access (WHO, 2020). To date, diagnostic and monitoring tests for NTDs such as visceral leishmaniasis are limited compared to diseases such as malaria, which receive greater treatment and research funding (Kosack *et al.,* 2017).

Understanding the biological and sociological causes of these discrepancies will be invaluable in improving malaria and visceral leishmaniasis diagnosis and have been reviewed here.

Box 1. Malaria

Plasmodium parasites are transmitted by the bite of female *Anopheles* mosquitoes, and cause the disease known as malaria. Mild symptoms include fever and chills, though without prompt treatment, the disease may progress to a more serious form which often leads to death. Severe symptoms include anaemia, respiratory distress, and cerebral malaria. WHO estimates 229 million cases in 2019 and 409,000 deaths, of which 94% were reported in the WHO Africa region. Children under 5 are at most risk of malaria infection, due to their lack of acquired immunity, and accounted for 67% of worldwide malaria deaths (WHO, 2020).

Box 2. Visceral leishmaniasis (VL or kala-azar)

VL is transmitted through the bite of female hematophagous sandflies, specifically phlebotomine species. Of the multiple diseases caused by *Leishmania* parasites, VL is considered the deadliest, with mortality rates as high as 100% within 2 years if treatment is not administered (WHO, 2020). Symptoms include fever, anaemia, and hepatosplenomegaly. The disease is responsible for the second largest number of deaths related to parasitic infection, after malaria (DebRoy *et al.,* 2017). VL is endemic in many regions across the world which have high population density settings, and where poverty is widespread. 90% of the global burden is concentrated to East Africa (Ethiopia, Kenya, South Sudan, and Sudan), Somalia, India, and Brazil (WHO, 2020). The cost of treatment and inability to work drives people in impoverished regions further into economic deprivation.

Clinical diagnostics

Clinical diagnosis is the determination of a disease via observation of the clinical symptoms presented by a patient. It is likely that clinical diagnostics are limited to resource-poor settings with inadequate health infrastructure, as this method is inexpensive and requires no laboratory equipment. It is worth noting that clinical diagnostics have largely been superseded by biological diagnostics, which are superior in both specificity and sensitivity. In fact, diagnosis by clinical symptoms alone is complicated by polyparasitism and co-endemicity of multiple diseases, as well as the non-specific nature of malaria symptoms.

Similarly, clinical diagnostics are not likely to be used unaided by other diagnostic techniques for the detection of visceral leishmaniasis, due to the costly, invasive, and toxic treatment that is required. Diagnosis is made through a combination of parasitological and serological tests, with clinical signs, such as hepatosplenomegaly, aiding identification (WHO, 2020).

Biological diagnostics - direct methods

Microscopy and culture

Malarial infection may be diagnosed microscopically by visualising *Plasmodium* parasites in thick and thin blood smears. Light microscopy remains the 'gold standard' diagnostic tool to which other diagnostics are compared, although important advances in diagnostic methods mean that there are tests which greatly exceed the sensitivity and specificity of blood smears. As an established method, there exists a huge amount of experience and knowledge in the field of microscopy and the results are highly specific (Rodulfo *et al.,* 2007).

Furthermore, microscopy allows for the quantification of parasite density, so that the efficacy of treatment may be monitored. In areas of polyparasitism, microscopy is an effective tool for diagnosing multiple coinfections at the same time, and so its importance as part of an integrated disease management plan should not be underestimated.

However, the quality of microscopy-based diagnosis is unpredictable, as it relies on the variable skill of technicians and the ability to identify species at low levels of parasitaemia or in mixed species infections (Tangpukdee *et al.,* 2009). Importantly, microscopy is often not 'deliverable to end users' in rural healthcare settings where there are no electricity or laboratory resources, and due to its laborious nature is not suitable for high throughput screening (Wangai *et al.,* 2011).

It is also possible to diagnose visceral leishmaniasis via light microscopy. This involves biopsy of relevant tissues, usually splenic aspirates, or bone marrow, followed by identification of the amastigotes of Leishmania species under microscopic examination. Though sensitivity is higher in diagnosis from splenic aspirates (95%) compared to bone marrow (60-85%), the aspiration procedure can occasionally lead to fatal haemorrhage (Sundar & Rai, 2002). Bone marrow extraction is much safer, but the procedure is resented by patients and requires adequate medical facilities. Culture of Leishmania is possible but is rarely necessary in routine diagnostics.

Antigen detection

An alternative approach to light microscopy centres on the biochemical detection of proteinaceous epitopes presented by a pathogen. Malaria rapid diagnostic tests (RDTs) detect specific antigens in the blood produced by *Plasmodium* parasites. RDTs currently target the histidine-rich protein-2 (HRP-2) antigen as well as two plasmodium enzyme-based detection assays: plasmodium lactate dehydrogenase (pLDH) and plasmodium aldolase.

The use of RDTs has expanded rapidly in recent years ahead of microscopy; from 40% of malaria tests in sub-Saharan Africa in 2010 to 75% in 2017, due to its simplicity, rapid receipt of results and inexpensiveness (Rapid Diagnostic Tests, 2019). As RDTs require little training or resources, they can be implemented on a community level, extending into rural areas and peripheral health posts. For this reason, they are extremely useful, ASSURED tools, as seen in Figure 2. However, RDTs are

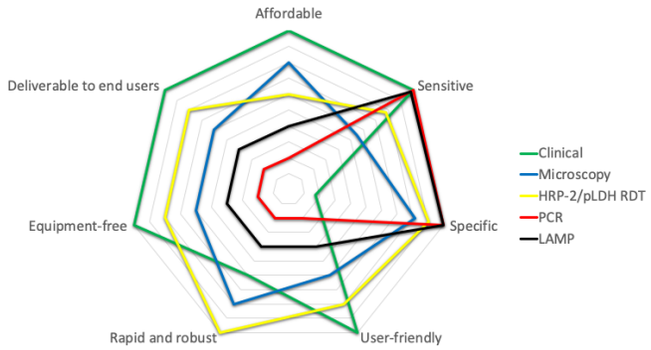


Figure 2. ASSURED criteria for each of the common malaria diagnostic method is represented on a radar chart. Quantitative data for specificity and sensitivity was extracted from Mfuh *et al.,* (2019), Berzosa *et al.,* (2018) and Sirichaisinthop *et al.,* (2011).

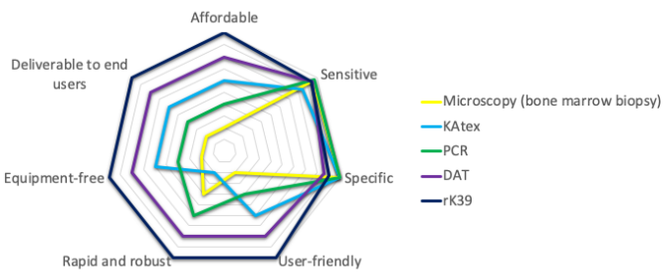


Figure 3. ASSURED criteria for each of the common visceral leishmaniasis diagnostic methods is represented on a radar chart. Quantitative data for specificity and sensitivity was extracted from Ghatei *et al.,* (2009) and Khatun *et al.,* (2017).

susceptible to stockout shortages, whereas the reagents required for microscopy last longer (Hasselback *et al.,* 2014).

There is also concern over persistent HRP-2 antigenemia after parasite clearance, the resultant false positives, and the inability of the test to monitor response to therapy (Iqbal *et al.,* 2004). Additionally, consideration must be given to the effectiveness and sensitivity of HRP-2 RDTs in areas with a high prevalence of pfhrp2/3 gene deletions. pLDH tests have reported higher specificity due to the short half-life of lactate dehydrogenase compared to HRP-2, but a purported lower sensitivity, which makes HRP-2 the preferred test in *P. falciparum* dominant areas (Coldiron *et al.,* 2019).

In the case of visceral leishmaniasis, there is urgent need for a rapid diagnostic test that maintains high specificity and sensitivity, with improved deliverability to end users. KAtex, a rapid latex agglutination test that detects urinary antigens in boiled urine, is effective for diagnosis in immunocompromised patients, unlike serological tests. However, sensitivity is too variable to be the ideal diagnostic test, and boiling urine is impractical and time-consuming (Sundar & Singh, 2018).

Molecular diagnostics

Despite its superior sensitivity and specificity, most resource-poor settings preclude the use of PCR, for reasons including limited financial resources and inadequate laboratory infrastructure (Hänscheid & Grobusch, 2002). It has however been argued that the expense of implementing PCR diagnostics, even in rural and peripheral healthcare settings, is less that the financial burden of misdiagnosis and the incorrect administration of antimalarials (Wangai *et al.,* 2011). An important strength of molecular diagnostic techniques is that they remain the only method of detecting drug resistant mutations, an advantage that may become more important as resistance threatens the efficacy of artemisinin-based combination therapy.

Molecular-level tools for visceral leishmaniasis diagnosis may be useful in areas that are approaching elimination goals, as more of the population becomes susceptible to infection. It will become increasingly important to identify all cases that maintain transmission with a highly sensitive and specific molecular diagnostic test (Sundar & Singh, 2018).

Biological diagnostics - indirect methods

Serological diagnostics

To date, a serological antibody test that detects the presence of *Plasmodium*, and observes ASSURED principles, has not yet been developed for point-of-care diagnostics. Serological testing is not an appropriate diagnostic tool due to the time it takes for antibodies to develop, as well as the persistence of antibodies after parasite clearance (Sulzer & Wilson, 1971). This makes antibody tests impractical for routine diagnosis of acute malaria, especially in endemic areas where a large proportion of the population is seropositive.

There are multiple well-developed serological tests for the detection of antileishmanial antibodies, notably enzyme-linked immunosorbent assay (ELISA) and direct agglutination test (DAT). DAT is not only expensive, but it requires trained technicians and has a subjective endpoint. To confirm diagnosis in patients with low DAT titres, splenic aspirates may need to be performed (Cañavate *et al.,* 2011). Consequently, several immunochromatographic tests which require minimal equipment and are more suited to decentralised diagnostics have been developed using the rK39 Leishmania antigen. Both tests have high sensitivity (DAT 94.8% and rK39 93.9%), but specificity is higher in rK39 (90.6% compared to 85.9%) (Cañavate *et al.,* 2011). For this reason, rk39-based diagnostics are excellent tools for accurate diagnoses in resource-poor settings. However, it should be noted that the test shows decreased sensitivity in East Africa (Bangert *et al.,* 2018).

Discussion

It will be effective to increase the use of RDTs in malaria diagnostics, especially in areas of high transmission and to reduce the burden in rural settings which lack the equipment and infrastructure for laboratory-based diagnostics. RDTs, due to their user-friendly nature, encourage active involvement from the community and provide an opportunity to move diagnostics away from the

laboratory and nearer to the patient. As a diagnostic tool, they are the most promising, as demonstrated by Figure 2.

However, there is a need for better investment into microscopy, which, when performed to a high standard, provides critical information for speciation, co-infection, and quantification of parasite burden. Though further investment should not detract from the implementation of RDTs in decentralised health posts, better training and facilities for existing healthcare services will provide greater opportunity for species-specific and polyparasitic treatment plans.

The current situation for visceral leishmaniasis diagnostics bears some similarities to malaria, such as the need for rapid, affordable, and deliverable tests to both urban and rural resource-poor settings. Serological tests, rather than antigen detection tests, such as rK39 are the most promising diagnostic that should be scaled up for high-throughput use, as shown in Figure 3.

Further research into the use of alternative molecular diagnostics should be conducted as multiple countries approach and set elimination goals. It is necessary to understand the contribution of sub-microscopic infections in malaria transmission, and which nucleic acid amplification-based diagnostic will be most cost effective to accelerate elimination.

References

Bangert, M., Flores-Chávez, M. D., Llanes-Acevedo, I. P., *et al.* 2018. Validation of rK39 immunochromatographic test and direct agglutination test for the diagnosis of Mediterranean visceral leishmaniasis in Spain. *PLoS Neglected Tropical Diseases*, 12(3), e0006277.

Berzosa, P., de Lucio, A., Romay-Barja, M., *et al.* 2018. Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea. *Malaria Journal*, 17(1), 333.

Cañavate, C., Herrero, M., Nieto, J., *et al.* 2011. Evaluation of two rK39 dipstick tests, direct agglutination test, and indirect fluorescent antibody test for diagnosis of visceral leishmaniasis in a new epidemic site in highland Ethiopia. *The American Journal of Tropical Medicine and Hygiene*, 84(1), 102–106.

Coldiron, M., Assao, B., Langendorf, C., *et al.* 2019. Clinical diagnostic evaluation of HRP2 and pLDH-based rapid diagnostic tests for malaria in an area receiving seasonal malaria chemoprevention in Niger. *Malaria Journal*, 18(1), 443.

DebRoy, S., Prosper, O., Mishoe, A. & Mubayi, A. 2017. Challenges in modeling complexity of neglected tropical diseases: a review of dynamics of visceral leishmaniasis in resource limited settings. *Emerging Themes in Epidemiology*, 14, 10.

Drugs for Neglected Disease Initiative. 2021. *Visceral Leishmaniais*. [online] Available at: <https://dndi.org/diseases/visceral-leishmaniasis/> [Accessed 22 February 2021].

Ghatei, M. A., Hatam, G. R., Hossini, M. H., & Sarkari, B. 2009. Performance of latex agglutination test (KAtex) in diagnosis of visceral leishmaniasis in Iran. *Iranian Journal of Immunology,* 6(4), 202–207.

Hänscheid, T. & Grobusch, M. (2002). How useful is PCR in the diagnosis of malaria? *Trends in Parasitology*, 18(9), 395-398.

Hasselback, L., Crawford, J., Chaluco, T., *et al.* 2014. Rapid diagnostic test supply chain and consumption study in Cabo Delgado, Mozambique: estimating stock shortages and identifying drivers of stock-outs. *Malaria Journal*, 13, 295.

Iqbal, J., Siddique, A., Jameel, M. & Hira, P. 2004. Persistent Histidine-Rich Protein 2, Parasite Lactate Dehydrogenase, and Panmalarial Antigen Reactivity after Clearance of *Plasmodium falciparum* Monoinfection. *Journal of Clinical Microbiology*, 42(9), 4237-4241.

Khatun, M., Alam, S., Khan, A., *et al.* 2017. Novel PCR primers to diagnose visceral leishmaniasis using peripheral blood, spleen or bone marrow aspirates. *Asian Pacific Journal of Tropical Medicine*, 10(8), 753-759.

Kosack, C., Page, A. & Klatser, P. 2017. A guide to aid the selection of diagnostic tests. *Bulletin of the World Health Organization*, 95(9), 639-645.

Mfuh, K., Achonduh-Atijegbe, O., Bekindaka, O., *et al.* 2019. A comparison of thick-film microscopy, rapid diagnostic test, and polymerase chain reaction for accurate diagnosis of *Plasmodium falciparum* malaria. *Malaria Journal*, 18(1).

Rodulfo, H, De Donato, M, Mora, R, González, L, & Contreras, C. E. 2007. Comparison of the diagnosis of malaria by microscopy, immunochromatography and PCR in endemic areas of Venezuela. *Brazilian Journal of Medical and Biological Research*, 40(4), 535-543.

Sirichaisinthop, J., Buates, S., Watanabe, R., *et al.* 2011. Evaluation of loop-mediated isothermal amplification (LAMP) for malaria diagnosis in a field setting. *The American Journal of Tropical Medicine and Hygiene*, 85(4), 594–596.

Sulzer, A. J., & Wilson, M. 1971. The indirect fluorescent antibody test for the detection of occult malaria in blood donors. *Bulletin of the World Health Organization*, 45(3), 375–379.

Sundar, S., & Rai, M. 2002. Laboratory diagnosis of visceral leishmaniasis. *Clinical and Diagnostic Laboratory Immunology*, 9(5), 951–958.

Sundar, S., & Singh, O. P. 2018. Molecular Diagnosis of Visceral Leishmaniasis. *Molecular Diagnosis & Therapy,* 22(4), 443–457.

Tangpukdee, N., Duangdee, C., Wilairatana, P., & Krudsood, S. 2009. Malaria diagnosis: a brief review. *The Korean Journal of Parasitology*, 47 (2), 93–102.

Wangai, L. N., Karau, M. G., Njiruh, P. N., *et al.* 2011. Sensitivity of microscopy compared to molecular diagnosis of *P. falciparum*: implications on malaria treatment in epidemic areas in Kenya. *African Journal of Infectious Diseases*, 5(1), 1–6.

Weber, M. W., Mulholland, E. K., Jaffar, S., *et al.* 1997. Evaluation of an algorithm for the integrated management of childhood illness in an area with seasonal malaria in the Gambia. *Bulletin of the World Health Organization*, 75(Suppl 1), 25–32.

World Health Organization. 2018. *Microscopy*. [online] Available at: <https://www.who.int/malaria/areas/diagnosis/microscopy/en/> [Accessed 4 November 2020].

World Health Organization. 2019. *Rapid Diagnostic Tests*. [online] Available at: <https://www.who.int/malaria/areas/diagnosis/rapid_diagnostic_tests/en/> [Accessed 16 November 2020].

World Health Organization. 2020. *Diagnosis, Detection And Surveillance*. [online] Available at: <https://www.who.int/leishmaniasis/surveillance/en/.> [Accessed 4 November 2020].

World Health Organization. 2020. *Innovative And Intensified Disease Management (IDM)*. [online] Available at: <https://www.who.int/neglected_diseases/disease_management/Innovative_Intensified_Disease_Management/en/> [Accessed 4 November 2020].

World Health Organization. 2020. *Malaria Fact Sheet*. [online] Available at: <https://www.who.int/news-room/fact-sheets/detail/malaria> [Accessed 22 February 2021].

World Health Organization. 2020. *Leishmaniasis.* [online] Available at: < https://www.who.int/health-topics/leishmaniasis#tab=tab_1> [Accessed 22 February 2021].

School Competition

Solving Modern Problems with Science

CONGRATULATIONS TO OUR WINNERS!

In Vitro Gametogenesis

Zizi Mpetha
Year 10, Liverpool College

Everyone is different, they have different needs, different biological functions and different attractions. We all have different desires for the future too, and that may include children. Even in increasingly chaotic times, the want for a family and settlement still runs rampant for generations to come as people still want babies. For many people having children can be a problem. It may be they're an LGBTQ+ couple (for people who are homosexual this proves to be a particular problem), they're infertile, past menopause, in a polyamorous relationship, or single and just can't find someone to have children with. There are a variety of different problems that come in the way of people and families. We need to find solutions to these problems, we need to hear the problems that different people face, rather than ignoring them like we have for so many years. Biologists should help overcome the societal norm of heterosexual, young, fertile couples. Because everyone, from many different walks of life, deserves to be happy and safe and for some that means having children.

There are orphanages and foster homes, which is one of the go-to options for same sex couples, but both of those things have something in common. The thing they have in common is that, while having any child in general is a wonderful thing to many, it isn't for everyone – some people are cut out for it and some people would just prefer to have their own biological child. IVF exists but that's only for infertile people, and not everyone else. My next suggestion would be to reduce the need for sperm and egg donors as a whole.

Which is why I think we should invest in IVG (*In Vitro* Gametogenesis) research. IVG is a process in development used to create babies with the DNA from each person, by turning adult stem cells into reproductive cells and fertilizing them. For same sex couples it means that babies are born with 50% of their DNA from each partner, which would be great because they have the chance to have a baby of their own without a third party or donor involved. They could have a biological family of their own. People who have gone through menopause no longer need to fear the 'closed window of fertility' as they'll be able to generate viable egg cells. This will benefit people who have gone through an early menopause but still want to have children – not being able to have a period will no longer have to influence your choice to have a child. People in polyamorous relationships, particularly those in fours, would be able to have children with a bit of every person's DNA in the baby – allowing their relationships to have the same validation as other couples. People on their own would be able to use their own stem cells and turn them into the respective reproductive cells, and they'd be able to fertilize themselves: so you wouldn't need a relationship to have children. This is one of the most inclusive breakthroughs in biology, considering everyone no matter their sexuality, age, or relationship. It ensures that people are able to have biological babies of their own, thus eliminating the need for egg or sperm donors. All of this is done with skin cells that are programmed to form pluripotent stem cells, so essentially anyone with skin would be able to have children (be it on their own, past menopause, in a same sex relationship, or in a polyamorous relationship).

People would still have children in the conventional way, that is to say with a uterus and penis should they want to, but in a few years' time it would benefit so many people to have biological children of their own. Imagine the possibilities! People would also still love to adopt children and foster them too – it would still be in high demand. It's just that this alternative process of having children, this different method of growing a family has been introduced as an equally beneficial method. No one stops wanting adopted children for good – as it stands, there are about 36 couples at most waiting for every one child who is placed for adoption. This just provides an option for people who are not able to adopt in the first place.

This process didn't get the media press and attention that it deserved, at least not enough of it. This was perhaps due to a few ethical concerns by which it may raise but I don't think that's fair. I don't think is fair because people deserve to know the endless possibilities that they may introduce to millions upon billions of couples around the world who are looking to have a child. All it entails is taking a few adult stem cells, or at that any cell around the body, from the couple and fertilising them. I think only the people who don't understand the struggle of not being able to have biological children would protest new ways of including everyone in the joys of children.

In conclusion, I think that our next motion forward is to investigate making the world a better, more inclusive place and that utopia starts with biology.

CONGRATULATIONS TO OUR WINNERS!

Biofuels as renewable energy sources

Harrison Darby
Year 10, Liverpool College

Soaring oil prices and global efforts to stave off the worst effects of climate change have made the search for clean, renewable fuels a new priority. Biofuels have been around longer than cars, but cheap petrol and diesel have long left them on the side-lines. The idea behind biofuels is to replace traditional fuels with renewable fuels made from plant or other sources. Today's transportation relies primarily on fossil fuels. Advances in biology show that our road trips, flights, and boat transportations account for approximately a quarter of the world's greenhouse gas emissions.

As fossil fuels start to run out, different solutions for fuels are required. There are two types of biofuels: biogas, and ethanol-based fuels. There are two ways to make ethanol, one of them is the hydration of ethene, however it is not a renewable source as it is made from crude oil so does not solve our problem. The second way is the fermentation of natural products. Ethanol is made when yeast breaks down glucose in anaerobic respiration, without oxygen. The ethanol is separated from the yeast and the remaining glucose by distillation. These ethanol-based fuels are quite effective. In places like Brazil, they commonly use ethanol mixed with petrol as it is a good fuel which is carbon neutral. This means that there is no net release of carbon into the atmosphere as there is a balance between the amount of carbon emitted and the amount of carbon absorbed. They also do not release any harmful gases when burned.

The other type of biofuel is biogas. Biogas is approximately 70% methane and 30% carbon dioxide and lots of different microorganisms are used to ferment plant and animal waste in anaerobic respiration. It can be made in a generator which is kept at a constant temperature so that the microorganisms are always respiring. The biogas cannot be stored as a liquid so it must be used immediately. However, this means that you can use it as a small biogas to generate gas for a family and the products which you are inputting are human, animal and food waste. The by-products released are not necessarily dangerous and can be used to fertilise crops. When the bacteria decompose, they produce methane as a waste product which is a flammable gas and can be used for fuel. Methane is a greenhouse gas but by using it as a fuel you are not releasing it into the atmosphere straight away.

There are different kinds of generators you can use to make biogas. Firstly, you can use a batch generator, which makes biogas into small batches. They are manually loaded and cheaper than continuous generators, but are less convenient as they continually need loading, emptying, and cleaning. Continuous generators make biogas all the time as waste is continually inputted, so this means it is more suited for large scale biogas projects. Generators should be kept well insulated to prevent heat loss because their optimum temperature for work is 35 degrees. Exothermic reactions occur which means they let off heat and they need to be kept away from houses because when you are using cow manure the smell is not the nicest.

Fossil fuels like oil, coal and natural gas are the remains of organisms from millions of years ago. They are mainly composed of carbon with varying amounts of hydrogen. There are some advantages to fossil fuels. Coal is formed from ferns, plants and trees which has hardened due to pressure and heat and it is relatively inexpensive. Oil is formed from small organisms like zoo plankton and algae where pressure has caused the more complex organic matter to decompose. It has a high specific energy and density. Natural gas is formed from the same process of oil only it was exposed to more heat and pressure causing it to further decompose into a gaseous form. It is also a relatively clean fuel and does not contribute to acid deposition. However, when these materials are burned the elements mentioned above are released into the atmosphere slowly causing the global temperature to rise. This is how greenhouse gases come to surround the earth. Greenhouse gases isolate the heat not allowing it to escape the earth’s atmosphere. By allowing the heat to rise, we are causing drastic allow consequences for our climate that we will not be able to reverse in the future such as glaciers melting which is leading to the rise of our ocean’s levels.

After analysing the advantages and disadvantages of biofuels, they are shown to be an efficient renewable energy source that can be extremely beneficial to our society if harnessed and used well. As opposed to non-renewable fossil fuels, biofuels do not produce any toxic gases. Ethanol is carbon neutral, its raw material is cheap, it is quite easily available, and the by-products can be used as fertiliser in biogas. Therefore, the next challenge for humanity to overcome would be to be able to utilise the modern-day biofuels to control the increasing issues associated with non-renewable fuels.

Careers

Getting a head start

Thinking about your future career can seem daunting, we know. Find out about the support available to you at Liverpool, how you can get a head start when thinking about your future career and what starting a PhD is like.

Top tips to improve your CV

Tierney Witty

Insider Imprint Student Ambassador and Master Student in Classics and Ancient History

Formatting your CV can be boring and hard work, especially if you are not sure on what to include. Your CV aims to offer potential employers a first impression of who you are, and it is important to strike the right balance between work experience, education, and personal information. Therefore, this page is designed to offer some extra tips to help improve your CV.

Length

Your CV should be no longer than two pages in length. Recruiters will receive hundreds of CVs for a position, keeping it concise at two pages helps to offer a good glance at your experience, without it looking overwhelming or as if you are over-explaining.

Strong Opening

Giving a strong first impression sets the tone for how the rest of your CV will be read. Make sure you tailor it to the position you are applying for, summarising your previous experience and showing how it is relevant.

'STAR' Method

Using the 'Situation, Task, Activity, Result' method helps you clearly showcase to the recruiter good examples of your previous experiences and emphasises you as an efficient worker.

Sell Yourself

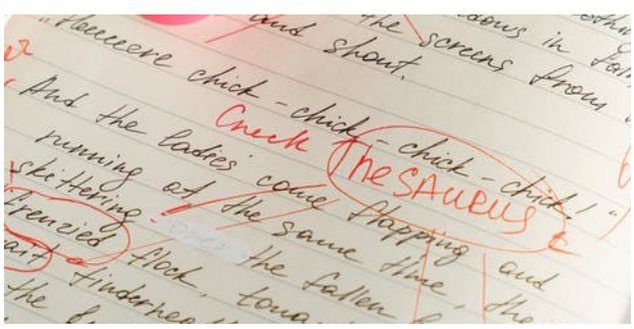
This one might sound a little obvious, but it is something that people fail to do time and time again. Your CV is your chance to big yourself up and really show-off the skills you have. Make sure you thoroughly highlight the different skills you have learnt from previous experiences.

Avoid Clichés

If you are using clichéd terms such as 'hard-working' or 'team player', it is essential you show the recruiter that you are these terms by using examples in previous experiences where you have demonstrated this.

Spelling and Grammar

Make sure your CV is triple checked for spelling and grammar, that it is professional and correct. This alone will show evidence of good communication and written skills to employers. No matter the industry or profession, having such skills is imperative to employers.



Simplicity

Simplicity in your formatting and design is the key to making things standout to recruiters, make sure your headings are bold and your text is organised and tidy to clearly separate information, making your CV easier to read.

LinkedIn

So, if you have not yet set up a LinkedIn page then you really need to do so. A social media platform used by working professionals, it is a place where you can go, talk about your experiences and network as much as possible with recruiters and potential employers. Right now, with everything being online, LinkedIn is vital.

If you need any more tips or guidance on constructing a CV for a job application, check out the University's career service. They have lots of excellent online resources, and you can now connect online to talk live with a careers coach or via messaging using the Handshake platform.

See the next pages to find out more!

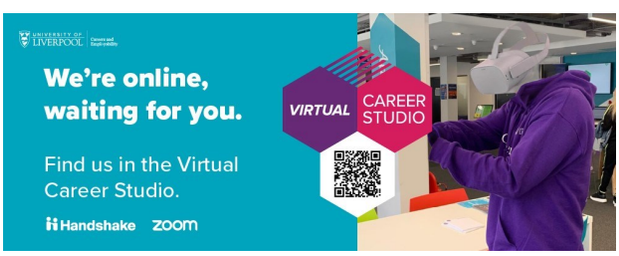
Your Careers & Employability Support

A Life Sciences Focus

At Careers & Employability, students are at the heart of everything we do, so much so that we have a dedicated faculty team to support life sciences students at every stage of your journey. We are committed to the University's vision of supporting students to become creative and culturally rich graduates, with the capacity to find employment that will enable them to be agents for change in a connected world. We work closely with our life sciences academic partners to ensure employability is at the heart of your student experience.

Our Approach

We know that employability is not just about 'getting a job', rather an ever-changing set of experiences, achievements, skill sets and values that is unique to you – your employability story! Data from the Department of Education shows students who engage in understanding and enhancing their own employability early on are most likely to be successful in securing the graduate job or post graduate opportunity they want. We can support you on this journey in different ways.



Virtual Career Studio

https://www.liverpool.ac.uk/careers/virtual-career-studio/

The Career Studio is our central support offer to all students, currently virtual via Zoom Monday- Friday from 11am- 2pm. It is an informal space, led by students, for students, empowered by career experts. There is no appointment needed, you can drop-in virtually to speak to a career coach at a time that suits, with coaches waiting online to help you to 'Explore, Connect, and Apply'. Students use the Studio for many reasons, and you can too! Drop in to co-explore what you can do with your life sciences degree, get your CV checked, develop your online profile, or learn about how you can connect with employers to expand your network and career related knowledge.

> "I am a Tropical Disease Biology Life Sciences student and a current Career Coach and would recommend that if you are a life sciences student you should use the career studio to help with all aspects of your career journey. From exploring career options to making connections and applying for roles, the career coaches are ready to help."
>
> ***Helen Elsworth, Tropical Disease Biology Undergraduate***

Handshake

https://liverpool.joinhandshake.co.uk/login

Handshake is the new online careers and employability platform connecting students, the careers and employability team and employers in one space. There are a range of benefits to logging on and developing your Handshake profile.

You will find life sciences related job opportunities on Handshake at undergraduate, graduate and post graduate level, and a wide variety of resources including 'Finding Life Sciences Related Work Experience' and '10 Steps to Choosing a Career'. You can connect with employers and other students that align to your interests. Log in with your university email address to complete your profile and access these careers resources, exclusive online events, and to start searching for graduate job opportunities.

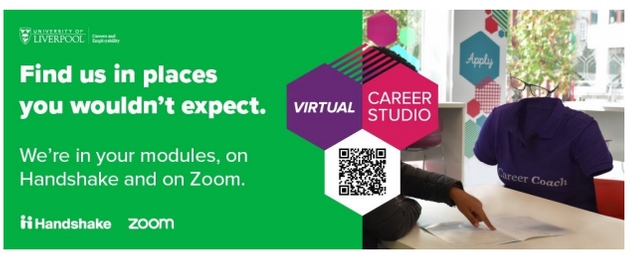
Embedding Employability & Employer Connections

https://liverpool.instructure.com/courses/28665

Careers & Employability work with academics to embed employability across modules within your specific programmes. This means that within your course you are enhancing your self-awareness, developing key employability skills and learning how to articulate your strengths in the recruitment process. We use up to date life sciences graduate employment data within the Life Sciences 2030 Skills Strategy and work closely with employers to create, deliver, and provide content to support student development.

Our dedicated Employer Connections Team https://www.liverpool.ac.uk/careers/employer-connections/student-zone/, brought 43 employers to connect with students

digitally as part of Life Sciences Employability Week and within module activity. We have had lectures from Hannah Randles, Health and Life Sciences Champion for the Liverpool city region and a variety of employers talking about the importance of commercial awareness for the life sciences as part of a key skills module (Life223) assessment. These employers range from large multi-national organisations such as GSK, to small local enterprises seeking to develop and attract UoL life sciences students. Much of this content is available for all life sciences students to access within the Health and Life Sciences employability CANVAS page.



Enterprise & Skills Enhancement – Building Digital Fluency

With a focus on connecting students with the best technology out there, we have a variety of skills enhancement opportunities and programmes to support you in your career development:

- **Design Your Future** – an online community with resources, events, and workshops designed for students looking to start a new enterprise or build entrepreneurial skills. https://www.liverpool.ac.uk/careers/enterprise/
- **CV360** – upload your CV for instant feedback and advice on how to improve it. https://ulms.careercentre.me/Members
- **ShortListMe** – practice video interviews with instant AI reviews, and the option for feedback from a Career Coach. https://go.shortlister.com/marketplace/uniofliverpool
- **LinkedIn Learning** - learn in-demand skills with online expert-led video tutorials. https://libguides.liverpool.ac.uk/online/linkedinlearning
- **Graduates First** – practice online tests and get exclusive insights into employer recruitment stages. https://www.graduatesfirst.com/university-career-services/liverpool/

Unlock Your Potential (UP) – Levelling the playing field for students.

https://news.liverpool.ac.uk/2020/10/15/sign-up-for-additional-careers-employability-support/

We know that all students face challenges as they plan for their future. We also know that for some of our students, there are additional challenges that can make this process even more difficult.

Our new UP initiative aims to level the playing field in as many ways as we can. Through a range of job and internship opportunities, exclusive career events, personalised support and a unique set of resources and tools, we hope to equip you with the skills, connections, and confidence to be successful.

You can also apply for our £300 Employability Support Award. These funds could be put towards many things that will enhance your employability, such as royal society student membership fees, conference/events tickets or help towards securing and maintaining internships/ placements and work experience that are not a compulsory part of your course or module.

All eligible undergraduate students* can sign up to the UP programme. **Eligible students include; recipients of University of Liverpool bursaries, those with disrupted education (including Care Experience, Young Carers, Estranged, and Asylum Seekers/Refugees), students who are BAME, LGBTQ+, disabled, mature, and those from areas where participation in higher education is low.*

"I was put in touch with the Unlock Your Potential Programme through the young carers team at the university. Jenny has kept in touch with me throughout my undergraduate degree and still after graduation. Without this support I would not have got as far as I have with my applications for dentistry and we continue to work together to explore the next stage of my career."

Joseph Ennis, Human Anatomy Graduate

"The Skills Enhancement Programme gave me the amazing opportunity to work within a small group to plan and manage all aspects of a virtual fundraiser whilst developing skills I wouldn't have developed in science-based experiences such as creativity and digital skills. Communicating and collaborating with the employer also developed my networking skills and confidence."

Phoebe Malin, MBiolSci Biological and Biomedical Sciences Undergraduate

Career in Toxicology:

How I got here and why I love it

Adam Wood

Pharmacology BSc Graduate

I completed the Pharmacology undergraduate programme at UoL in 2018, before moving onto, and completing the MRes programme the following year.

After my studies I moved into the consumer goods industry as a toxicologist. My first job was with a large high street chain, which owns several popular cosmetic and personal care brands. Recently, I have moved jobs to work for one of the world's largest consumer goods companies whose products are used by >2.5 billion people per day!

What does my job entail?

Essentially, my role involves performing a risk assessment to answer a question such as “can we safely use ingredient x at y% of product z?”.

To answer this question, I have to consider a number of different questions which include:

1) Does ingredient x have the potential to cause toxicity such as allergy and reproductive toxicity? If so, at what concentration?

2) When a consumer uses product z containing ingredient x at y%, what is the predicted dose that the consumer is exposed to?

3) Is the dose the consumer is exposed to higher or lower than the amount needed to cause toxicity? If it is lower, then how much lower? Do I have enough certainty to say that this difference is acceptable?

Why do I enjoy my job?

I enjoy that I get to use many of the skills I developed at university (both the in depth pharmacology knowledge as well as the more general skills like data interpretation and presentation skills).

I also love my job because if the assessment concludes that ingredient x is safe at y% in product z, it is immensely satisfying to see those products safely and successfully introduced onto the market.

But mostly I enjoy it because I get to make a difference in the toxicological community. Traditionally, in order for a chemical to have a full package of safety data i.e. we know its potential to cause allergy, reproductive toxicity (and many other types of toxicity) this requires thousands

of animal lives, costs potentially millions of pounds and can take several years. However, there has been significant investment in the development of non-animal approaches, and I am now part of a team at the forefront of improving the development, communication and legislative uptake of these approaches, which I am very proud to be a part of.

If the above sounds exciting, then a career in toxicology may be your cup of tea (or maybe your laundry powder or shampoo!).

From MSc at Liverpool to PhD in Singapore

Brian Hanotte

MSc Graduate - Advanced Biological Sciences (Cell Signalling)

I graduated from the University of Liverpool with an MSc as part of the class of 2019. I am now a PhD student in a world leading evolution and development (evo-devo) research lab at the National University of Singapore (NUS). Upon arrival in Singapore, I initially underwent a two-week quarantine period before moving into my permanent accommodation, the NUS halls of residence. Singapore is a very exciting and dynamic place to be a PhD student and even during the pandemic I have my hands full both with research and non-research activities. From the outset, my lab team were very friendly and welcoming, this made settling in and integrating easy. Life in Singapore is fast paced, however after a few months I feel like I have integrated and now call this island my new home.

My project is looking at the evolution and development of patterns found on the wings of Saturniid moths. My project is a wet lab-based project which involves transcriptome, genome and transgenic work. Currently I am designing guides for a CRISPR experiment. The aim of this experiment is to create a transgenic line through the knockout of certain key genes. The lab already has a few successful transgenic butterfly lines such as the ''yellow'' knockout butterfly line and I hope to add to one of these successful transgenic projects. The purpose of these knockout experiments is to determine gene function and regulation *in vivo*.

Figure 1. A fifth instar *Bicyclus anynana* caterpillar and pupae on a petri dish. The pupae will take a week to emerge into the adult butterfly.

This allows us to see what pathways are controlled by the ''knocked out gene'' and whether genetic co-option is available for these knocked out genes. This work gives us a deeper insight into the evolution and development of phenotypes and genotypes in Lepidoptera (butterflies and moths). When not in the main lab, my responsibilities include tending to the caterpillars as well as the adult moths and butterflies we have in the insectary. I also need to tend to the food plants which are kept in the green house and feed the butterflies, moths and caterpillars when required.

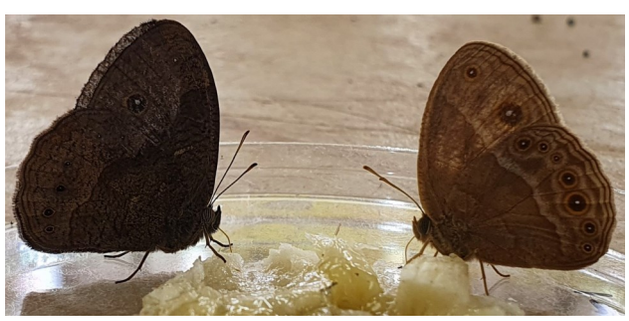


Figure 2. A dry season wild type and CRISPR yellow knockout *Bicyclus anynana* (African bush brown) enjoying some banana.

Outside of the lab other responsibilities I have as a PhD student are to attend lectures for PhD level modules and work as a teaching assistant (TA) for undergraduate modules. Being a TA is very enjoyable because as well as teaching the students you can go on field trips to special destinations within Singapore. As a TA I have found that I learn as much from the students as they learn from me. I have also been able to meet other TA's and professors from different labs within the biological sciences department, allowing me to make friends and expand my scientific circle. Currently I am a TA for the undergraduate second year biodiversity module and I have been fortunate enough to teach in several of the different Singaporean nature reserves, describing and observing the different animals and plants of the forests/mangroves which are found on the island.

Chasing my Dream

How I finally got my dream PhD

Jack McElhinney

MSc in Advanced Biological Sciences Graduate

After finishing my MSc in Advanced Biological Sciences, I knew I wanted to carry on looking into animal physiology, specifically in respiratory physiology. However, there were very few PhD programmes on offer that were even slightly related to what I wanted to study. I applied for PhD studentships in projects that I knew I wasn't completely engaged in and some had absolutely nothing to do with what I wanted to study. I just felt compelled to complete a Doctorate, any PhD course, I had to stay in research. After many failed attempts to get onto a course, I made a drastic decision. With no assurance of entering the scientific industry, I moved to where the animals I wanted to study are, Australia.

Before my MSc, I worked aboard research vessels looking at cetaceans. I'm fascinated by marine animals, but I'm obsessed with one group, in particular, sharks. I feel as though they are possibly the most misunderstood animals on the planet. Sharks are so unjustly demonized by Hollywood and the media to the point where masses of people have irrational fears of giant great white sharks in public swimming pools, c'est la vie. Instead of resigning myself to defeat, I decided to double down on my dream of becoming a marine biologist and booked a one-way plane ticket down under.

In February of 2018, I left Liverpool and arrived in Perth's summer. I found a job in a bar to keep me going while I tried to figure out where my life was going. I contacted many universities around the state, and I did some volunteer work with Bottlenose dolphins with a university research group. While out on a cetacean boat survey, we travelled past a sandy bay and a marina full of boats. The bay was part of the University of Western Australia (UWA) main campus, WA's most prestigious university, I knew that's where I needed to go.

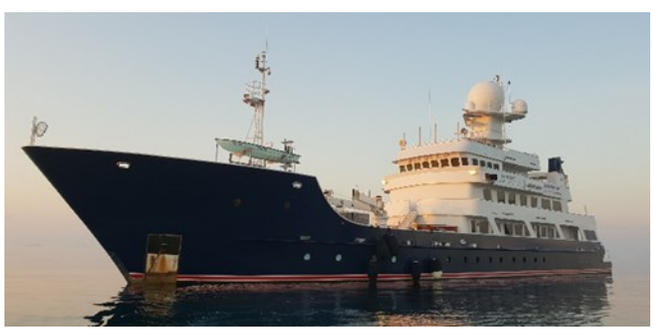
The thought of applying to the state's top university was daunting and I was aware of the research produced by this institution, being a shark fanatic myself. Nevertheless, I was inspired and I contacted everyone I could in the marine biology and oceans institute. I eventually got to meet Professor Jessica Meeuwig, and I presented my proposal for my research. Within one month of our meeting, I was spending the beginning of August in the Indian Ocean working for Professor Meeuwig's 'Marine Futures Lab'. Throughout this time I had no access to my emails, and I was waiting to find out if my PhD application was successful. Fortunately, this time it was.

I'm currently in my final year as a PhD candidate at UWA in the Marine Futures Lab. I've been researching how the world's warming oceans affect fish (including sharks), and how we can predict the severity of the outcome. I have some of the smartest minds in the world of marine ecology and biology on my committee which I'm extremely grateful for. However, I'd be lying if I said it's been easy. The PhD experience is different for each candidate, however, the unifying trial we all go through seems to be 'failure', research is not easy. As failure is a universal experience to PhD candidates, then resilience must be a trait in successful candidates. It was resilience, I later learned, that earned me a place in the lab.

The past few years have been so extraordinary. I have spent several weeks at sea aboard research vessels, swimming with and taking blood samples from sharks. I have landed in a seaplane in the middle of the ocean, spent a month aboard a superyacht, stayed overnight on

an airstrip in the bush, and I'm the first scouser to reach a small island north of Australia consisting of a tiny airstrip, surrounded by a reef! I also figured out sleeping is not considered a priority when in the field with some 16-hour workdays, you nap when you get the chance!

I love what I do, it is everything I've ever wanted - It feels cool to say that. So, I guess if you're in the same position I was in, when everything seems against you, don't give up. If you know what you want, don't stop until you've got it. Be resilient.



BiotechYES

Biotechnology Young Entrepreneur Scheme

Kiran Riasat[1], Stuart Gaines[2], John Gostage[2] and Daniel Hayman[3]

[1]PhD student, University of Liverpool; [2]PhD student, University of Sheffield; [3]PhD student, University of Newcastle

The BiotechYES competition is run for all Medical Research Council (MRC) funded students as an entrepreneurial scheme designed to give post-graduate students a real insight into business. The scheme runs yearly and each year there are three themes; i) Biomedical, ii) Energy, Engineering and environmental, iii) Plant, microbial and environmental. Each theme then contains within it a subcategory, and we applied to enter the biomedical theme focusing on diagnostics, drug delivery and discovery, healthy ageing and new tools for therapies. Our team consisted of four CIMA (Centre for Integrated research into Musculoskeletal Ageing) students Stuart Gaines, John Gostage, Dan Hayman and myself, based at the universities of Sheffield, Newcastle and Liverpool respectively.

The competition is highly centred around business innovation and therefore the idea you present doesn't have to be business ready, rather a well thought through concept. The competition offered us many opportunities to attend business seminars around pitching, finances, commercialisation and intellectual property. Our idea for this competition centred around a fitness app, which would collect data on users and we would then transparently sell this data to research institutes, enhancing research within healthy ageing and exercise. We developed a team name and brand "Lunate health" and really wanted to focus on the app idea as we believe, given the current climate, data and online privacy are a major concern for many individuals.

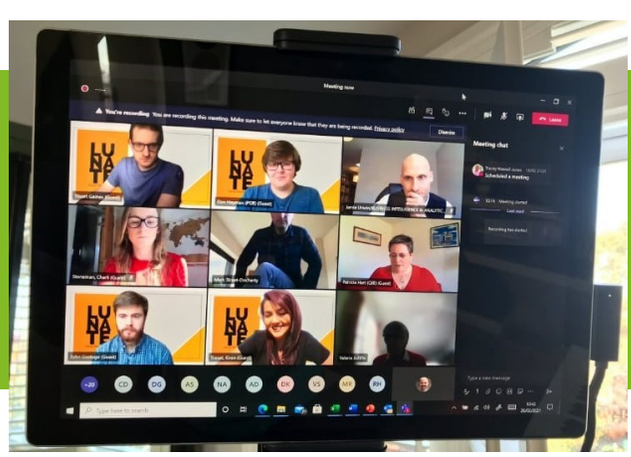
The competition, under normal circumstances, would have comprised of a residential visit to the designated theme lead business partner; for us this would have been at AstraZeneca's Stevenage office, but was all online due to the pandemic. We were still able to engage in mentorship and received valuable constructive advice on our idea. For prospective students we would highly encourage adapting your idea as you go along and always be open to the advice mentors give you. Some of the advice we received was to really consider the financial aspect of the business and understand that this competition is designed around the same theme of 'Dragons Den'.

The 'final product' is a 10-minute pitch to a panel of prospective investors; your idea has to be the most attractive in concept as well as returns in investment, and you could ask for as much investment as you want! We were asked questions around the business and the need for such a business considering the other fitness apps available, and we were also asked about the financial aspects of the business. We were not successful in this round and did not get to go to the final. The winning team was from Oxford and had designed an innovative point of care device that could sense cancer.

This experience was honestly an invaluable one, especially as a group of postgraduate students, from the mentorship and advice right through to the seminars we got to attend off the back of the competition. The work that went into the conceptualisation of the business was quite demanding especially as it was mostly online for us this year. We are very proud of our hypothetical business and would encourage others to take part in this scheme (recruitment for 2021 is now live!).

YES Unleash your ingenuity
YOUNG ENTREPRENEURS SCHEME

For more information visit www.yescompetitions.co.uk.



Want to get involved with Insider Imprint?

Tips from the Insider Imprint Team

There are lots of opportunities to get involved with Insider Imprint. So many in fact, that you might not know where to start! Here are some tips and suggestions to get you off the starting line.

"If you want to write for us but you are not sure what we publish, look at our previous issues on our website so see what we have published before for inspiration"

~ Fabia

"Think about what you are really interested in - it is much easier to write about something you are excited about"

~ Emily

"Don't forget we publish lots of different articles types, from shorter reflections to longer commentaries, as well as artwork. Also, we are always open to publishing something we have not had in the journal before, so if you have an idea, just ask us!"

~ Manohar

"You can listen to our podcast - you will find interviews with previous authors, so you can find out more about publishing with us from the authors themselves!"

~ Cat

"Thinking about becoming an ambassador but you are not sure? Contact us and one of the team can chat to you about it, no problem."

~ Heather

"Remember to look at our publish with us page on our website, here you will find out about our publishing process and you will find our author guidelines to help you get started."

~ Natalie

"If you have any questions about publishing with us, just drop us an email at insiderimprint@liverpool.ac.uk. We will be happy to help!"

~ Liam

Wellbeing

The importance of mental health

Emotional health can affect all aspects of life and can affect how you feel, think and behave. Being able to talk about any issues that concern you will make you feel empowered and help you find strategies to cope better.

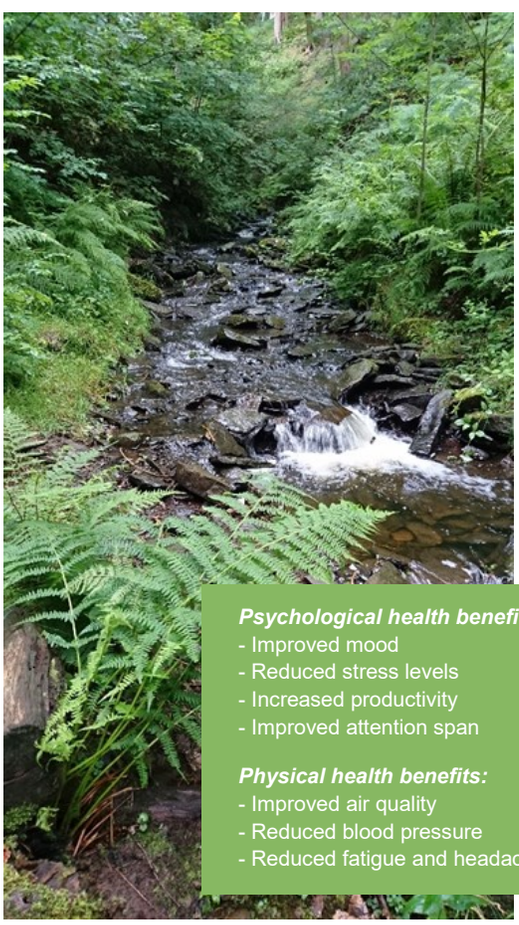
In the next few pages you can find some advice, suggestions of activities to help you relax and contacts if you need someone to chat to.

Getting back to nature

Megan Harrickie

3rd year Genetics BSc

It is common knowledge that plants have been shown to improve our psychological wellbeing and physical health. Looking after our mental wellbeing has become as important now in our current environment than ever before. Mental wellbeing does not have one definitive definition but can be interpreted in many ways; how we feel, how are we coping within our daily life, how we are coping with the ups and downs of the every day.



Psychological health benefits:
- Improved mood
- Reduced stress levels
- Increased productivity
- Improved attention span

Physical health benefits:
- Improved air quality
- Reduced blood pressure
- Reduced fatigue and headaches

In life, students can experience different stresses and worries both at university and at home. I live away from home in student accommodation with other people who I have never seen or met until I moved in. I study Genetics which can be a busy course especially in the third year with your project which needs to be researched in depth and written up, alongside constantly working on other modules with deadlines piling up. It can be hard to balance time to focus on everything, and breaks are needed. Having a plant in your room or around the house can be extremely beneficial as it encourages you to take a breath and focus on something else for a bit.

Personally, I have always been interested in plants but over the summer during lockdown, since my internship was cancelled and the world was a bit crazy, I had the time to focus my mind and improve my mental health by working on my garden at home. It provided me with the opportunity to create a living wall which I have always loved the idea of. I went for a mixture of climbers, evergreens and flowering plants so it won't always look the same, it will change with the season.

If you don't think you can keep a plant alive there are a variety of different low maintenance house plants that can easily help solve this and are affordable. I currently have two on my windowsill and they are absolutely thriving.

A huge tip with any plant indoor or outdoor, would be to consider the importance of drainage. Excess water needs somewhere to go, this is why the majority of house plants die because people often over water them and they end up sitting in water, killing the roots. If you are planting outside put holes in the bottom of the planter. For indoor planters you can keep the plant in the plastic pot it has been bought in and then just place it into a nice ceramic pot you have found. This also allows you to remove the plant for easy watering. If the top of the soil feels dry try and get two inches into the soil to be sure it is dry before watering.

Here are 3 examples of low maintenance plants I own. It is best to buy your plant in person to check the health and condition. I recommend a little local independent plant shop called Roots located in Liverpool Bluecoat.

1. *Microsorum musifolium* = Crocodyllus

This particular plant got the name Crocodyllus due to the scaly reptilian pattern on the leaves. It requires a temperature around 18°C-23°C and high humidity; this can be achieved by misting but what I do is put the plant in the bathroom when the shower is on and I even water it with the shower head on a warm temperature to get the same affect. When this plant produces new leaves, they are tightly curled up and over time you can see it slowly unravelling.

2. *Pilea peperomioides* = Chinese money plant or friendship plant

Supposedly, this plant provides good fortune and abundance to their owners getting its name money plant due to the shape of the leaves being circular and flat looking like coins. But it is known that this plant is a productive air purifier. It prefers a temperature between 15°C-30°C. A normal room humidity is good for this plant so it doesn't need to be in the bathroom and simple watering under the tap is fine. As this plant matures it can lose the lower leaves and begin to look more like a tree. It can also be propagated later on in life so you can have plenty of luck. (Propagation is the process of creating a new plant from a cutting or by separating the plant during repotting.)

3. Schlumbergera = Christmas cactus

A low maintenance flowering plant, so called the Christmas cactus because it flowers late November to late January making it an ideal Christmas gift apparently living for hundred years. So far, I`ve had mine for three years and it is still going strong producing pink flowers that bud at the end. As the name also suggests it's a cactus, so not a lot of water needed during the winter but you can increase watering during summer months. This plant may need a bit of pruning if it starts drooping too much, most likely after a couple of years but fear not if you put the cuttings into some compost soil, in sun light and water sparingly it should take root within three to twelve weeks.

All of these plants aren't a fan of direct sunlight; it can burn the leaves so another tip is to turn them around throughout the day if where you have put them gets constant sunlight. Even if they are not in direct sunlight, rotating allows even growth. Bonus, all the plants mentioned are nontoxic to humans, cats and dogs.

If these three plants aren't your style there are plenty out there, go into your local plant shop and ask questions as they are more than happy to help. Alternatively, there are many books, such as '*The little book of house plants and other greenery*' by Emma Sibley.

It is a fact that plants are also beneficial for the environment. Having plants in your garden will attract pollinators such as birds, insects, bees, butterflies etc. Besides the environmental importance of these amazing animals, they are also visually pleasing to watch in outdoor spaces.

At the University of Liverpool, we are very fortunate to have the Green Guild that enables students to get back to nature and help the environment. The Green Guild have a number of initiatives including beehives, fruit and vegetables growing on the guild roof, the living wall on the side of the guild. The Green Guild offer many opportunities that are advertised on social media throughout the year that students can get involved in, to help students get their hands dirty by planting or helping out with the beehives.

Now, I appreciate plants are not for everyone, but there are numerous alternative activities that help with personal wellbeing and mental health. Turning off your electronic devices, walking around campus or Sefton Park (I enjoy walking around the Royal Albert Dock if I want to get away), exercise, socialising via zoom, reading, listening to a podcast etc.

Most importantly it won't be perfect at first, but it is worth its weight in gold prioritising self-care and mental wellbeing. You just have to find activities and hobbies to fit around your lifestyle and interests. You may even discover more about yourself along the way!

Wellbeing Reminders

From One Student to Another

Rebecca Court

2nd year Zoology BSc

With the marriage of the 'best years of your life' with the perfect storm for loneliness, mental health and wellbeing should rightly be brought to the forefront of any student's concern during a global pandemic. All aspects of our lives have recently become much harder to maintain and upkeep, from routine to healthy eating. Instead of the usual tips and tricks, I wanted to provide a few reminders you should tell yourself as we continue the battle of holding onto a life buoy of positivity in what feels like a hurricane of anxiety.

Let yourself feel

Be honest with yourself and how you're feeling: take note of your emotions. We all too often ignore or push past negative feelings, telling ourselves that things are just temporary and will improve 'soon'. But it's so important to notice when you are feeling low and to let it all sink in. Life is rubbish at the minute, and we can't expect ourselves to put on a brave face each day. Somewhat conversely, don't try to work your feelings out; not everything has a solution.

University takes a lot of time and energy so remember, if you're not feeling it, give yourself some time off. No one is productive when they're not in the mood to be! There is very little point in forcing yourself to sit at your desk when it will just lead to a vicious cycle of stress. We've all needed to hear this at some point.

Exercise

We know we should exercise, but we can't all be fitness influencers. Home workouts are definitely not for everybody and it's absolutely okay to struggle to find the motivation to even move off your bed. But the benefits for our mental health and fitness that exercise bring cannot be ignored, particularly when a lot of movement is restricted right now. A walk outside (I'm sure you've been on a few), dancing about or doing some morning stretches is enough to get yourself away from the dastardly desk and bed duo, to move your body and to give yourself some me-time. Alternatively, check out any fundraisers going on for charities at the moment to boost both your heart rate and charity pots.

You're a person, too

Often with assignments and big, long to-do lists we can forget that university is only a portion of our lives. Think about what your current life would be like without COVID restrictions (without shedding a tear) and try to recreate some of the situations, as allowed.

Get in touch with old friends and/or do something you KNOW will cheer you up. Reading permits a fictional escape to worry about someone else's life, small crafts (remember sequin art?) can keep you feeling accomplished whilst binge-watching, and cooking or baking will always reap great rewards. Keeping busy is an important factor in acknowledging our self-worth, not to mention passing the time whilst waiting for normality to return.

You are missed

Lockdown and so much time to ourselves means we're craving seeing familiar faces and being close to the ones we love. But this is not a one-way street: you are that person who others are missing too. Others are thinking of you and can't wait to see you, so you are not alone in that feeling, guaranteed. Let people know you're thinking of them and don't be surprised when you hear it back.

So, as we face more remote learning and balancing student life with attempted inhibition of grey hair growth, remember not to expect too much from yourself, but to keep as proactive as you can and always call on someone for company when you need it.

We all know these things inside, sometimes we just need to hear - or read - it.

Peer Mentoring

Provide a helping hand to every student who is about to embark on a new and exciting chapter of their life

Kate Murray

Peer Assisted Learning and Wellbeing Influencer Lead, Student Success Projects Officer

Are you keen to add value to your University experience? **Why not try mentoring and support your fellow students with their wellbeing?** Not only is this a great opportunity for you to engage further with your peers, it's also an excellent addition to your CV. The central peer mentoring programme has now evolved into two strands: The Faculty offer will comprise of course-specific peer assisted learning, and the central offer will centre on a 'wellbeing influencers' scheme. We will be appointing students for both schemes during the summer months and the roles will be publicised on all relevant University platforms. However, if you are keen to find out about activities you can support with right now, please contact us directly at peermentors@liverpool.ac.uk.

So, mentoring - what is it?

The Wellbeing Influencers will sit within Student Success which is part of the University's Careers and Employability team, whilst all peer assisted learning activity will be Faculty led. The previous mentoring programme was an excellent opportunity for cross collaboration and the Student Success Team have initiated a peer mentor community of practice whereby mentoring plans are discussed with colleagues across the wider University. The vision is to instil a culture that ensures every student always has a friendly face to go to.

More about the Wellbeing Influencer role and embedding a sense of belonging to every student's experience…

Wellbeing Influencers will undertake training so you can support students with a wide variety of areas. You will play an integral role in ensuring a sense of belonging is embedded into every student experience, and to decrease feelings of isolation. As Professor Liz Thomas wrote in the final report from the 'What Works? Student Retention & Success programme', this should be a high priority for all programmes, departments and institutions.

*'…based on evidence from across seven higher education institutions of all types a significant minority of students consider withdrawing, and thus improving student belonging should be a priority for all programmes, departments and institutions. …students who think about leaving are more likely to do so than those who have not considered withdrawing' (*Thomas, 2012).

What can you expect from our wellbeing influencers? How can you reach out to one?

Previously, mentors were available via Handshake to answer any queries, and we will look to use the same platform for our influencers. However, they will have a wider reach and will support with various social media channels across the University, as well as continuing to provide useful resources on wellbeing.

Want to hear more about wellbeing?

You can also watch our full suite of Peer Mentors Recommend sessions at a time that is convenient for you. Here, our previous cohort of mentors cover everything from books, podcasts and recipes, to tips on useful ways to work from home.

University of Liverpool Wellbeing Influencers stand for diversity, equality and inclusion…

Now, more than ever, we need to come together as a community to support one another and represent every student voice at the University. When we consider a sense of belonging, it is essential to ensure all activities exemplify a diverse, equal and inclusive approach. This is, of course, integral to all University activity but as a Wellbeing Influencers you will play a major role in sharing this message with other students. During our recruitment process, and through all mentor events, we will reach out to students who are underrepresented so that our cohort embodies every student voice. We will ensure that every student is aware of the mentor support that is available to them through continually reaching out to all University societies during the

Thomas, L. 2012. *Building student engagement and belonging in Higher Education at a time of change: final report from the What Works? Student Retention & Success programme.* Available from: https://www.advance-he.ac.uk/knowledge-hub/building-student-engagement-and-belonging-higher-education-time-change-final-report

recruitment process, and by sharing all Peer Mentor events on as many platforms as we possibly can.

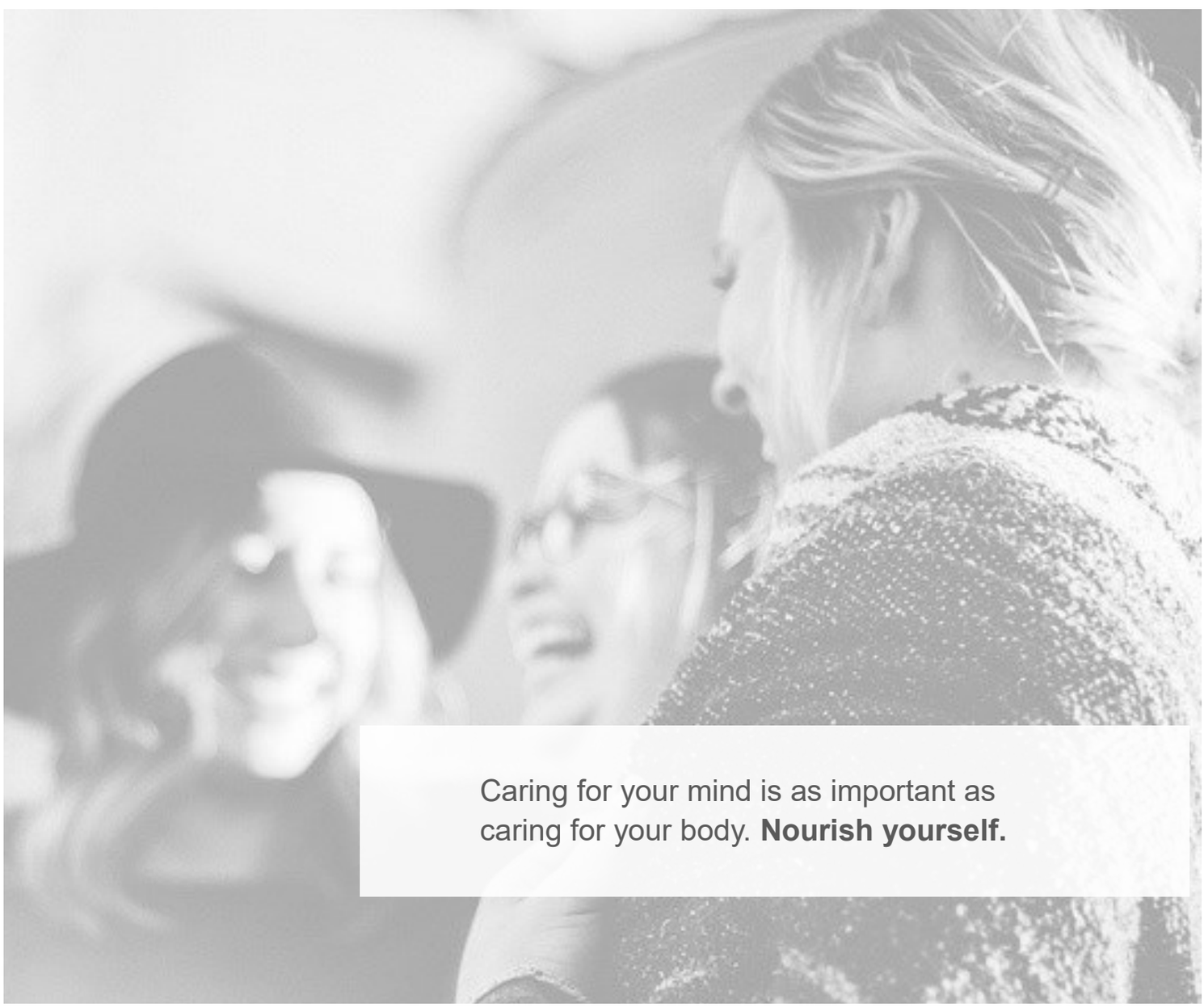
We can always learn more! Please get in touch…

The ability to recognise and be open to new and improved ways of working is essential and we can always listen and learn more. It is for this reason that we want to hear from you! We welcome your feedback so, please reach out to us at peermentors@liverpool.ac.uk if you would like to put forward any ideas or suggestions on anything we can add to the scheme, or if you think there is anything we can do differently.

Other ways to keep up to date…

You can also keep up to date with all of our activity on our **Twitter page** @livunimentors. And, why not visit our **webpage** https://student.liverpool.ac.uk/road-to-success/peer-mentoring/.

We look forward to beholding the excellent support you will provide to your peers!



Student Support Services at Liverpool

Student Welfare Advice and Guidance are part of Student Services at the University and are based in the Alsop Building on University Square. Our role is to provide students with advice, support and information, particularly around financial issues (**Money Advice and Guidance**), disability (**Disability Advice and Guidance**), international queries (**International Advice and Guidance**) and welfare issues (**Advice and Guidance**). Through our reception service we act as a general point of referral to other services both within and outside the University. Individual staff with specialist knowledge and experience see students by

About the Wellbeing Team

As part of Advice and Guidance, the Wellbeing Team provides information and ongoing advice and support to students around any issues that may be affecting their day-to-day life or having an impact on their studies. Wellbeing Advisers offer confidential support via email and one-to-one appointments, allowing students the opportunity to discuss these issues in a safe space and offering advice on how they can make changes to overcome their difficulties. In some cases this will be looking at the thoughts and perspective that someone is bringing to their situation, at other times students may need guidance around adapting their routine and patterns of work and rest, or support in implementing a new way of doing things. The Wellbeing Advisers can help set manageable goals and provide follow-up appointments to check on progress, identify barriers and pitfalls, and keep students on the right track to make the changes that they want to see for themselves. The advisors can also talk with a student about ways of improving wellbeing and mental health.

For students that may require specialist support in a particular area of their life, the team can help a student to identify suitable options of support and make decisions about the right approach to take. Often students will have a range of issues affecting them at the same time, the team can help to disentangle and prioritise individual difficulties so that they are less overwhelming and allow a student to choose a clear path forward. The Wellbeing Team can also discuss the suitability of further mental health support and refer students to single session therapy with the Counselling Team at the University or the Mental Health Advisory Service. Single Session Therapy is designed to address students presenting concerns within one session. This appointment with a counsellor is usually sufficient for many students. At the end the single session, the counsellor may provide students some further resources to read through in their own time. This is a chance to explore different coping techniques and sources of support. If appropriate additional counselling sessions can be put into place for students.

The Wellbeing Team also offer a range of self-help materials and resources, all of which are available for free and easily accessible through our website. Some of these include; links to external organisations where students can find further specialist support, access to SilverCloud, a programme based on cognitive behavioural therapy with modules on managing anxiety, stress and depression and more information on Fika, a mental fitness tool to help stay motivated, productive, focused and connected during this period of remote work and study. Recently, the Wellbeing Team have produced a Newsletter and so far covered topics of loneliness and isolation, self-care and motivation with further subjects due to be discussed in the coming months, as well as presentations for students to attend to find out some useful tips to manage their wellbeing and make the most of their experience at University. The Wellbeing Team also have in place a named contact for Local Authority Care Leavers and provide support for students who are estranged from their parents. The contact can liaise with funding bodies in cases where a student is trying to establish independent status on the basis of estrangement. In order to contact the Wellbeing Team, students can email advice@liverpool.ac.uk

About Money Advice and Guidance

Money Advice and Guidance (MAG) is part of Student Welfare Advice & Guidance and provides support and advice to both current and prospective students on a range of financial matters. The team offers discretionary financial help through the University Hardship Fund for students experiencing financial difficulty, advice on statutory funding for full & part-time undergraduates, budgeting sessions aimed at improving money management, debt advice service, advice on funding for EEA migrant workers and other EU students who may qualify for UK funding, eligibility and calculation of welfare benefits and advice and support for students who are carers. If a student has any previous higher education study, or if they are planning to withdraw, suspend, transfer or repeat part of their programme, their entitlement to funding may be reduced, please contact the team for further information: money@liverpool.ac.uk

About Disability Advice & Guidance

Disability Advice & Guidance (DAG) is responsible for the co-ordination of individual support for disabled students. The team provides a specialist guidance and support service for all prospective and current students. The support offered includes confidential one-to-one appointments, daily appointments, assistance with obtaining funding to support disability related study needs (e.g. Disabled Students' Allowance), arrangements for study needs assessments, loan of specialist equipment and software, liaison with academic and other University departments to communicate & implement reasonable adjustments, access to Independent Specialist Support Tutors, provision of study assistance (e.g. note takers, mentors, library assistants) and development of a Student Support Document to identify appropriate reasonable adjustments. Students can contact the team on disteam@liverpool.ac.uk

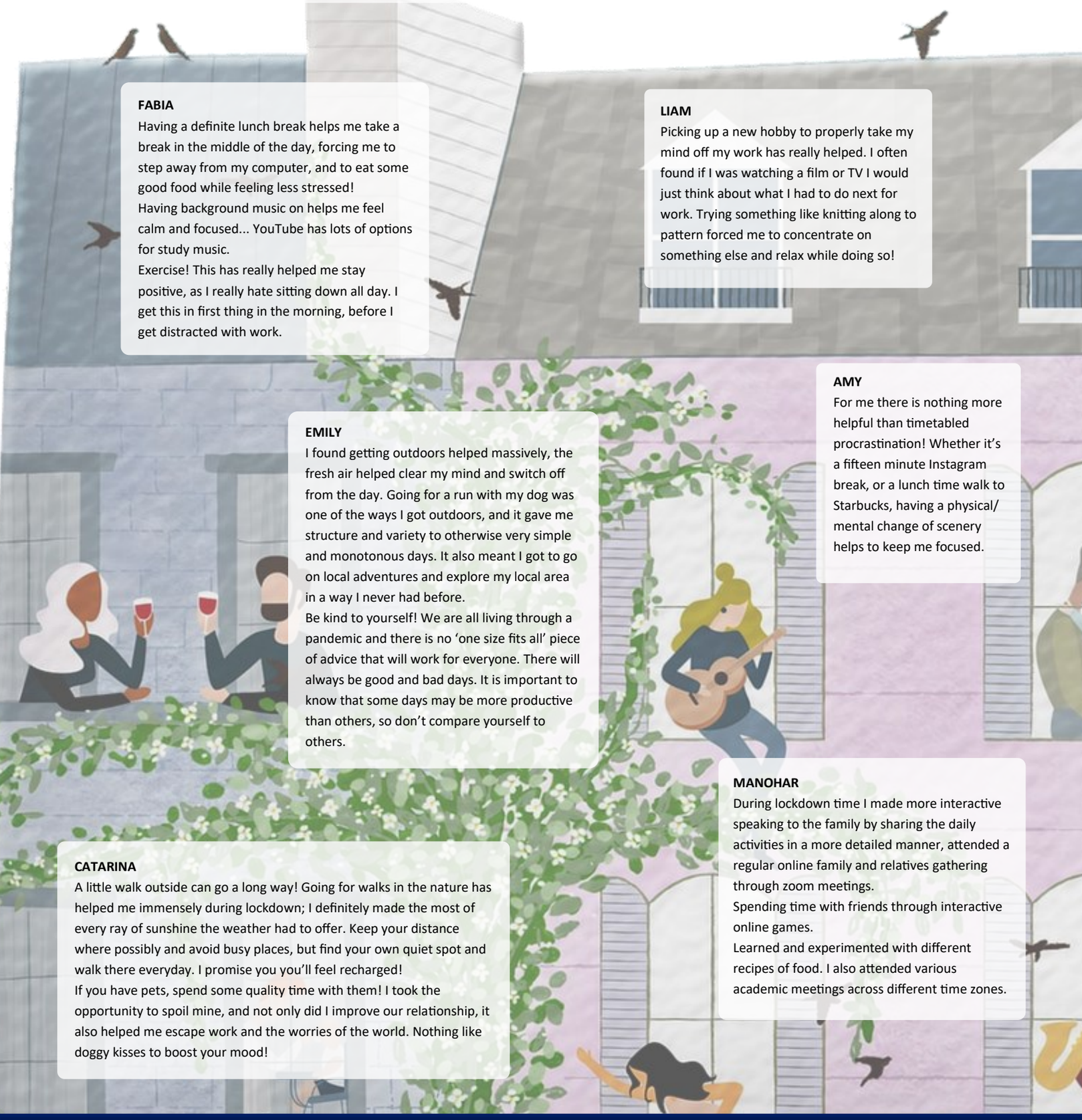
About International Advice and Guidance

International Advice and Guidance (IAG) is the central point of contact for international and European students throughout their study at the University. IAG provides support for international students to assist with a range of issues including immigration, finance, safety and welfare; specialist immigration advice including sessions on how to extend your student visa; presentations and workshops throughout the year on a range of topics including 'Working in the UK After Your Studies'; regular updates on current international student issues; opportunities for students to enhance all aspects of their student experience; comprehensive web based information; information and assistance for families of international students. To contact IAG, students can email iagteam@liverpool.ac.uk

Remember, there is lots of help available for students - visit Liverpool Student Support Services: www.liverpool.ac.uk/studentsupport/

Surviving lockdown

Tips from your editors & ambassadors



FABIA

Having a definite lunch break helps me take a break in the middle of the day, forcing me to step away from my computer, and to eat some good food while feeling less stressed!

Having background music on helps me feel calm and focused... YouTube has lots of options for study music.

Exercise! This has really helped me stay positive, as I really hate sitting down all day. I get this in first thing in the morning, before I get distracted with work.

LIAM

Picking up a new hobby to properly take my mind off my work has really helped. I often found if I was watching a film or TV I would just think about what I had to do next for work. Trying something like knitting along to pattern forced me to concentrate on something else and relax while doing so!

AMY

For me there is nothing more helpful than timetabled procrastination! Whether it's a fifteen minute Instagram break, or a lunch time walk to Starbucks, having a physical/ mental change of scenery helps to keep me focused.

EMILY

I found getting outdoors helped massively, the fresh air helped clear my mind and switch off from the day. Going for a run with my dog was one of the ways I got outdoors, and it gave me structure and variety to otherwise very simple and monotonous days. It also meant I got to go on local adventures and explore my local area in a way I never had before.

Be kind to yourself! We are all living through a pandemic and there is no 'one size fits all' piece of advice that will work for everyone. There will always be good and bad days. It is important to know that some days may be more productive than others, so don't compare yourself to others.

MANOHAR

During lockdown time I made more interactive speaking to the family by sharing the daily activities in a more detailed manner, attended a regular online family and relatives gathering through zoom meetings.

Spending time with friends through interactive online games.

Learned and experimented with different recipes of food. I also attended various academic meetings across different time zones.

CATARINA

A little walk outside can go a long way! Going for walks in the nature has helped me immensely during lockdown; I definitely made the most of every ray of sunshine the weather had to offer. Keep your distance where possibly and avoid busy places, but find your own quiet spot and walk there everyday. I promise you you'll feel recharged!

If you have pets, spend some quality time with them! I took the opportunity to spoil mine, and not only did I improve our relationship, it also helped me escape work and the worries of the world. Nothing like doggy kisses to boost your mood!

HEATHER

Don't be so hard on yourself, working remotely is difficult and it's hard to be as productive as you would if we weren't living through a global pandemic. If I find myself procrastinating or unable to focus then I take a break from my computer and do something else for 15 minutes (like getting some fresh air or having a cup of tea). I find this helps me to come back to my computer with a fresh perspective.

PRAKRITI

Listening to songs or low beat background music while working calms me down, going on walks, or short aerobic/ dance session, making good food, or reading something other than coursework like some novel. If sometimes I feel overwhelmed, I write in my journal all the thoughts. Also I recently started meditating, and I am finding this really useful.

NATALIE

Having a timetable has really helped me, including short- and long-term goals, I plan my week out in advanced making sure to include some free time.

As difficult as it can be to motivate myself exercising can help especially when it is outside, even if it is just for a walk, it really helps with my mental health.

Do something fun, it helps to break the week up by having scheduled activities, I like to have games night every Friday with friends over Zoom.

MEGAN

I found keeping in touch with friends extremely important throughout lockdown, especially for my mental health. Whether university course mates or friends from home, having evening plans to video call and finding various things we could do virtually helped me to still feel connected even if we were all stuck indoors. It also allowed me to take a break from university assignments and deadlines, look forward to something and have a bit of fun!

I also found podcasts super useful when needing a small break, or when I went for daily walks. Whether it was a funny, educational or documentary podcast, it kept my brain thinking but in a different way to how I was doing university work. This kept me sane in an unpredictable time such as lockdown.

TIERNEY

My main survival tip for lockdown is to stick to a sleep pattern as best as possible. Lockdown has been pretty unpredictable at times, but I find sticking to a sleep pattern has helped manage that and allows me to be as organized with my day as I can be, even if I don't end up doing much work, if at all.

Secondly, don't be too hard on yourself. Take breaks and time away from your work if you need to. Go watch a new show on Netflix, listen to a new podcast, or pick up that book that you'd forgotten you were reading. Go for a run, workout, take up yoga. Lockdown has shown how important mental health is for all of us, and at times we forget that we can take days away from work to look after our mental wellbeing.

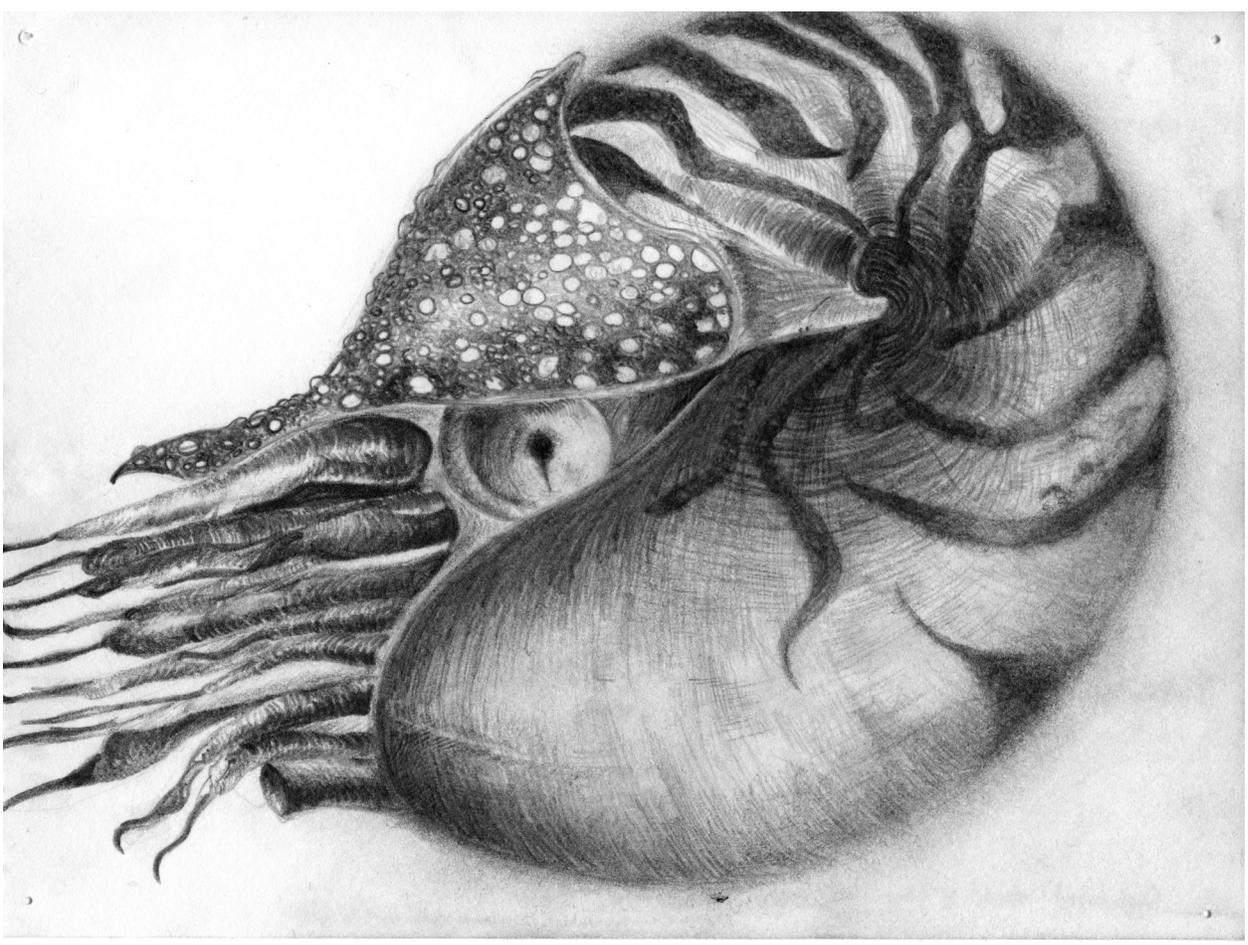
Artwork

When art meets science

Often seen as opposites, science and art can complement each other. Check out our students' amazing artwork!

The Chambered Nautilus

– a "living fossil"



“One of the oldest surviving cephalopods, the Chambered nautilus (*Nautilus pompilius*) is a curious organism distinguished by its Fibonacci spiral, tens of appendages and an intricately connected system of chambers inside the shell to control its buoyancy and position in the water column. Whereas its locomotory strategy by jet propulsion is similar to other species in the same class, its cognitive function is significantly reduced, and the underdeveloped eyes lack corneas. This more primitive physiology of the *Nautilus pompilius* is similar to its fossilised ancestors from 500 million years ago, showing this “living fossil” has undergone little evolution since the Paleozoic era.

This Chambered nautilus remains understudied; now the species is also in drastic decline due to overharvesting, as its shells are deemed valuable hunting trophies. Due to the low migratory ability and the isolated populations of the nautilus, further decreases to its population size will endanger its survival; and without the immediate implementation of conservation strategies, the *Chambered nautilus* may be lost forever. This illustration is an effort to introduce this peculiar organism to a wider audience, promote its conservation efforts, but also increase awareness of the detrimental effects of overharvesting on the nautilus, along with other organisms.”

Erika Kupyrova

3rd year Zoology BSc

Dead lamb

“This impressionist oil painting was created as part of a project exploring the darker realities of farming lives. It depicts a still born lamb with Schmallenberg; the body and limbs deformed and blood seeping from the joints, mouth, and nose. I wanted to convey the juxtaposition between the warmth and joy of bringing new life into the world, something I learnt to do as a young child, and the cool tones associated with death and disease, something also taught young in my community. Lambing is often a lovely time of year (despite the later nights and earlier mornings) with few casualties and representing this with the iodine wash background seemed fitting in the context of the piece, as iodine is used to prevent ambilocal infections and expresses the care for animal’s welfare. The represented trauma of the Schmallenberg outbreak contrasts with this background by use of subjective representation through surreal impressionism, the obvious distressing imagery, and the exposed neck of the still born highlighting our vulnerability to tragedy and need for compassion. While there are many cases where livestock and their carers die in tragic circumstances, I felt it important to draw attention to this example particularly due to the cultural significance of lambs. They are a species as old as the landscapes we farm and a common symbol, even in modern day, of life, youth and innocence, something that disease takes from individuals, livestock or not. Death is undoubtably a part of life, but the deformed dead lamb drives home that it can often be out of our control, hard to come to terms with and a distinctly uncomfortable truth.”

Jemima Western

Final Year MBiolSci (Biological Sciences)


Insider
Imprint