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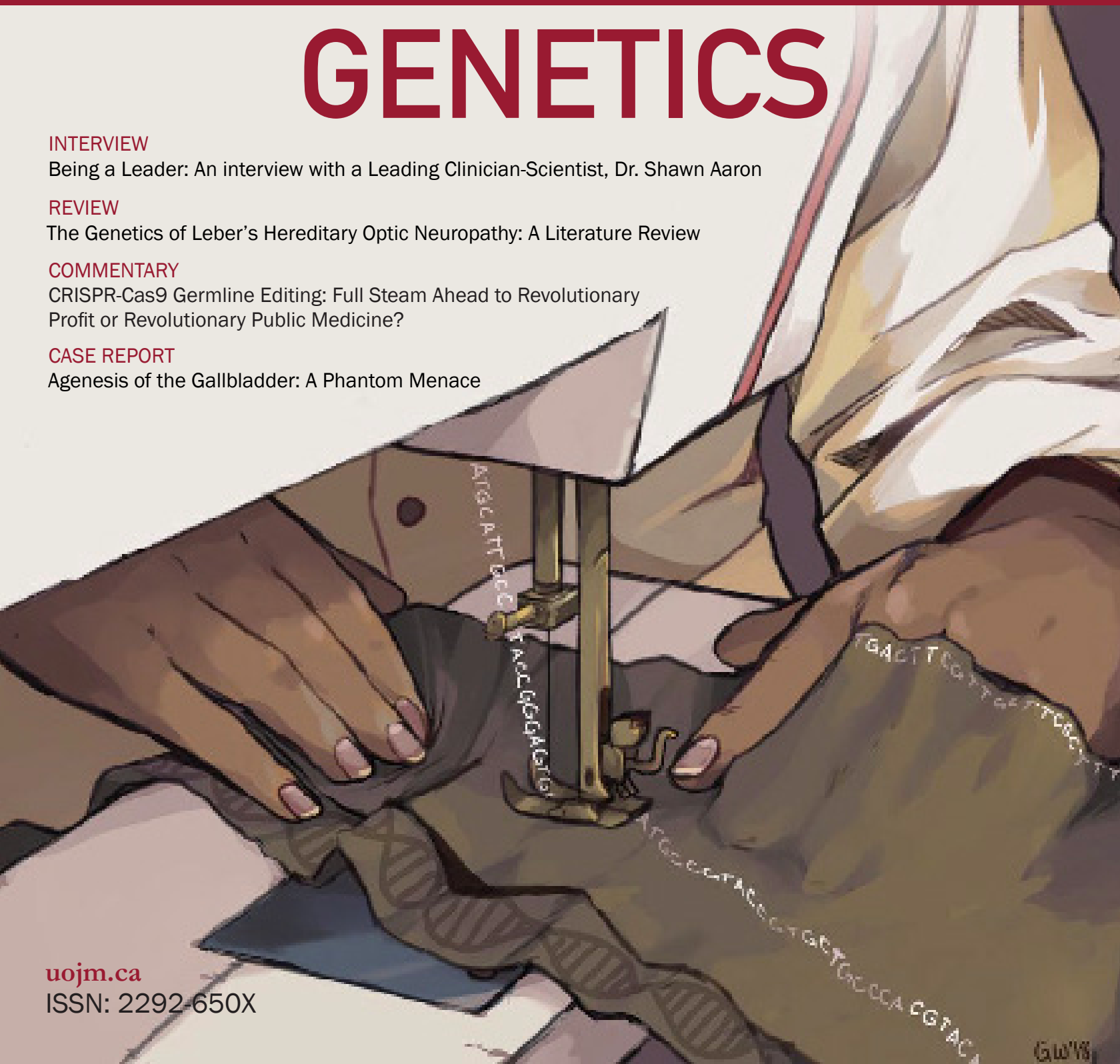

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ABOUT US

UOJM is an international peer-reviewed journal led and published by the students of the Faculty of Medicine. We welcome submissions in a variety of areas in biomedical research and feature original research, review articles, news and commentaries, case reports and opinion pieces. Our articles are written in both English and French, and represent the only bilingual medical journal in Canada run by students.

Le JMUO est un journal revu, édité et publié par les étudiants de la Faculté de médecine. Nous encourageons les soumissions d'une variété de différents domaines en recherche biomédicale et publions des articles de recherche originale, des articles de revue, des nouvelles et commentaires, des rapports de cas et des pièces d'opinion. Nos articles sont écrits en français et en anglais et représentent le seul journal médical bilingue géré par les étudiants au Canada.

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UOJM: Preface

Now at the end of its 8th cycle, the University of Ottawa Journal of Medicine continues to proudly showcase the research and perspectives of graduate and medical students within Canada, as well as internationally. A reflection of this, the UOJM has sustained a dedicated readership and contributor base as Canada's only bilingual, academic medical journal. During 2018, our presence at the Ontario Medical Students' Weekend, the 6th Annual Action Global Health Network Conference, and the Interdisciplinary Student Research Conference on Healthcare has allowed us to reach students with backgrounds in medicine and basic science, as well as those completing degrees in Law, Nursing, and Human Kinetics, to name a few. Furthermore, we continue to host seminars focused on peer-review, academic writing, and bench-to-bedside research, ensuring ample educational opportunities for students of all Faculties.

UOJM 8.2: Genetics explores the advances, as well as the inherent barriers to progress in the dynamic and rapidly-growing fields of basic and clinical genetics. Beyond the building blocks of DNA itself, our understanding of the human genome at a structural level has allowed us to tackle the broader domains of human health, including immunology, cancer, neuroscience, chronic illnesses, and much more. Thus, this issue focuses on the implementation of gene editing technologies to understand and effectively fight inheritable genetic diseases, vector-borne illnesses, and psychological disorders.

Looking forward, we are excited to announce our **Spring 2019 issue on Medical Innovation.** Every year, healthcare professionals and scientists revolutionize the study and practice of medicine: from breakthroughs in diagnostic and imaging software, to multinational drug trials and novel transformative therapies for disease management. In the past decade alone, society has been witness to a paradigm shift in how medicine fights back against invading cancer cells, how statistics can predict the course of a disease prior to its first symptom, how artificial intelligence can be used for image-recognition in radiology, and how surgeons can use wireless networking to perform operations from across the Atlantic. This innovative spirit is what drives patient care and what will inspire future individuals to pursue a career in healthcare. Thus, Issue 9.1 will serve as a love letter to the innovative and passionate medical minds who push boundaries and tirelessly strive to improve the lives of those around them.

The submission deadline for our **Spring 2019 issue is March 1, 2019.** High-quality writing will be recognized with an honorarium award. Submissions can be made online, and details regarding article formatting and the submission process can be found on our website at www.uojm.ca.

We hope you enjoy the UOJM's Genetics issue!

Senior Editors-in-Chief
Danny Jomaa
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Junior Editors-in-Chief
Linda Fei
Phillip Staibano

JMUO: Préface

Maintenant dans son 8e cycle, le Journal médical de l'université d'Ottawa continue à fièrement mettre en exergue la recherche et les perspectives des étudiants de troisième cycle et de médecine, au Canada, ainsi qu'internationalement. En réflexion de ça, le JMUO a soutenu un lectorat et des contributeurs dévoués comme le seul journal scolaire médical bilingue au Canada. En 2018, notre présence à la fin de semaine des étudiants de médecine de l'Ontario (OMSW), à la 6e conférence annuelle Réseau santé mondiale action, et à la conférence de recherche estudiantine multidisciplinaire sur les soins, nous a permis de rejoindre des étudiants de médecine et de sciences, ainsi que ceux qui complètent des études dans le droit, les sciences infirmières et les sciences de l'activité physique, entre autres. En plus, nous continuons à héberger des séminaires sur les revues par les paires, l'écriture scolaire, et la recherche du « banc au chevet », en assurant de nombreuses occasions éducationnelles pour les étudiants de toutes les facultés.

UOJM 8.2 La génétique explore les avancements, ainsi que les barrières inhérentes, dans la progression des champs dynamiques et croissants de la génétique de base et clinique. Au-delà des composantes de base de l'ADN, notre compréhension du génome humain à un niveau structural nous permet d'adresser les vastes domaines de la santé humaine, y inclus l'immunologie, le cancer, la neuroscience, les maladies chroniques et d'autres. Ainsi, ce numéro vise l'implémentation de technologies éditrices de gènes pour comprendre et combattre effectivement les maladies génétiques héréditaires, les maladies menées par les vecteurs, et les désordres psychologiques.

Prochainement, nous avons le plaisir à vous annoncer notre numéro de **printemps 2019 : Les innovations médicales.** Chaque année, des professionnels de la santé et des scientifiques révolutionnent l'étude et la pratique de la médecine : dès les percées en logiciels diagnostiques et d'imagerie, aux essais de drogue multinationaux et thérapies importantes qui révolutionnent fondamentalement la gérance des maladies. Dans la dernière décennie seule, la société a témoigné un changement de paradigme comment la médecine se bat contre des cellules cancéreuses envahissantes, comment les statistiques peuvent prédire l'évolution d'une maladie avant ses premiers symptômes, comment l'intelligence artificielle peut être utilisée pour la reconnaissance d'une image en radiologie, et comment les chirurgiens peuvent utiliser des réseaux sans fil pour opérer à travers de l'Atlantique. Cet esprit innovateur pousse les soins et sera l'inspiration aux individus futurs qui désirent poursuivre une carrière dans le domaine de la santé. Ainsi, le numéro 9.1 se servira comme une déclaration d'amour pour tous les esprits médicaux innovateurs et passionnés qui poussent constamment les frontières et s'efforcent sans relâche d'améliorer la vie de tous ceux qui les entourent.

La date limite de soumission pour notre numéro de **printemps 2019 est le 1er mars 2019.** L'écriture de haute qualité sera récompensée avec un prix d'honneur. Les soumissions peuvent être faites en ligne, et les détails sur le format des articles et le processus de soumission peuvent se trouver sur notre site web www.uojm.ca.

Nous espérons que vous aimez le numéro du JMUO sur La génétique !

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An Interview with a Leading Clinician-Scientist, Dr. Shawn Aaron

Rashi Raju Hiranandani*[1], Mohammad Ali Faraz*[1]

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Dr. Shawn Aaron

ABSTRACT

Dr. Shawn Aaron is a professor of medicine, senior scientist, principal investigator at the University of Ottawa, and the Chief of the Division of Respirology at The Ottawa General Hospital. He is also the Director of the Canadian Respiratory Research Network, a Canadian Institutes of Health Research (CIHR) Emerging Research Network. Dr. Aaron's clinical and research interests include chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis (CF). We had the pleasure to speak with Dr. Aaron about his clinical and research experiences. He shared information about his medical, academic, and leadership roles. Finally, he also provided some advice for medical trainees in their path towards practicing medicine.

RÉSUMÉ

Dr. Shawn Aaron est un professeur de médecine, un scientifique chevronné et un chercheur principal à l'Université d'Ottawa, et le chef de la Division de pneumologie à l'Hôpital d'Ottawa. Il est également le directeur du Réseau canadien de recherche respiratoire, un réseau pour la recherche émergente des Instituts de recherche en santé du Canada (CIHR). Les champs d'intérêt cliniques et de recherche du Dr. Aaron incluent la maladie pulmonaire obstructive chronique (MPOC), l'asthme et la fibrose kystique (FK). Nous avons eu le plaisir de discuter avec Dr. Aaron au sujet de ses expériences cliniques et de recherche. Il a discuté de ses rôles médicaux, académiques et de leadership. Finalement, il a aussi offert quelques conseils pour les étudiants en médecine, en ce qui concerne leur cheminement vers la pratique médicale.

Could you tell us a bit about yourself and your academic background?

I completed my undergraduate and medical degrees at McGill, after which I did an Internal Medicine residency at the University of Toronto. I then pursued a combined Respirology and Critical Care fellowship, also at the University of Toronto. After that, I came to Ottawa and decided I wanted to do clinical research. I started my practice at the Ottawa Hospital and at the same time completed a Master's of Epidemiology at the University of Ottawa. The master's taught me how to perform research. I completed my master's when I was about 35-36 years old, at which point I officially became staff and an assistant professor at the University of Ottawa and started doing my own research.

How did you pick respirology as a career?

The thing I really loved about respirology was that you did not have to memorize anything. Everything could be figured out from going back to the first principles of medicine and physiology. I really liked the physiology and the fact that the lungs and breathing were logical and made sense when you thought of them. So that is why I chose respirology, because I loved the fact you can figure things out without having to memorize!

Can you tell us about your roles as a clinician, researcher, professor and administrator and any other roles that you may have?

You are right. Right now, I do hold all of those roles. It can be challenging because you have to be doing a lot of different things in the same day. As you said, I am a clinician so I spend about 30% of my week seeing patients. The exception is that when I am on service, like I am now, I spend about 90% of the week seeing patients. The administration involves running the division and that is a big job; it takes about 20% of my time. I have to make sure that the division as a whole is meeting its clinical objectives, its educational objectives, and its research objectives. So there is a lot to take care of. We have to make sure we are providing good respiratory care to the citizens of the region, but we also have to make sure we are providing what the hospital needs, which is obviously a lot of service; a lot of taking care of patients within the hospital. We also have to make sure we are meeting the university requirements. This involves educating the students and the residents, and making

Keywords: Asthma; COPD; Cystic fibrosis

sure the divisional members are producing quality research. The other 50% of my time is spent teaching and doing research. There is a lot we juggle, and if you are a young person you might think this is overwhelming, but my life is never boring; I am doing something different everyday. I love that part of my job. Everything is fresh, exciting, and fascinating. It never gets boring!

Considering that you are a very busy person, how do you manage your work-life balance?

It is easily managed because I just have to say that when I am at work, I am going to concentrate on my work. I work as hard as I can in order to be able to get everything done and go home at a decent time. I find that if you work hard and don't waste your time, you can get an awful lot done in a day and still get home at 6 pm. I am not superman nor am I smarter than anyone else. I come in to the hospital and start working as soon as I can. I also try to not get distracted; I don't go out for lunches during the daytime and I don't spend hours at the coffee shop. Also, I try not to bring my work home. It's not always easy, some nights I do have to work at home, but not most nights. Most nights, I get to spend time with my family and do other things that are important to me. So don't be discouraged, every doctor should be able to manage their work-life requirements assuming they work smart. Don't work longer, work harder.

Can you tell us a bit about your research in asthma?

The reason I chose these particular diseases (asthma, COPD, CF) to research is because they are chronic lung diseases that have devastating impacts on patients who suffer from them; I wanted to do things that could potentially improve their lives. I have had a really fun research career trying to do big studies to try to improve our care for these diseases. When I say big studies, I mean 700 patient studies where we try to answer an important question about the disease. One of the studies I am doing now, which I think might be important, is to examine how well people are being diagnosed with chronic lung disease in our communities. I get a lot of referrals to my clinic from family physicians because the patients are being referred for "asthma" and it turns out they do not have asthma, but they have something else. The question is: why are physicians sometimes over-diagnosing asthma or mistreating other diseases and calling them asthma? We are doing a big study right now to see what is happening across Canada. This was a study where we enrolled 700 patients across the country, from Halifax to Vancouver, who were recently diagnosed with asthma. We are testing the study participants to see if they actually have asthma, and if they do not we are linking them to a study respirologist to figure out what they actually have. These patients were randomly recruited from the community. There is a large minority that does not have asthma, more than 30%, and some of them have very serious conditions that have gone misdiagnosed (1). So we are picking up people with very serious conditions who have been treated inappropriately with asthma medications when they should have been treated with something else. Ultimately, the aim is to try to improve care for everybody, while educating physicians across the country and the world to diagnose these conditions appropriately.

What recommendations would you make to physicians to appropriately diagnose asthma?

The most important thing is that asthma is a disease that causes typical symptoms such as shortness of breath, wheezing, and coughing. But the diagnosis can't be made without first confirming it with spirometry or a lung function test (the same goes for COPD). The problem is that in 50% of the cases in Canada, the disease is not being confirmed with these tests (2). The equivalent would be diagnosing diabetes and putting someone on insulin without measuring a blood sugar; that would never be done. We would never put someone on an hypertensive medicines without measuring their blood pressure. But for some reason in Canada, we are putting people on inhaled corticosteroids and other asthma medicines and labeling them as having asthma without ever testing their lung functions. It does not make any sense. So basically, the bottom line is that we should have objective measures, preferably before we make the diagnosis and start the treatment. It is not always easy, I understand that. But even if you can't get the tests before you treat, you should treat and then get the tests. So that is what I would advise doctors, let's start choosing wisely and doing the proper test in the proper context.

Could you tell us a bit about your research in CF?

One of the important questions that I have researched in the past and one of the important steps in CF management is to try to predict survival when the patient is getting so sick that they need to be referred for lung transplantation. As you could imagine, this is a very big and dangerous surgery that is not suggested until the patient is at the end stage of disease with an expected survival of less than 2 years. We are doing several

studies now, trying to develop models to predict survival pre-transplant and also to predict survival for patients post-transplant. Our plan is to develop an online application for physicians and patients to use so that they can input their particular data into the application, such as their lung function, their sex, their genetics, and their bacteria, and then get the application to spit out the expected survival without lung transplantation and the expected survival if they go ahead with the transplant. This application would help the patients and physicians decide when is the appropriate time to go through this incredibly life-changing, dangerous surgery. So, that is one of the things I am working on trying to improve care for patients with cystic fibrosis with end stage, severe disease. I think in a year or two the application will become available for use by patients and physicians. It is all based on mathematical models of predicting survival in patients based on their current stage and we have done a lot of research on it. It is mathematics and statistics and it is lots of fun, particularly because it can be applied to help people and doctors make better decisions.

Could you also tell us a bit about your research in COPD?

Regarding COPD, I have done a lot of research in the past, way back in the dark ages when I did my master's degree in late 1990s and early 2000s. I started my master's thesis project at the University of Ottawa to determine whether prednisone was useful for COPD exacerbations. Believe it or not, back then we didn't have clinical data to show whether prednisone was useful in this context. So I designed a study in which patients who came to the Emergency Department (ED) were treated with either antibiotics and prednisone or antibiotics and placebo, to determine which group did better once discharged from the ED. The study started as my master's thesis with just about 25 patients. But then, because it was looking so interesting, I got a grant from the CIHR to do a bigger study which ended up growing to hundreds of patients, showing that prednisone with antibiotics clearly protected the patients with COPD exacerbations. The study ended up getting published in the New England Journal of Medicine (3), and it, along with those from other groups, provided the basis for how we practice medicine today (3-5). So for those of you in medical school, when you rotate through general medicine, family medicine, or emergency medicine, you will see that everybody who is admitted with, or goes home from the ED with COPD exacerbations is prescribed prednisone. Everybody thinks that of course we use prednisone, but twenty years ago this wasn't the case; we didn't know whether we should use this medication or not. So that's how you can change the face of medicine through research. In this way, you can impact patient care not only locally in your own practice, but globally, and that is incredibly exciting and rewarding.

As a respirologist, what public health measures would you suggest or hope to see for smoking cessation and prevention?

It is clear that smoking cessation and prevention are extremely important public health initiatives especially for lung diseases but even for cardiovascular diseases, cancer, and other illnesses. Smoking predisposes you to every cancer out there. Thus far, I think that we have done an excellent job in Canada to decrease smoking rates from 40%, 25 years ago, to 16-18% now. I think what I would like to see is more strict control. If at all possible, my dream would be to see tobacco become illegal in this country. I don't know whether that will ever happen, but at the very least we can continue to spread the message to Canadians that tobacco is a dangerous product and we want to be limiting access to tobacco in any way that we can. For example, we should be using plain [cigarette] packaging. I don't think tobacco companies should ever be allowed to advertise or put pictures on their cigarette packages. Cigarettes should be packaged with a plain grey label, with a big warning that cigarettes cause lung cancers, emphysema, bladder cancers and the message has to keep on going to the young people. We have to keep educating young people on the evils of tobacco smoke and try to make smoking as uncool as possible. If we can show people that it is not attractive to smoke, we can keep young people from smoking. Also, we have to target the populations that are most at risk, because unfortunately those who tend to smoke are the people of lower socioeconomic status, people in some minority groups, and Aboriginal Canadians. We want to target those groups especially hard to try to convince them not to take up this habit.

As the final question, what advice would you give students who want to pursue a career in respirology?

Well, my advice would be that while respirology is a great field, they shouldn't necessarily pursue respirology and exclude other choices. Pursue what you are really interested in and what you think you are going to love. I think medicine right now is at an incredibly interesting me. The scientific and medical communities are developing amazing biological and molecular therapies to treat all sorts of chronic and acute diseases. So my advice is to do what you think is really going to interest you. The important thing is that you want to stay passionate about things and stay interested. My other bit of advice to young

people is to get as much training as you can. Don't be afraid of the next residency, or the next research fellowship. Don't be afraid to start training in research, because an academic career where you do research and clinical work, as well as teach, is extremely desirable and interesting. So my advice to you is not to be afraid to challenge yourself. Go out and get advanced training in whatever field interests you, whether it is clinical, research, or education. Get as many years of education as you can, as much training as you can, because that makes you incredibly marketable for a job; you become interesting to other academic doctors if you have a master's degree, a PhD, a fellowship, or clinical training in some area, you become marketable and employable. And the other thing about the academic job that many people don't realize is that because of what you do, you get invited to give talks all over the world. I am not saying this to brag, but it can be exciting when you get to go to China to give a talk to the Chinese Respiratory Society and they pay for you to stay in a fancy hotel, and even take you out to a dinner! So, the bottom line is that you should have a job that will bring you joy, as well as keep you engaged and interested throughout a long career.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Aaron for taking his valuable time to answer all of our questions. He also helped edit the text in the article, and for that we thank him.

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Antisense Oligonucleotide Gene Therapy for Neuromuscular Disorders

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ABSTRACT

Antisense oligonucleotides (ASOs) are synthetic, single-stranded DNA molecules that can bind to specific mRNA sequences and alter protein expression. ASO gene therapies are leading to breakthroughs in the treatment of once intractable neuromuscular disorders. In 2016, ASOs became the first FDA-approved drugs for treating spinal muscular atrophy and Duchenne muscular dystrophy. Recent trials also suggest ASOs may be effective in combating Huntington's disease, amyotrophic lateral sclerosis and hereditary transthyretin amyloidosis. This article highlights ASOs' mechanism of action, their use in treating neuromuscular disease and future obstacles the gene therapy must overcome, providing an update on the state of ASO technology.

RÉSUMÉ

Les oligonucléotides anti-sens (ASO) sont des molécules synthétiques de l'ADN à simple brin, qui peuvent se lier à une séquence spécifique de l'ARNm et altérer l'expression des protéines. La thérapie génique par ASO mène des percées importantes dans le traitement des désordres neuromusculaires antérieurement intraitables. En 2016, les ASO sont devenues les premières drogues approuvées par la FDA pour le traitement de l'amyotrophie spinale et la myopathie de Duchenne. Des essais récents suggèrent que les ASO pourraient également être efficaces dans le traitement de la maladie de Huntington, de la sclérose latérale amyotrophique et de l'amylose de la transthyrétine héréditaire. Cet article souligne le mécanisme d'action des ASO, leurs usages dans le traitement des maladies neuromusculaires, ainsi que les obstacles futurs que la thérapie génique doit surmonter, donnant une mise à jour sur l'état de la technologie des ASO.

Antisense oligonucleotides (ASOs) are an emerging form of gene therapy that promise to transform the treatment of neuromuscular disorders. ASOs are synthetic, single-stranded DNA molecules, usually no more than 30 nucleotides long, that utilize the complementary nature of nucleotide base pairing to hybridize with a specific mRNA sequence and alter protein expression (1). The first antisense drug was approved by the US Food and Drug Administration (FDA) in 1998 to treat cytomegalovirus retinitis and ASOs have also been developed to combat familial hypercholesterolemia and age-related macular degeneration (2). ASOs significantly slowed the progression of each of these diseases and researchers have been eager to replicate this success in other disorders (2). Perhaps most exciting has been the success of ASOs in treating previously intractable neuromuscular illnesses. In 2016 the FDA approved two antisense therapies for spinal muscular atrophy and Duchenne muscular dystrophy (2). In addition, ongoing clinical trials are testing ASOs in Huntington's disease, amyotrophic lateral sclerosis, and other neurological disorders. With the recent completion of a trial demonstrating an ASO's efficacy in treating hereditary transthyretin amyloidosis, this article provides a timely update on how antisense drugs are quickly becoming an essential component in the neurologist's toolkit.

MECHANISM OF ACTION

The two primary mechanisms by which ASOs can impact protein expression are by targeting an mRNA transcript for degradation or by altering how the transcript is processed (3). The hybridization of an ASO with mRNA to form a DNA-RNA complex recruits the ribonuclease H (RNase H) enzyme to hydrolyze the RNA strand (3). An advantage of these ASOs is that cleavage of the mRNA and downregulation of protein expression will occur regardless of the segment of mRNA that is targeted **(Figure 1A)**. In contrast, ASOs that cannot recruit RNase H, but instead alter aspects of RNA processing, must be carefully targeted to specific mRNA segments to elicit their effects. Binding at the start codon can inhibit ribosomal assembly **(Figure 1B)**, preventing mRNA translation. Alternatively, the association with intron-exon junctions can stimulate the inclusion or exclusion of introns and exons **(Figure 1C)** and form novel protein variants with altered functionality (4,5). Whether an ASO works through RNase H mediated cleavage or by influencing transcript processing depends on the chemical backbone of the drug

Keywords: Antisense oligonucleotides; ASO; Neuromuscular disorders; Gene therapy

APPLICATIONS IN DISEASE

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by mutations in the SMN1 gene (6). SMA presents with degeneration of motor neurons, muscle atrophy and weakness. In infantile-onset, SMA typically leads to severe weakness by 6 months and respiratory failure by 2 years (7). The SMN1 gene encodes survival motor neuron (SMN), a protein necessary for motor neuron health, and mutations reduce the levels of functional SMN. The SMN2 gene can also code for SMN but due to alternative splicing, most of the gene's mRNA transcripts are lacking exon 7 and instead produce a non-functional SMN variant that is rapidly degraded (8).

Rather than attempt to treat the SMN1 mutation, researchers ingeniously targeted the alternative splicing of SMN2 using the ASO nusinersen. Nusinersen binds to a downstream intron of exon 7, modulating the splicing of SMN2's mRNA to include exon 7 and produce functional SMN (9). In a 13-month double-blind randomized controlled trial, nusinersen was intrathecally administered to 121 infants with SMA (10). The nusinersen arm of the trial had a 47% lower risk of death or use of permanent assisted ventilation, compared to the control group. In addition, 51% of the infants receiving therapy were able to reach motor milestones such as sitting upright without aid, standing and rolling. However, no infants in the control group achieved these milestones. These results led to nusinersen receiving FDA approval in late 2016 and Health Canada approval in 2017, making this ASO the first treatment capable of slowing the progression of SMA.

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular disorder that leads to the breakdown and weakness of muscle (11). DMD is caused by a mutation in the gene encoding dystrophin, a structural protein that holds the cytoskeleton and plasma membrane together. Absence or dysfunction of this protein compromises muscle stability. DMD patients usually lose the ability to walk by age 12 and experience respiratory failure in their early twenties (11). About 13% of DMD cases are specifically caused by a nonsense mutation in exon 51 where an inappropriately placed stop codon produces a truncated, dysfunctional form of dystrophin (12).

The ASO eteplirsen binds to exon 51 and stimulates its excision, allowing for production of functional, albeit slightly shortened, dystrophin (13). In a double-blind placebo-controlled trial involving 12 patients, eteplirsen was intravenously infused for 48 weeks after which muscle biopsies were taken (14). Eteplirsen increased the percentage of dystrophin-positive fibers to 52% of normal. The drug also allowed DMD patients to walk an additional 67 meters, compared to placebo, in a 6-minute walk test. Despite these modest improvements, the FDA review team recommended against approving eteplirsen, citing the small sample size and questioning whether dystrophin levels had been enhanced enough to observe a true clinical benefit (15). Controversially, the FDA's director of the Center for Drug Evaluation and Research overruled the review team and granted eteplirsen accelerated approval, making it the first drug approved in the United States to treat DMD. A larger phase III trial involving about 110 patients is currently underway and a clearer answer on eteplirsen's efficacy is expected in 2020 (16).

Huntington's disease

Huntington's disease (HD) is a neurodegenerative disorder caused by expansion mutations in the huntingtin gene (17). The mutation stimulates the huntingtin protein to accumulate in toxic aggregates that induce degeneration of the striatum along with progressive motor deficits and cognitive dysfunction. Many in the HD community suspect the progression of the disease may be slowed by lowering levels of the mutant huntingtin protein. Indeed, genetic mutations that naturally reduce the expression of mutant huntingtin can delay disease onset by as much as 9.3 years (18). There is currently no treatment for HD though ASO technology may soon lead to a breakthrough. In a phase I/II trial, the ASO IONIS-HTTRX was intrathecally administered over 13 weeks to 46 HD patients (19). Unlike nusinersen and eteplirsen, IONIS-HTTRX alters protein expression through RNase H mediated cleavage (20). After 3 months, there was a 40%-60% reduction of mutant huntingtin in the cerebrospinal fluid (21). Most importantly, no serious adverse events were reported, with none of the participants dropping out of the study. A phase II trial to further assess the safety and tolerability of IONIS-HTTRX in treating HD is ongoing (22).

Amyotrophic lateral sclerosis

Brought to prominence by Stephen Hawking, amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease (23). ALS is caused by progressive degeneration of motor neurons, leading to muscle wasting, weakness and ultimately respiratory failure (23). Life expectancy after disease onset is only 3 to 5 years and current therapies have poor efficacy. Most ALS cases are sporadic, with no known cause. However, about 20% of familial ALS cases are caused by a mutation in the superoxide dismutase 1 (SOD1) gene (23).

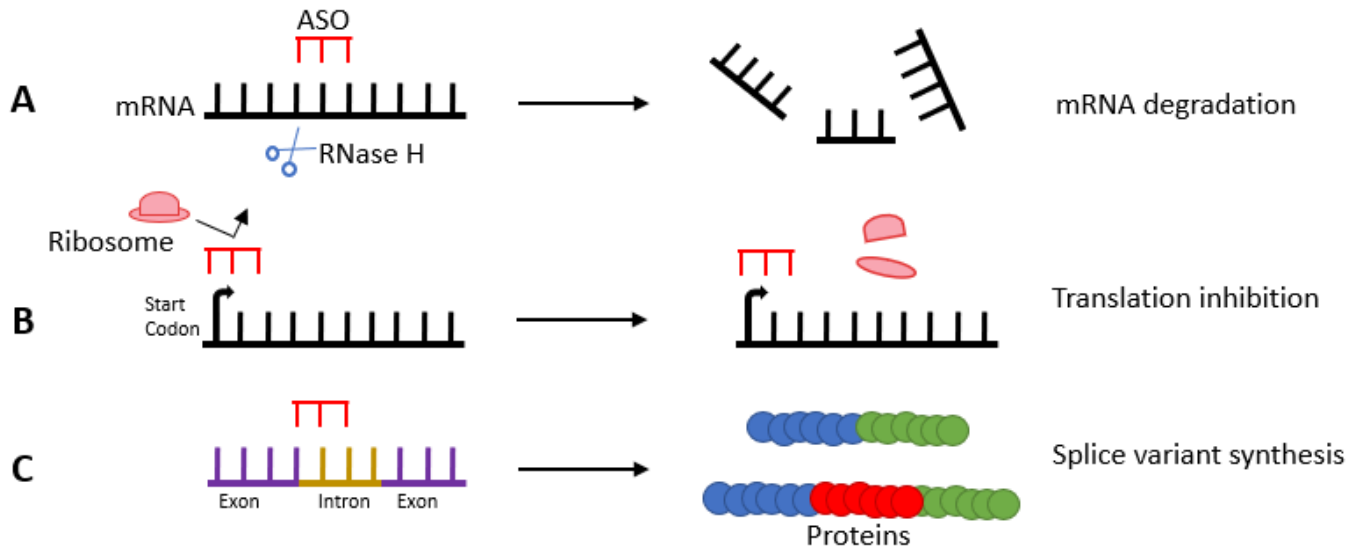

Figure 1: Antisense oligonucleotide (ASO) mechanisms of action – ASOs can impact protein expression by stimulating mRNA degradation or altering how the transcript is processed. (A) Binding between an ASO and mRNA can stimulate ribonuclease H (RNase H) to cleave the mRNA, reducing protein expression. (B) An ASO can also bind at the start codon, preventing ribosomal assembly and lowering protein expression. (C) ASOs can also impact splicing of an mRNA transcript by binding at an intron-exon junction, altering the protein produced through translation.

SOD1 is an antioxidant enzyme that, when mutated, misfolds into toxic aggregates within motor neurons. There is hope that ASOs, which lower SOD1 levels may improve the disease. The feasibility of this approach was investigated in a phase I study where 32 patients with SOD1-positive ALS received intrathecal infusions of the ASO IONIS-SOD1Rx (24). Despite the ASO concentrations being too low to impact SOD1, no serious adverse events were reported. Another phase I trial investigating the safety and tolerability of the ASO is underway (25).

Hereditary transthyretin amyloidosis

Hereditary transthyretin amyloidosis (hATTR) is an autosomal dominant polyneuropathy caused by mutations in the transthyretin (TTR) encoding gene (26). Upon mutation, TTR misfolds to form insoluble amyloid fibrils that accumulate and impair various organ systems. Neurons are particularly impacted, and patients typically present with motor deficits alongside sensory and autonomic dysfunction (26). As the disease progresses, walking becomes increasingly difficult until a wheelchair is required. The average life expectancy is a mere 10 years after onset of symptoms. Since over 95% of mutant TTR is synthesized in the liver, liver transplantation is the standard of care though nerve function rarely improves after the surgery (26).

The ASO inotersen offers a far less invasive means of combating hATTR. Inotersen binds with TTR's mRNA transcript, stimulating cleavage by RNase H and reduction of protein levels (27). The ASO was tested in a double-blind, randomized, placebo-controlled phase III trial, where 172 hATTR patients received weekly subcutaneous injections of inotersen over a 15 month period (28). Average levels of TTR fell by 74% and the rate of neuropathy progression slowed within the inotersen group. Remarkably some patients even reported reversal of their neuropathy symptoms (28). Unfortunately, there were several serious adverse events with 54% of patients in the inotersen group experiencing thrombocytopenia and one patient dying from an intracranial haemorrhage. However, no serious adverse events occurred once enhanced monitoring was implemented (28). These results culminated with inotersen receiving drug approval within the European Union in July of 2018. The drug is currently under review by the FDA and Health Canada.

FUTURE OBSTACLES

A major drawback of ASO therapies is their exorbitant price. For instance, nusinersen costs $125000 per injection (29). With the Canadian Agency for Drugs and Technology in Health recommending that health insurance only cover nusinersen for patients not requiring permanent invasive ventilation, it is pivotal that ASO therapies become less expensive (30).

Another limitation of ASOs is their poor blood-brain barrier permeability (31). Whenever the central nervous system needs to be targeted, as in the case of nusinersen and IONIS-HTTRX, intrathecal injections are necessary and this can serve as a deterrent to starting therapy. Efforts are underway to enhance ASO blood-brain barrier permeability by encapsulating the ASO in extracellular vesicles or by tagging them with cell-penetrating peptides (32). These techniques also serve to improve the bioavailability of the drug. Finally, adverse reactions are a concern for any new class of drug and though nusinersen and eteplirsen appear safe in trials, inoserten induced serious adverse events. In addition, there is always the possibility of unintended off-target genetic effects.

CONCLUSION

ASOs are revolutionizing the management of SMA and DMD, and commit to do the same for HD, ALS and hATTR. By altering the expression of genes directly implicated in disease, ASOs can target the cause of an illness far more successfully than conventional drugs. They represent a promising new frontier in genetic therapy for helping patients with previously untreatable conditions. However, challenges remain particularly in the form of excessive prices, poor blood-brain barrier permeability and coping with adverse reactions. As the technology continues to advance we can expect many more exciting breakthroughs in the treatment of neuromuscular diseases.

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CRISPR-Cas9 Germline Editing: Full Steam Ahead to Revolutionary Profit or Revolutionary Public Medicine?

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ABSTRACT

The CRISPR-Cas9 system uses precise germline genome editing, which enables the mutagenesis of disease sequences in embryonic DNA, thus enabling the birth of healthy individuals who would otherwise inherit genetic diseases. In 2012, the scientific community rushed to claim intellectual property in anticipation of CRISPR's future economic potential. As the war for commercial territory forges ahead, the question of public accessibility and affordability has largely gone unaddressed. The current drive towards total CRISPR-Cas9 commercialization will lead to exorbitant costs of accessing life-giving treatment, especially in regulatory frameworks that prohibit federal funding of germline editing research.

RÉSUMÉ

Le système CRIPSPR-Cas9 utilise l'édition génique de la lignée germinale qui permet la mutagenèse des séquences malades de l'ADN embryonnaire, permettant ainsi de naître un individu en santé qui aurait autrement hérité des maladies génétiques. En 2012, la communauté scientifique se pressait pour le droit à la propriété intellectuelle, en anticipant le potentiel économique de CRISPR au futur. Face à la guerre pour le territoire commercial, la question de caractère abordable et d'accès pour le grand public n'a pas été abordée en gros. Le mouvement courant vers la commercialisation totale de CRISPR-Cas9 mènera à des coûts exorbitants pour l'accès à un traitement vivifiant, notamment avec des cadres régulatrices qui prohibassent le financement fédéral de recherche dans l'édition de la lignée germinale.

In the modern era of medical research, CRISPR-Cas9 gene editing is one of the most promising therapeutic technologies for the advancement of human health through disease prevention. Although CRISPR-Cas9 gene editing research has not yet progressed to human clinical trials in earnest, scientists are cautiously beginning to study the technology in more complex animal models. For example, CRISPR-Cas9 was recently used to successfully restore an essential muscle protein called dystrophin in a canine model of Duchenne Muscular Dystrophy (1). However, the current battle over the intellectual property rights to CRISPR raises the question of whether this potentially life-saving technology will be accessible to everyone when the dust has settled. While scientists continue to refine the efficacy and safety of the technology for human therapeutics, there has already been considerable movement among research institutions to pre-emptively establish territory for commercialization. However, in this rush to claim commercial rights, meaningful ethical discourse has fallen to the wayside, particularly regarding the implications for global health and accessibility. The unfortunate reality may be that while CRISPR picks up momentum in labs and in the media, scientists are becoming increasingly fixed upon a path that will lead to financial payoff at the high cost of equitable public access to revolutionary medical technology.

The rise of CRISPR-Cas9 in the biomedical research community has been meteoric, and for good reason. "CRISPR" stands for Clustered Regularly Interspaced Short Palindromic Repeats, which is a mechanism of genome cutting found initially in bacteria (2). Specifically, bacteria recognize and copy the DNA sequences of invading viruses, thus creating CRISPR arrays which match up to and target viral DNA if similar viral strains invade again. The Cas9 enzyme then deactivates the virus by cutting apart the virus' DNA. Researchers have exploited this mechanism by creating their own targeting sequences, which binds to a known segment of a genome and then uses the Cas9 enzyme to mutate the targeted segment (2). The unlimited application to genetic modification comes into play when the mutated segment is replaced by a customized DNA sequence.

THE PATENT BATTLE

This powerful technique and its wide application were first described by Jennifer Doudna from the University of California (UC), Berkeley, and Emmanuelle Charpentier from

Keywords: CRISPR-cas9; Germline editing; Patents; Gene editing

the Helmholtz Centre for Infection Research in Germany in a landmark 2012 study in Science (3). Another high-profile leader in the CRISPR field is the Broad Institute's Feng Zhang, who has gone toe-to-toe with UC Berkeley since the technology first came to attention in 2012. Although Doudna first demonstrated in publication that the CRISPR-Cas9 system works to cut DNA in a test tube, Zhang published an article six months later showing that the system could be applied to human cells, thus staking for Zhang a major intellectual landmark for the application of CRISPR-Cas9 in humans (4). Zhang and the Broad Institute of Harvard and MIT were the first to win a patent in 2014 for their application of CRISPR-Cas9 in human cells (5).

Zhang's claim was contested by UC Berkeley, who argued that they were the first to show that the CRISPR-Cas9 system can be harnessed to edit genes in all cell types, and that their discoveries are separately patentable. Indeed, the USPTO's Patient Trial and Appeal Board (PTAB) ruled that UC Berkeley's claim to CRISPR-Cas9's application in any setting does not directly compete with the Broad Institute's specific application of CRISPR-Cas9 in mammalian cells, even though the board has yet to grant UC Berkeley's patent (6).

As the patent battle between UC Berkeley and the Broad Institute rages on, other research institutions have not lain idle. As of August 2018, the USPTO has granted more than 90 patents that advance, vary or use the CRISPR-Cas9 technique (7). As CRISPR-Cas9 is refined, modified, and adapted for different applications, more patents will be granted, thus necessitating the purchase of multiple licenses to fully use the latest versions of the technique. Biotech companies will have to pay licensing and sub-licensing fees to a multitude of institutions. The barrier to access the complete toolbox of CRISPR-Cas9 could become prohibitively high for anyone except the most financially flush biotech companies.

IMPLICATIONS FOR AFFORDABILITY

The field of CRISPR research is undoubtedly exciting because of its potential to widely and drastically revolutionize medicine, but evidently researchers are as much driven by the promise of a massive financial payoff for those who can lay claim to bigger pieces of the intellectual property pie. If all patent holders demand licenses to allow others to use their technological variations, only a handful of commercial enterprises will be able to afford to harness and commercialize the technology. However, this problem has not gone completely ignored. In April 2017, the patent packaging company MPEG LA invited CRISPR patent holders to submit their patented technology into a pool that users could buy into as part of a non-exclusive licensing agreement with the patent owners (8). The goal is to help smaller companies more quickly and affordably obtain more pieces of the CRISPR technology in one package, rather than buying the individual licenses in a piecemeal manner. As of October 2017, 22 patents from the Broad Institute, Rockefeller University, Harvard, and MIT have been submitted for consideration into the pool, though UC Berkeley has been silent on their intention to participate (8). Given that the drive behind commercial investment in any developing human therapeutic is the promise of market monopoly through exclusive licenses, these proposed patent pools are perhaps too idealistic. Clinical trials are extremely expensive, so for biotech companies to take on the immense cost of developing human therapeutics, they must have the incentive of a future larger market share. All of the major academic institutions and their leading researchers have created their own start-ups to field exclusive licences coming out of their academic labs. UC Berkeley established Caribou Biosciences, Intellia Therapeutics, and CRISPR Therapeutics, while the Broad Institute established Editas, all of which already have exclusive rights to their respective institutes' CRISPR technologies, enabling these enterprises to sub-license out their foundational techniques to other entities. The rush to claim licensing territory has defined the impetus behind CRISPR development as one of commercial profit rather than public benefit. Lisa Larrimore Ouellette, a law professor at Stanford University, expressed this concern by stating "[t]here is not enough attention being paid to whether research from public institutions, funded by public money, is licensed in a manner that serves public interest." (8)

All signs point to the future birth of the first clinically-proven germline editing therapies in the handful of commercial labs who were able to afford exclusive licenses. These labs could therefore command large portions of the therapeutic market by demanding exorbitant prices to access literally life-changing treatments. Since these biotech enterprises would likely be the scant few able to advance the technology, they would also be the few who would hold most patent rights on more advanced CRISPR tech down the line. To complicate the picture even more, major institutions currently have varying IP footholds in other jurisdictions. China's State Intellectual Property Office (SIPO) and the European Patent Office (EPO) have both granted UC Berkeley its original patent for general CRISPR application, whereas the USPTO still has not. These regional variations would subject biotech companies around the world to different

licensing costs, depending on who owns the patent rights under a particular framework.

Even as CRISPR-based human therapeutics advances at breakneck speed, there still exists a strong ethical stance against germline editing, generally at the international level, and in varying degrees between nations (9). Even now, as investors funnel huge amounts of money into biotech in anticipation of a future CRISPR therapeutics boom, ethical opposition from the public and within legal frameworks is still a limiting factor against advancement and may increasingly bias the development of CRISPR research within the private sector. For example, in the US, the transition of CRISPR research into human embryos is impeded by the fact that studies using gene-editing on human embryos are ineligible for federal funding from the NIH, a prohibition passed down from Congress based on public aversion against the use of taxpayer money to fund embryo-destroying research (9). The direct effect of this federal funding ban is a heavy reliance on private sector funding, which further pressures US researchers to develop CRISPR technology for commercial benefit instead of public benefit.

THE REGULATORY LANDSCAPE

Even if CRISPR germline editing technology were to become available as a safe human therapeutic today, there would still be international and national regulatory instruments that would make universal accessibility extremely difficult. UNESCO's Universal Declaration on the Human Genome and Human Rights characterizes germline editing as a human rights issue, specifically that germline interventions are "contrary to human dignity" (10). Although UN Declarations are not legally binding, they still express "political commitment on matters of global significance" from the states that vote in favour of the Declaration (11). This supposed universal sentiment of caution and moral opposition toward germline editing has largely been overridden by the current push in significant swaths of the scientific community to develop germline interventions through CRISPR technology, especially in the US. There are two possible, interrelated explanations for this disconnect: one is simply that the Declaration is outdated, because it was adopted at a time when safe and accurate germline editing was not yet scientifically possible. Another possible explanation is that commercial incentives encourage nations to flout unenforceable "soft laws" like the Universal Declaration on the Human Genome and Human Rights.

Currently, the research community seems to have mired itself in a short-sighted approach that fails to truly take into account public interest on a national and international level. The unpopular question must be asked whether CRISPR should be patented at all if such technology potentially confers a huge global health benefit. A mindset against CRISPR patents and commercialization exists within the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), whose objective is to harmonize global IP rights. TRIPS protects patent rights for inventions in all fields of technology, but allows World Trade Organization member states to block the commercial exploitation of inventions necessary to protect human life or health (12). This broad goal could be connected to the more specific statement in the 1998 Directive of the European Parliament on the legal protection of biotechnological inventions: "...whereas it is therefore important to exclude unequivocally from patentability processes for modifying the germ line genetic identity of human beings..." (13). Despite these long-standing international expressions against the patentability of human germline editing, they have largely been ignored as patent offices continue to grant patents for CRISPR technology. The current trajectory of CRISPR development is undoubtedly driven by commercial incentive, which spells trouble for the affordability of future therapeutics. A CRISPR revolution with a high price tag would likely keep the revolutionary medical advancement out of reach to everyone but the wealthiest, thus widening the socioeconomic gap as the poor remain handicapped by preventable and curable genetic diseases.

FUTURE APPROACHES

The advent of CRISPR-Cas9 germline therapeutics should be accompanied by a concerted effort on the part of governments, regulatory bodies, research institutions and scientists to ensure such revolutionary medicine is available and affordable for everyone. For instance, a more serious commitment to negotiating and building patent pools could help reduce the development costs for more biotech companies, thus increasing competition and reducing the price of the final product. Public and non-profit health sectors could also intervene by buying CRISPR-Cas9 therapeutics in bulk as part of special contracts called advance market commitments. These advance agreements enable care providers to negotiate for lower prices by guaranteeing a viable market to manufacturers. This approach was used by UNICEF to secure pneumococcal vaccines for developing countries, ultimately saving almost $800 million since 2011 (14). Through multiple strategies to mitigate the development and market costs of CRISPR-Cas9 therapeutics, the private and public sectors could share the future triumph over genetic diseases

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Cannabis Therapy Knowledge Study: Toward Establishing a Pedagogical

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ABSTRACT

Upcoming legalization of cannabis calls for physicians to increase knowledge on medical and recreational cannabis use. We analyzed physician knowledge and opinions on i) mechanism of the endocannabinoid system, ii) current training with cannabis, iii) risks associated with cannabis use, iv) creating effective treatment plans using cannabis and v) future training needs. Physician knowledge and opinions on cannabis are limited and divided. Physicians support integration of cannabis training through webinars, in person training, peer reviewed literature and clinical guidelines. A curriculum must be developed for current and future physicians to create a standard of care as it relates to cannabis.

RÉSUMÉ

La légalisation imminente du cannabis fait appel aux médecins à augmenter leurs connaissances de l'usage médicinal et récréatif du cannabis. Nous avons analysé les connaissances et les opinions des médecins sur i) le mécanisme du système endocannabinoïde, ii) l'entrainement actuel avec le cannabis, iii) les risques associés à l'usage du cannabis, iv) la création de plans de traitement efficaces par le cannabis et v) les besoins futurs d'entrainement. Les connaissances et opinions des médecins sur le cannabis sont limitées et divisées. Les médecins appuient l'entrainement sur le cannabis par des webinaires, par l'entrainement face à face, par littérature révisée par les pairs ou par lignes directrices cliniques. Un programme doit être développé pour les médecins actuels et futurs pour créer une norme de soins pour le cannabis.

The legalization of recreational cannabis remains a popular topic of conversation in Canada. The Liberal Party of Canada has set Fall 2018 as the tentative deadline for the legalization of recreational cannabis. Although the implications of the increased access to cannabis are yet to be determined, the move towards legalization represents a shift in public attitude and increase in the tolerance of cannabis use (1). From the physician's perspective, this shift requires an increase in awareness regarding the laws, health risks and safety factors, training on medical indications, and an eventual modification of clinical practice as it relates to cannabis.

Under the Access to Cannabis for Medical Purposes Regulations (ACMPR), Health Canada designates physicians as decision makers in the prescription of medical cannabis (2). Following this decision, physician groups such as the Canadian Medical Association expressed concern at the lack of education and scientific literature to help decision-making around cannabis (3). These concerns are justified, as physicians report using news media, patients, friends/family and dispensary owners as sources of education regarding cannabis (4). This lack of education has led to highly variable physician opinions regarding cannabis. Some physicians do not believe it has any medical indications due to perceived variability in dosing, lack of evidence of efficacy, concerns about potency and methods of administration (5). Others are excited by new research showing therapeutic evidence for cannabis in neuropathic pain, nausea and vomiting secondary to chemotherapy, spasticity, and anorexia associated with Acquired Immunodeficiency Syndrome (AIDS) (6). Lastly, some physicians admit that they lack a clear understanding on the indication for cannabis and are open to being educated (6). Regardless of whether cannabis is indicated for medical therapy or not, it is critical to educate physicians in the hope of unifying opinions and creating a standard of care as it relates to cannabis.

METHOD

A search was conducted on EMBASE, Medline and PubMed databases to identify 210 articles using the terms "physician", "knowledge" and "cannabis". Of the identified studies, 206 were

Keywords: Cannabis; Medical education; Physician education; Knowledge Study

excluded using the following criteria: i) duplicate articles, ii) irrelevant to topic of interest, iii) studies outside of North America, iv) studies that did not collect primary data and v) studies that did not survey physicians. Four studies that conducted anonymous surveys of physician knowledge and opinions regarding medical cannabis were selected. These were the only North American studies, to our knowledge, that collected primary data from the physicians on prescription of medical cannabis. Three of these studies were conducted in the United States and one was a national needs assessment in Canada. Independently, each of these studies identified a gap among physicians' current understanding and comfort level in various topics related to medical cannabis (7-10). However, the discussions of each study recognized that sample size, variation by location and physician demographics make it difficult to draw any conclusive evidence based on their respective results (7-10). The number of participants, location, mean age of respondents, mean number of years in practice and field of practice for each study are reported in **Table 1**. The findings of these studies must be collectively analyzed to draw evidence that can be used to develop an educational tool for both current and future physicians.

Questions with similar themes were identified across the four chosen studies, and their combined results were analyzed by taking weighted averages of physicians' responses and presenting them as percentages. All of the studies used similar, Likert type scales, to gather data. The combined sample size from the four studies was 1542 physicians, but not all studies included each of the questions that were analyzed. **Table 2** represents the statements that can be further categorized as: i) physician knowledge and current training on cannabis, ii) physician comfort in discussing and prescribing cannabis to patients, iii) physician opinions on integration of medical cannabis training at various levels of education.

DISCUSSION

Physician knowledge on cannabis

Based on the results, respondents indicated a lack of current knowledge and training with medical cannabis (**Table 2**). This was because only 46.5% of respondents indicated feeling trained about medical cannabis and 56% were aware of the mechanism of action of the endocannabinoid system. Further, only 68.5% of respondents felt knowledgeable when asked about the risks associated with cannabis use. The lack of knowledge can be attributed to the absence of high-quality literature on medical cannabis and relating health measures (7). In addition, since some physician organizations (e.g., Canadian Medical Association) have taken a stance against medical cannabis, independent practitioners may be less likely to seek out information. Regardless of individual opinions on the use of medical cannabis, physicians must be educated to avoid the risk of misleading patients. To ensure consistency in education, a resource outlining the known medical indications, risks associated with short-term and long-term use of cannabis (e.g., second hand smoke) and safety concerns (e.g., driving while

Table 1. Demographics of survey respondents.

First author, year (Ref.)	Number of Participants	Location	Mean age of respondents (years)	Mean number of years in practice	Field of Practice
Brooks, 2017 (6)	114	Colorado, United States	(Not specified)	(Not specified)	(Not specified)
Carlini, 2016 (7)	494	Washington, United States	~45	(Not specified)	Family Medicine (267), Other / Not specified (227)
Ziemianski, 2015 (8)	426	Canada	(Not specified)	~17.8	Family Medicine (189), Specialists (219), Other / Not specified (18)
Kondrad, 2013 (9)	508	Colorado, United States	~47.6	(Not specified)	Family Medicine (508)

impaired) must be developed.

Physician comfort in discussing & prescribing cannabis

Respondent comfort in discussing medical cannabis with patients as a therapeutic option and creating an effective treatment plan was similarly low. Only 46% of respondents felt comfortable initiating a conversation regarding medical cannabis with patients and 41.5% indicated that they would be able to create an effective treatment plan (**Table 2**). Physicians may be reluctant to discuss medical cannabis as a therapeutic option, as there is no standardized set of guidelines on which to base their recommendations. Instead, respondents reported using sources such as news media, patient requests, and other non-reliable sources to guide their beliefs on medical cannabis (8). Cannabis prescribing may also be limited due to the stigma that exists against patient groups for whom it may be beneficial (e.g., chronic pain, HIV/AIDS, mental health) (11). Dosing for medical cannabis presents another challenge, as cannabis may be consumed by patients in a variety of different forms (e.g., smoking, edibles, vaping, oils, etc.) and is available in numerous strains, each with different potencies (11). Unclear practice guidelines may compromise patient care through either incorrect prescribing of medical cannabis or withholding a therapeutically beneficial drug. This lack of clarity presents an opportunity to educate physicians on how to correctly prescribe medical cannabis and reduce stigmas that surround the subject.

Physician opinion on integrating cannabis education

There is an agreement amongst respondents that training regarding medical cannabis should be implemented in medical education. Physicians were 90.4%, 90.1%, and 86.2% in agreement that medical cannabis training should be incorporated into ongoing education for physicians, family medicine residency curricula, and medical school curricula, respectively (**Table 2**). The high demand for introduction of a medical cannabis curriculum indicates that if developed, there would be high utility for such resources. **Table 3** presents the preferred formats for receiving education on medical cannabis.

When considering different formats cannabis education may be delivered in, physicians preferred webinars (58.1%), in-person training (56.1%), peer-reviewed literature (55%) and clinical guidelines (53.7%) more than symposia/conferences (44%), scripts to guide patient conversations (43.8%), expert speaker tours (35%) and grand rounds (33%) (**Table 3**). It is important to deliver education in physicians' preferred formats to ensure the highest rate of uptake of knowledge. With the current trend of e-learning in medical education, online training may be a superior method of delivering education on medical cannabis (12). The development of online modules would also be more cost-effective, more accessible to all physicians in Canada and easier to update as evidence and guidelines evolve. However, it is important to recognize that this sample was only limited to 540 physicians. Physician demographics such as age, number

Table 2. Survey statements and mean number of respondents that agreed with each statement.

Statement	Mean number of respondents who agreed (%)	Studies from which this data was obtained
Feel adequately trained regarding cannabis	46.5	6, 7, 8 (N = 1034)
Feel knowledgeable on the mechanism of action of cannabis (ECS)	56.0	8 (N = 426)
Feel knowledgeable about the potential risks associated with cannabis use	68.5	6, 8, 9 (N = 1048)
Feel comfortable initiating a conversation about cannabis use with a patient	46.0	6, 7 (N = 608)
Feel comfortable creating an effective dosing and treatment plan for patients using cannabis	41.5	7, 8 (N = 920)
Training about medical cannabis should be incorporated into medical school curricula	86.2	7, 9 (N = 1002)
Training about medical cannabis should be incorporated into family medicine residency curricula	90.1	7, 9 (N = 1002)
Clinicians should receive training prior to recommending cannabis	90.4	7, 9 (N = 1002)

Table 3. Preferred formats of cannabis education.

Format	*Mean number of respondents who agreed (%)
Webinar	58.1
Small Group Sessions	56.1
Peer reviewed literature	55.0
Clinical guidelines	53.7
Symposia/conferences	44.0
Scripts to guide patient conversations	43.8
Expert speaker tours	35.0
Grand rounds	33.0

*Data was obtained from reference 10 and 11 (N = 540)

of years in practice, location and previous experience with cannabis may all influence the preferred format of education. Moreover, there was only a minor difference between preferences for webinars, in-person training, peer-reviewed literature and clinical guidelines. As a result, all of these options should be considered while developing an educational tool.

Family physicians and psychiatrists would provide an excellent starting point for education on cannabis. Both of these specialties are centered around continuity of care and developing longitudinal relationships with patients. Patients may feel more open to speaking with these physicians regarding the use of medical cannabis or potential implications of recreational cannabis use. Many of the diagnoses for which cannabis is indicated are also managed by these two specialties. As a result, these clinicians may carry less bias against patients seeking medical cannabis.

CONCLUSION

An in-depth review of the literature has highlighted the gaps in physicians' knowledge and comfort in working with medical cannabis. It has also been identified that most physicians agree with the integration of education around medical cannabis at various stages of medical education. The preferred formats of education were webinars, in-person training, peer-reviewed literature and clinical guidelines. Our commentary highlights that action is required to develop a tool to uniformly educate Canadian physicians on the medical indications, safety concerns, appropriate treatment plans and ongoing monitoring as it relates to cannabis. The development of this tool would greatly increase physicians' ability to inform patients regarding cannabis and act as a step towards establishing a standard of practice as it relates to cannabis.

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Point-of-Care Ultrasound in Undergraduate Medical Education: a Probe into its Feasibility

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ABSTRACT

Point of care ultrasonography (POCUS) has had its applications expand rapidly over recent years and across several medical specialties. Enough so that is has become an essential skill in most residency training programs across Canada. Despite this, there is little to no structured POCUS training at the medical undergraduate level. The goal of this commentary is to briefly introduce the value of POCUS in medical education; the feasibility of its integration; current barriers to its introduction; and the potential for students to be a possible solution until faculty can introduce a formal undergraduate POCUS curriculum.

RÉSUMÉ

L'échographie ciblée a vu ses utilisations agrandir rapidement dans les dernières années et parmi plusieurs spécialités médicales. Jusqu'au point où elle est devenue une habileté essentielle dans la plupart des programmes de résidence à travers du Canada. Malgré ça, il y a très peu d'enseignement sur l'échographie ciblée dans le domaine de l'éducation médicale du premier cycle. Le but de ce commentaire est d'introduire brièvement la valeur de l'échographie ciblée dans l'éducation médicale; la faisabilité de son intégration; les obstacles courants à son lancement; et le potentiel que les étudiants en prennent charge jusqu'au moment où la faculté peut introduire un programme formel d'échographie ciblée pour l'éducation médicale du premier cycle.

Expertise with point of care ultrasound (POCUS) is becoming a sought-after skill for new residents entering their training since a widening range of specialties are using it more frequently to aid in clinical diagnoses and guide procedures (1). The use of ultrasound for viewing heart, lung and abdominal pathology is commonplace, while central and peripheral venous access, biopsies, joint aspirations, nerve blocks and lumbar punctures are being done under ultrasound guidance with increasing frequency. This shift towards POCUS in clinical practice is imposing the need for medical student education and training in the technology. Attempts to incorporate POCUS into undergraduate medical education have been made, however resources and training are limited and there are significant instructional inconsistencies among the programs (1-8). Currently at the University of Ottawa, there is no formal ultrasound training in the undergraduate medical curriculum. As the use of this technology quickly evolves in modern medicine, it will be important that our medical education keeps pace. To maintain the clinical competence of medical students both at the clerkship level and as they enter residency, POCUS training will need to be incorporated into the undergraduate medical curriculum at uOttawa.

Keywords: Point of care ultrasound; Medical education

Basic POCUS competency requires only a limited number of sessions; students have reported a quick learning curve and demonstrated proficiency with the most clinically relevant ultrasound practices after only 5 instructional sessions (5, 9-11). At uOttawa, the addition of basic education in the procedural and diagnostic fundamentals of cardiac, thoracic and abdominal exams has been proven feasible without necessitating cuts to the current curriculum (12). One reason is its natural positioning within an anatomy syllabus; POCUS is described as an excellent anatomical educational tool, highly effective at facilitating the learning of anatomy and the physiologic and pathologic characteristics of many organ systems (13-16).

Our Faculty currently acknowledges the value of POCUS and its integration into other medical school curriculums. Initial thoughts on its integration at uOttawa will target the current anatomy syllabus. In anticipation, the Department of Anatomy purchased 9 ultrasound machines this year and ran two "pilot" Anatomy POCUS boot camps during the summer of 2018 to test possible methods of POCUS integration into its existing curriculum. Results showed that the 9 machines are sufficient to educate our class size using a rotatory schedule similar to what is already in place for anatomy classes. However despite these encouraging results, the development of a robust infrastructure for POCUS training will not be immediate; some barriers remain, slowing its integration.

One major barrier to the incorporation of ultrasound training into most medical undergraduate curricula is the relatively small number of available instructors compared to the number of students. Competency in POCUS techniques is developed through supervised hands-on training, which requires more teachers than traditional didactic lecturing. With typical institutional limitations with finances and physician availability slowing the development of a formal POCUS educational infrastructure, a more creative temporizing solution for student education is necessary in the interim: training upper year students as instructors to lower year students. In this set-up, the problem of having a large number of students to teach can solve the problem of not having enough instructors (17). Several universities have validated the effectiveness of "peer mentoring" and its ability to deliver ultrasound training to large groups of students with a significantly reduced need for faculty resources (18–20). Demonstrations of this system show a single physician instructor can supervise up to four student-instructor-led groups and effectively provide coaching and feedback with the consequent self-directed learning even providing added long-term benefits (21). There is also a definite benefit for student instructors; they get a valuable opportunity to develop teaching skills, which not only help to solidify understanding of the concepts but are also preparatory for future teaching roles encountered during residency training (22).

This broadening use of POCUS in medicine dictates that incoming residents should have some knowledge of ultrasound. However POCUS training has lagged behind at the undergraduate level, since traditional educational systems have substantial limitations in available instructors and time; therefore, students' participation in the teaching process could provide a solution to this problem. Results from other institutions suggest that peer mentoring can facilitate the large-scale implementation of ultrasound education in undergraduate medical curricula (17). Studies out of Canada, the UK, Australia and Spain all report that a small number of physician-trained students can successfully teach POCUS skills to lower year students with good results (18-20).

Experiences at our own institution from pilot trials of POCUS education echo what has been demonstrated at these other sites. Thus, peer mentoring is a valuable resource worth considering to integrate basic ultrasound education into the uOttawa undergraduate medical curriculum, at least as a temporizing measure until more robust educational infrastructure can be developed.

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Interventional 3D Augmented Reality in Orthopedic, Trauma and Vascular Surgery

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ABSTRACT

The Medical Education, Training and Computer Assisted Interventions (METRICS) Laboratory aims to integrate novel mixed-reality technologies with application in computer assisted interventions. We showcase two technologies with specific aims at optimizing surgical workflow and minimizing radiation exposure in orthopedic, trauma, and vascular surgeries. The first is an Augmented Reality C-arm fluoroscope, which provides intuitive real-time visualization by accurately overlaying X-ray to video images. The second is a 'Desired-views' user interface which resolves the challenges involved in the optimal control of C-arm fluoroscopes for their constant repositioning during surgery by either the interventionalist or the surgical team.

RÉSUMÉ

Le laboratoire d'éducation médicale, de formation et d'intervention assistée par ordinateur (METRICS) vise à intégrer de nouvelles technologies à réalité mixte à des interventions assistées par ordinateur. Nous présentons deux technologies ayant des objectifs spécifiques pour optimiser le flux de travail chirurgical et minimiser l'exposition aux rayonnements dans les chirurgies orthopédiques, traumatologiques et vasculaires. La première est un fluoroscope C-arm à réalité augmentée, qui fournit une visualisation intuitive en temps réel en superposant avec précision les rayons X aux images vidéo. La seconde est une interface utilisateur «Desired-views» qui résout les problèmes liés au contrôle optimal des fluoroscopes C-arm pour leur repositionnement constant pendant la chirurgie, soit par l'interventionniste, soit par l'équipe chirurgicale.

AUGMENTED REALITY C-ARM FLUOROSCOPY IN ORTHOPEDIC AND TRAUMA SURGERIES

It is expected that by 2050, the number of patients undergoing hip and spine surgery will reach 40 million, which will make up a global market share estimated to reach $30 billion (1,2). Hip and spine surgery data show that 90% of these occur in older adults (ages 65 and older), usually as a consequence of osteoporosis or a low-energy mechanism such as a fall from a standing height (1,2). All the more alarming is that hip and spine surgeries account for most of the total radiation exposure in all orthopedic and trauma types of procedures, with the risk of contracting cancer being up to eight times more for a surgeon and their patients when compared to other workers having a non-clinical background (3). The reason is that surgeons often place emphasis on acquiring the 'perfect picture' using C-arm fluoroscopes (X-ray devices) in order to complete surgery efficiently, which may lead to excess from a standing height (1,2). All the more alarming is that hip and spine surgeries account for most of the total radiation exposure in all orthopedic and trauma types of procedures, with the risk of contracting cancer being up to eight times more for a surgeon and their patients when compared to other workers having a non-clinical background (3). The reason is that surgeons often place emphasis on acquiring the 'perfect picture' using C-arm fluoroscopes (X-ray devices) in order to complete surgery efficiently, which may lead to excesive use of X-rays. As an alternative to C-arm fluoroscopes, 3D navigation systems are commercially available. These systems use pre-operative CT scans, external optical tracking systems, and tracked markers as reference landmarks affixed onto both the medical instruments and the patient (4). The 3D navigation system then provides information during the surgery on the spatial relation between the medical instruments, patient, and the pre-operative CT scan which, in theory, facilitates the completion of many surgical tasks without X-ray acquisition (4). However as of 2016, it is now documented by major medical policy makers (Blue Cross Blue Shield Association, United HealthCare, etc.) that these systems are costly, are common in only some

Keywords: Augmented Reality; Virtual Reality; Computer Assisted Surgery; User interfaces

surgeries, showed no increased benefit to patient outcomes when compared to traditional C-arm fluoroscopes, and have yet to find a niche in orthopedic and trauma operating rooms worldwide. Consequently, surgeons fall back to the traditional C-arm devices to perform surgeries (4).

The President of the Radiation Safety Institute of Canada has reported that cancers of various types are a potential outcome of occupational overexposure to radiation. The Health Economics Review published a report in early 2017 titled "Costs of Productivity Loss Due to Occupational Cancer in Canada (…)", which highlighted the estimated total cost of cancer to the Workers' Compensation System in Canada, due to productivity losses alone, to be $1.2 billion (5).

With respect to risks associated to healthcare providers, reports have documented the dosage of radiation among interventionalists as the greatest registered among any medical staff working with C-arm fluoroscopes (6). As highlighted by Picano et al. (7), cumulative doses after 30 years of working life are in the range of 50 to 200 mSv, corresponding to a whole-body dose equivalent of 2,500 to 10,000 chest X-rays. In the case of patients, the benefits of a proper usage of C-arm fluoroscopy devices outweighs the experienced radiation risks, especially in the older age groups (8). The patient is exposed to primary radiation, namely radiation between the X-ray source and the image intensifier. Short-term risks are radiation induced skin damages (erythema, epilation and even dermal necrosis), which result from acute radiation doses beyond 2 Gy (9). Thus, there is an urgent need to develop technologies that rectify radiation exposure in the surgery room.

The METRICS lab (Medical Education, Training, And Computer Assisted Interventions) aims to break new ground on optimizing the current surgical workflows in orthopedic and trauma surgeries while alleviating the effects of radiation exposure. An Augmented Reality (AR) C-arm fluoroscope provides intuitive real-time visualization for surgeons by overlaying the 2D X-ray images to the 2D video images obtained by affixing one camera (or multiple depth sensors) on the C-arm device (10,11). Thanks to a one-time calibration of the AR C-arm, the camera centre and the X-ray source centre are virtually aligned, thus providing a geometrically correct 2D/2D overlay (12). The precision of the overlay is less than 0.5mm (11). **Figure 1a** shows the AR C-arm fluoroscope and **Figure 1b** shows an example of the 2D X-ray overlaying a 2D video image. Advanced multi-modal visualization is also a possibility when applying machine learning algorithms to the X-ray and video data (**Figure 2**). Our publications conclusively demonstrate that the video image overlay has the benefit of facilitating many surgical tasks without the acquisition of an X-ray (13–19).

As mentioned previously, it is clinically accepted that some C-arm device positions may generate up to six times more radiation exposure to highly sensitive body-parts such as thyroid, hands, eyes and head, and should be avoided (20). Modeling of X-ray photon propagation is complex and not often possible ton achieve analytically. To visualize the amount of radiation dosage during surgery, we consider Monte Carlo (MC) simulations, which are algorithmic methods that approximate radiation exposure by simulating the X-ray imaging process, and tracking the resulting scattered particles towards clinicians and patients during surgery (20,21). Optimizing the position of any C-arm device (to minimize radiation exposure) is challenging since the context, the imaging parameters, and the patient's and staff's positioning need to be considered in the MC simulations. As in Bott et al. (22), the real C-arm imaging parameters (tube kilovoltage, filtration, aperture angle and beam projection), together with the X-ray photon scattering process, are all considered by the simulation. These can be extracted from the C-arm device application programming interface (API). The patient and surgeon's positioning is known from the camera or multiple sensors affixed to the C-arm. The representation of the surgeon and patient is created and their surface (tessellation) is visualized in 3D in real-time. The radiation dose at the surface is visualized using color coding. The color values for each triangle corner of both the surgeon and patient tessellation are computed by interpolating the dose distribution information of the dose accumulation (**Figure 3**). With the advent of modern mixed-reality head-mounted displays (HMDs), the typical challenges encountered by mobile AR technologies have now been solved, and commercial devices suitable for medical applications are now available. Indeed, a recent study showed that the Microsoft's HoloLens HMDs are now suitable enough in terms of contrast perception, task load and frame rate, during surgical interventions (23). Thus, the HMD not only provides an accurate feedback about the current radiation exposure to surgeon and patient, but also correlates such an exposure to the underlying workflow tasks taking place. Collecting the dose generated at each step of the surgery can help generate exposure statistics. Such statistics will be computed by considering each person's record of previous exposures to radiation, which would help to reduce the risks of long-term negative effects of exposure. To conclude,

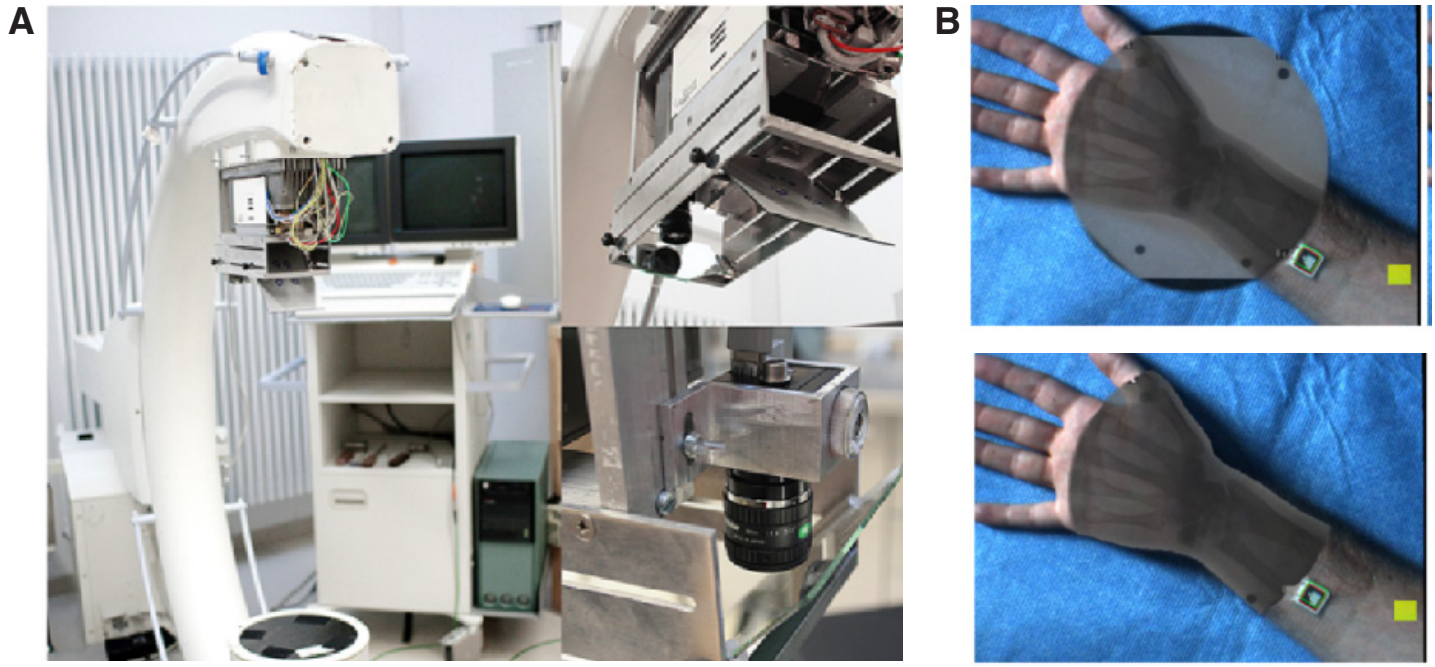

Figure 1. Augmented Reality Fluoroscope. A) A C-arm augmented by video camera, and B) 2D X-Ray images correctly aligned to 2D Video images.

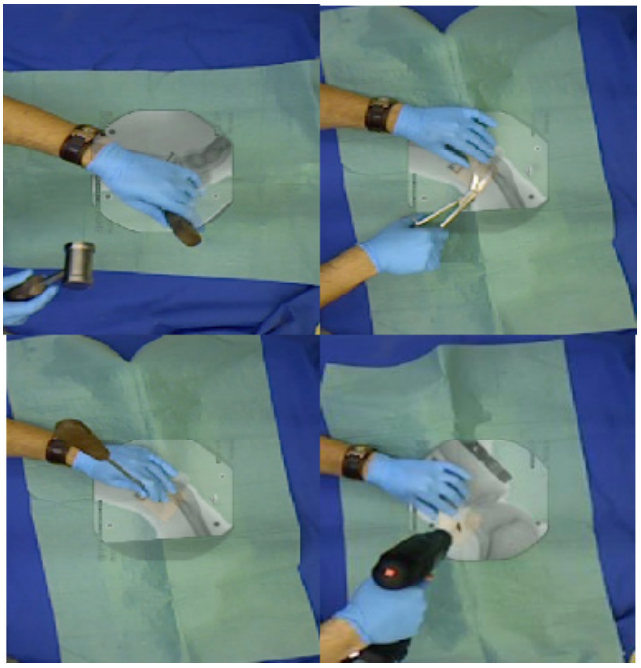

Figure 2. Example visualizations between X-ray and video images demonstrating center punching, drilling and clamping tasks.

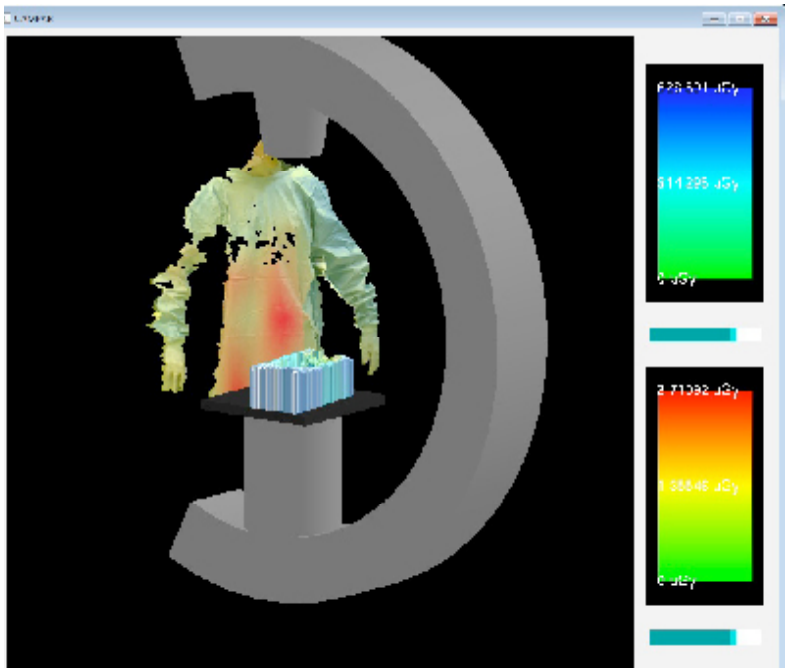

Figure 3. Radiation exposure visualization. Red colors demonstrate areas of high dosage whereas blue colors demonstrate areas of low dosage.

the development of the AR C-arm was necessary as it has the potential of transforming orthopedic and trauma surgeries at a relatively low cost (i.e. equivalent to the cost of affixing a camera or several RGB-D sensors on the C-arm grantry) in order to maximize patient outcomes and minimize radiation exposure.

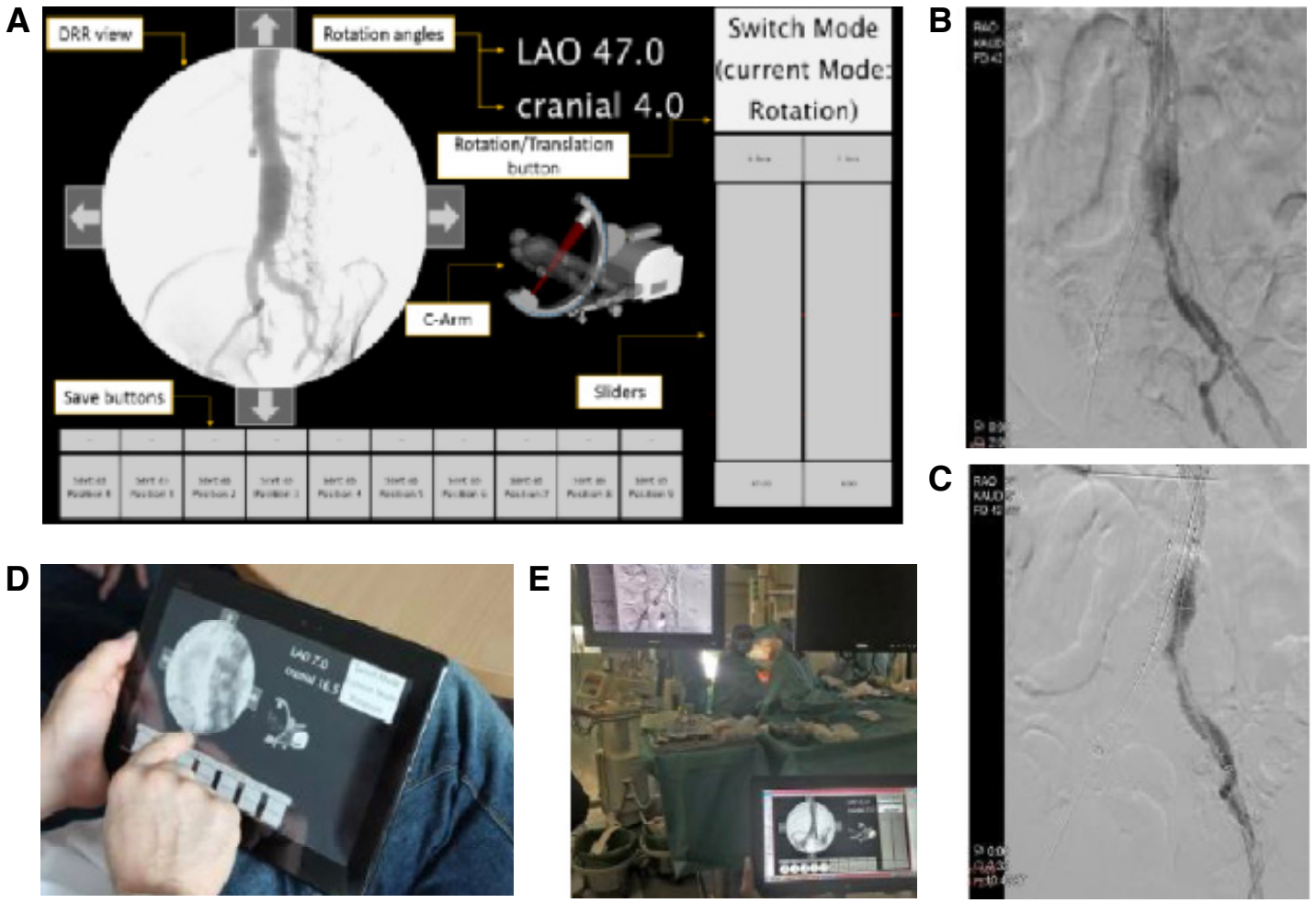

Figure 4. "Desired-views" user interface. A) The user interface, B) Iliacs optimal view vs. C) Iliacs non-optimal view, D) Defining optimal view before surgery, and E) Using predefined optimal view.

A preliminary and unpublished study was undertaken to evaluate this interface in a real clinical scenario. Twelve patients underwent EVAR surgery at our clinical partner site. The study consisted of comparing the planned C-arm positions using our 'Desired-views' interface prior to intervention, to the ones during intervention when navigating through the left and right iliacs of each patient. For all patient cases, we received the relevant intra-operative X-ray images, which enabled us to identify any deviations of the intra-operative C-arm parameters from the pre-operatively planned 'desired-views'. Results demonstrated that for all twelve patient surgeries the C-arm was positioned optimally on the first try, which leads us to conclude that the proposed work has potential to increase the accuracy while decreasing radiation exposure, contrast intake, and duration of future patient surgeries.

CONCLUSIONS

The METRICS lab hosts many junior and senior researchers aiming to redefine the design and development of physiological sensing, virtual and augmented reality immersion, and advanced user interactions. This is made possible with the collaboration of clinicians, computer scientists and industry, for the effective translation of our new developments inside the operating rooms of tomorrow. Two key projects involve the development and validation of an Augmented Reality C-arm device and a 'Desired-views' user interface in orthopedic, trauma, and vascular surgery setting. The Augmented Reality C-arm device will be evaluated during the next five years as the principle investigator is the recipient of an Early Career Research Award titled: Interventional 3D Augmented Reality. Additionally, the validation of the 'Desired-views' user interface will continue with partners in the Faculty of Surgery upon ethics board approval.

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The Genetics of Leber's Hereditary Optic Neuropathy: A Literature Review

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ABSTRACT

Leber's hereditary optic neuropathy (LHON) is a mitochondrial disease characterized by a painless, acute loss of central vision, with 95% of affected individuals harbouring one of three pathogenic mutations (G11778GA, G3460A, or T14484C). The purpose of this review is to highlight the distinguishing clinical presentations of the disease with respect to the mutation subtype and present our recent understanding of two unique features of the disease: male predominance and incomplete penetrance. We also review recent advancements made in the diagnosis and treatment of this rare mitochondrial disease and their implications for genetic counselling.

RÉSUMÉ

La neuropathie optique de Leber est une maladie mitochondriale caractérisée par une perte aiguë, et indolore de vision centrale, avec 95 % des individus affectés possédant une de trois mutations pathogéniques (G11778GA, G3460A, ou T14484C). Le but de cette revue est de surligner les aspects distincts de la présentation clinique de la maladie selon le sous-type de la mutation et de présenter sur nos compréhensions récentes de deux traits uniques de la maladie : la prédominance mâle et la pénétrance incomplète. Nous allons également revoir les avancements récents dans le diagnostic et traitement de cette maladie mitochondriale rare et leurs implications pour le conseil génétique.

Leber's hereditary optic neuropathy (LHON) is a rare mitochondrial disease with a male predominance that results in painless central vision loss (1,2). More than 90% of patients with LHON carry one of three primary mutations affecting complex I (NADH dehydrogenase) of the mitochondrial electron transport chain (ETC). These mutations are G11778A, G3460A, and T14484C (3). Affected individuals typically experience significantly reduced visual acuity, blurring, central or cecocentral scotoma, and diminished colour vision (2). Visual deficits have been associated with dysfunction of retinal ganglion cells (RGCs) and their axonal transport system (4). RGCs, particularly the papillomacular bundle, are vulnerable to disruptions of the mitochondrial bioenergetics as a result of their structural and energy constraints (4). Moreover, histopathological studies of individuals with LHON show progressive degeneration of myelin and axoplasms at the level of the optic nerve, but early changes can be noted on fundoscopy and include peripapillary retinal nerve fiber layer (RNFL) edema and telangiectasia of the retinal vessels (3).

LHON has a disease prevalence of approximately 1 in 50 000, making it one of the most commonly inherited optic neuropathies (5). The majority of patients are affected prior to the age of 60, with an average age of onset ranging from 15–35 years (5). During the acute phase of the disease, patients present with a painless blurring of vision. Vision loss is bilateral in 25% of cases and sequential in 75% of cases, with an inter-eye delay interval of approximately eight weeks (3). A nadir in visual acuity is often reached by 4–6 weeks following onset of disease and can worsen to less than 6/60 (3). In the acute phase, fundoscopic examination is typically unremarkable; however, it may also demonstrate disc pseudoedema, tortuous vessels, and peripapillary telangiectasias (5). The characteristic visual field finding of central or cecocentral scotoma may be observed on Goldmann perimetry (6). In the more chronic phase of the disease, optic atrophy can be noted in the form of optic nerve head pallor as a result of selective degeneration of the RGCs (6).

MOLECULAR MECHANISM

The causative factor for disease in LHON is a missense mutation in NADH dehydrogenase, a co-enzyme responsible for the production of ATP and cellular energy (7). This results in decreased ATP synthesis, elevated oxidative stress, and disruptions to the transport of glutamate, ultimately leading to

Keywords: LHON; Leber's Hereditary Optic Neuropathy

a degeneration of the RGCs and eventual cell death via apoptosis (7). However, the majority of the proposed mechanisms have been established through mitochondrial cybrid cell lines. One study by Lin et al. employed a mouse model of LHON and introduced the P25L mutation—which results in LHON in humans—into the ND6 subcomponent of the respiratory chain (8). The authors subsequently concluded that oxidative stress is the primary mediator of RGC cell death while ATP production has been relatively spared. Mechanistically, it has been observed that the transport of electrons along the ETC can be disrupted as a result of a mutated complex I, allowing more free electrons to react with molecular oxygen. This process leads to the conversion of superoxide and subsequently increased oxidative stress (9). Interestingly, it is rare for patients to have systemic symptoms. Moreover, no other neuronal cell lines apart from the ganglion cells—including photoreceptors which also require high energy demand and mitochondrial load—appear to be affected (7). A recent study by Levin examined the role of superoxide production in RGC-5 cell line and found superoxide production to occur at significantly reduced levels in RGC compared to mitochondria in neuronal cell lines (10). This has been hypothesized to be due to the regulatory effect of superoxide dismutase (SOD-2), an enzyme that converts the toxic superoxide into nontoxic components (10). Pioneering work by Guy and Qi et al. has also demonstrated that knockdowns of SOD-2 in RGCs result in optic neuropathy, while overexpression of SOD-2 improve optic neuropathy (11, 12). Despite having the SOD-2 system to modulate the amount of superoxide production, mutations in complex I (as observed in LHON) could lead to high levels of superoxide production that may overwhelm this regulatory pathway, manifesting in eventual ROS release and cellular apoptosis (10).

CLINICAL CHARACTERISTICS OF LHON

Features of Pathogenic Mutations

Although there are a multitude of primary mutations responsible for LHON, over 90% of the LHON pedigrees harbour one of three primary mutations in the mitochondrial genome at nucleotide positions 3460, 11778, and 14484 (1). The mutations affect the ETC chain complex I genes at the ND1 (3460), ND4 (11778) and ND6 (14484) subcomponents (1).

There are several distinguishing characteristics based on demographic factors and clinical manifestations across the three mutations. G11778A is the most prevalent mutation subtype, reported to account for approximately 70% of Northern European patients and 90% of Asian patients with LHON (1). T14484C and G3460A are approximately equal in prevalence, but there is a higher representation of the T14484C mutation among French Canadians due to a founder effect (13). With respect to the age of disease onset, the T14484C mutation exhibits the lowest age of onset at approximately 19 years compared to the other primary mutations G11778A (26 years) and G3460A (21 years) (14). Clinically, visual prognosis differs according to the mutation subtype such that the rate of spontaneous visual recovery is highest for the T14484C mutation. Between 37% and 58% of patients with the T14484C mutation experience recovery compared to only 4% of patients with the G11778A mutation and 20% of patients with the G3460A mutation (1). Moreover, the G3460A mutation is associated with more severe disease biochemical phenotype (15).

Though all three mutations present with simultaneous mutations, the percentage of simultaneous versus sequential onset is significantly different (14). There is a greater predilection for simultaneous compared to sequential onset for T14484C. Moreover, as previously observed among LHON mutations with sequential onset, the T14484C mutation subtype also has a more restricted distribution with inter-eye onset occurring at less than 44 weeks; in contrast, the G11778A and G3460A mutations can have onset ranges of up to 2016 weeks and 816 weeks, respectively (14). This is interesting to observe given that T14484C is also the mutation that harbours the mildest defect and greatest potential for recovery. Patient populations affected at a younger age, especially individuals harbouring the T14484C mutation, may be more genetically predetermined to becoming affected and thus demonstrate bilateral involvement at initial presentation.

Male Predominance

One unique characteristic of LHON is its male predominance; previous pedigrees have reported that 80–90% of affected family members are male (1). One hypothesis proposed by Bu et al. has been related to an X-linked recessive susceptibility gene that interacts with the mitochondrial genome which could account for the increased disease prevalence in males (17). Female carriers who become affected are thought to be homozygous at the X-linked locus or display inactivation of the X chromosome (17). Although recent linkage analyses have identified a possible region that may be suggestive of this susceptibility gene in the long arm of the X chromosome, the exact gene remains to be elucidated. Another hypothesis that could account for the disparity in disease representation

between males and females is the influence of hormones and the protective role of estrogen in females. Estrogen has been implicated in modifying the severity of mitochondrial dysfunction including dysregulated ATP synthesis, oxidative stress, and cellular apoptosis. Indeed, estrogen receptors are abundant within the ganglion cells, and their subsequent stimulation has been associated with increased activity of SOD-2 and reduction in ROS (18).

Incomplete Penetrance

A second intriguing characteristic of this mitochondrial disease is its rate of incomplete penetrance, with only 50% of male and 10% of female carriers expressing the disease phenotype during their lifetime (2). Genetic factors cannot completely account for this reduced penetrance. Based on results from five reported monozygotic twin studies (19-24), two pairs had one sibling that did not become affected by the disease during long-term follow-up (21,23). It is, therefore, more likely that LHON is a multifactorial disease whereby the presentation is influenced by the interactions of the primary mutations with environmental influences. Indeed, several prior investigations suggest an increased rate of disease conversion in LHON carriers with increased alcohol and tobacco consumption (25). The effect of smoking has also been examined by Kirkman et al. in a multicentre study of 206 unaffected and 196 affected carriers (25). Their study demonstrated that not only is smoking associated with an increased risk of visual symptoms in carriers of LHON, but that heavy smokers were more likely to manifest the disease phenotype compared to light smokers, lending support for a potential dose-response relationship. Furthermore, it is suggested that cigarette smoke can reduce the efficiency of complex I and, in turn, increase production of ROS. Several other reports have noted other precipitating factors that may contribute to disease conversion including trauma, nutritional deficiencies, toxin exposure, and psychological stress; however, the strength of these relationships require further evaluation.

ADVANCEMENTS IN DIAGONISTICS & MANAGEMENT

Diagnosis

Research in optical coherence tomography (OCT) has also shown changes in the retinal ganglion cell complex (GCC) relative to the RNFL over time. Barboni et al. discovered that there was a thickening of RNFL on OCT in early LHON (<6 months) and disc atrophy in chronic LHON (>6 months). The temporal fibers of the papillomacular bundle were the first and most severely affected areas, whereas there appeared to be nasal fiber sparing of the optic nerve until more advanced stages of the disease (26). Savini et al. investigated RNFL thickness in unaffected carriers of LHON and found that there was an apparent thickening of temporal fibers in all subgroups of LHON carriers (27).

Additionally, visual electrophysiological recordings including visual evoked potentials (VEPs) and pattern electroretinograms (PERGs), which provides a more objective measure of ganglion cell function, have been employed. For example, it has been documented that LHON patients demonstrate characteristic changes such as increased VEP latencies, waveform distortions, and decreased VEP amplitudes during the acute stage (28,29). Additionally, reductions in PERG amplitude have been noted in LHON carriers despite normal visual acuity, visual field, and RNFL thickness (30). However, VEP elicits potentials from the visual cortex and, as a result, does not directly measure RGC function. While PERGs have the ability to measure RGC activity, their utility is primarily limited to the inner retina and necessitates compensation of the patient's refractive prescription and accurate foveal placement (31).

Recently, it was observed that RGCs generate a slow negative wave signal observable on the electroretinogram (ERG) referred to as the photopic negative response (PhNR). Numerous studies have evaluated the predictability of disease pathology such as glaucoma, various optic neuropathies, retinal vascular diseases, and idiopathic intracranial hypertension using the PhNR (32-37). In full-field ERG, the PhNR amplitude reflects cone-related RGC function and is significantly reduced with advancing visual field loss and reduced RNFL thickness as seen on OCT (38). The thinning of the RNFL typically follows the decreased PhNR amplitude, suggesting that functional changes precede structural abnormalities in the ganglions cells in LHON (38). In summary, there is good evidence to suggest the use of clinical ERG including the PhNR and ocular imaging such as OCT in aiding the diagnosis and monitoring the severity of disease in patients with LHON.

Treatment

Presently, there is no definitive medical treatment for LHON. Nevertheless, there is theoretical benefit in the use of anti-oxidants including Coenzyme-Q10 in mitigating stress from ROS (39). Recent trials have also been conducted on idebenone, a short-chain benzoquinone related to Coenzyme-Q10 which allows electrons to proceed from complex I directly to complex III in the ETC, bypassing complex II (40). The Rescue of Hereditary Optic Disease Outpatient Study (RHOSOS) recruited 84 patients with primary LHON mutations (40). Patients were randomized and either received high dose idebenone for 24 weeks or

placebo. Post-hoc analyses revealed improvement in visual acuity in the treatment group, particularly those with G11778A and G3460A mutations. Investigations are currently being conducted for EPI-743, a more potent para-benzoquinone compared to idebenone, and have shown some encouraging results in smaller cohort studies (41).

Gene therapy has become an exciting avenue for current and continued research in advancing treatments for LHON. Exogenous DNA can be inserted into the nuclear DNA through adenovirus-associated virus (AAV) type 2. Given the defect in LHON is within the mitochondrial genome, allotopic rescue has been employed. In this process, genetic material is incorporated into the nuclear genome and is then targeted to the mitochondria (11). Preliminary results have been demonstrated in a mouse model injected with an AAV vector containing ND4 (the protein affected by the LHON 11778 mutation); it was successful in preventing vision loss, stabilizing ATP synthesis, and rescuing ganglion cells from cellular apoptosis (41). Apart from ND4 viral vectors, the NDI1—a yeast nuclear gene which encodes an equivalent of complex I—can be delivered directly as demonstrated by a study by Marella et al. (42). Through gene therapy, the NDI1 gene was successfully delivered to the optical layer within the superior colliculus in a LHON rat model. Subsequently, rats with previously impaired vision that were treated with the NDI1 gene experienced complete improvement in vision compared to animals injected with green fluorescent protein (GFP) controls.

Additionally, gene therapy holds promising potential and has been part of a Phase 1 and 2 dose-escalation trials of GS010, an intravitreal gene therapy for LHON (43). The early phase recruited 14 patients with the ND4 mutation who received a one-time dose of GS010 in their more severely affected eye. Follow-up at 96 weeks revealed that the eyes treated with AAV improved by an average of 21 ETDRS letters from their initial baseline. The treatment was also found to be most effective in 5 patients who had experienced vision loss within 2 years prior to enrollment. Though these findings are promising, continued follow-up is required to assess the long-term outcome of these patients.

Genetic Counseling

In many inherited diseases, with LHON being no exception, the role of genetic counselling is vital for patients to be informed of the risk of disease and chance of development in their children and relatives. More than 85% of individuals with LHON are homoplasmic, meaning that all of their mitochondria express the pathogenic DNA mutation (2). Due to maternal inheritance, men who are carriers of the mitochondrial mutation cannot pass it on to their offspring, while all children of a female carrier will harbour the mutation. However, as noted above, LHON demonstrates incomplete penetrance with only 50% of male and 10% of female carriers becoming affected; the difficulty in genetic counselling lies in the fact that genetic testing cannot predict the severity or rate of progression of disease carriers. LHON is a disease that can have significant visual consequences and impact quality of life—thus, a referral to a low vision specialist is warranted to educate patients on techniques to help preserve and improve reading and mobility. Lastly, given the multifactorial nature of the disease, it would be beneficial to also counsel on risk factors for disease conversion including tobacco and alcohol use.

CONCLUSION

LHON is a rare but well-studied mitochondrial disease that results in bilateral central vision loss and is a result of one of three pathogenic mutations (G11778A, G3460A, and T14484C). Currently, there is no proven treatment. However, given our growing understanding of the disease mechanism and ongoing research and trials being conducted on idebenone, EPI743, and gene therapy, there remains hope for emerging solutions for this devastating visual disease.

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Pharmacogenetics of Major Depressive Disorder: Progress in Two Serotonergic Targets

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ABSTRACT

Major depressive disorder (MDD) is a multifaceted, debilitating condition affecting over 300 million people worldwide. It contributes significantly to social, psychological and economic burdens on individuals and on society at large. Currently, the most widely prescribed antidepressant medications are selective serotonin reuptake inhibitors (SSRIs), which act by blocking serotonin (5-HT) reuptake into presynaptic neurons, thereby increasing the extracellular 5-HT concentration in the brain. However, response to SSRIs and other psychotropic medications used to treat depression is highly variable, with only about one third of patients responding to treatment with an SSRI. This may reflect, at least in part, the genetic heterogeneity of depressed individuals. Studies investigating the genetic components of depression aim to improve treatment outcomes and possibly pave the way for personalized medicine in which the first medication prescribed is the one most likely to result in remission. This review presents the results of several studies on two 5-HT related genes: *SLC6A4* and *HTR2A*, which encode for the serotonin transporter and the serotonin-2A receptor, respectively. Extensive studies have demonstrated that possessing two copies of the long allele (L/L) of the *SLC6A4* gene can predict better responses to the SSRI Escitalopram. However, this finding was significant only in the Caucasian population. In addition to this, several single nucleotide polymorphisms in the *HTR2A* gene also predict clinical outcome, although molecular mechanisms remain unclear. Hence, the results indicate that while there is significant potential for predicting treatment response associated with these and other genetic targets, there is much work left to be done to establish conclusive evidence for and feasibility of pharmacogenetic testing.

RÉSUMÉ

La dépression majeure est une condition variée et débilitante qui affecte plus de 300 millions-personne mondialement. Elle contribue de façon significative à des fardeaux sociaux, psychologiques et économiques sur les individus ainsi que sur la société en général. Actuellement, les médicaments antidépresseurs les plus prescrits sont les inhibiteurs sélectifs de la recapture de la sérotonine (ISRS), qui agissent en bloquant la recapture de la sérotonine (5-HT) aux neurones présynaptiques, afin d'augmenter la concentration extracellulaire de la 5-HT dans le cerveau. Cependant, la réponse aux ISRS et aux autres médicaments psychotropes utilisés pour traiter la dépression est très variable, avec seulement un tiers des patients qui répondent à un traitement de IRSR. C'est un reflet, en part, de l'hétérogénéité des individus déprimés. Les études examinant les composants génétiques de la dépression visent à améliorer les résultats des traitements et à ouvrir la voie si possible à la médecine personnalisée, dans laquelle le premier médicament prescrit est le plus probable à causer la rémission. Cette revue présente les résultats de plusieurs études sur deux gènes reliés à la 5-HT : *SLC6A4* et *HTR2A*, qui encodent le transporteur de la sérotonine et le récepteur sérotoninergique-2A respectivement. Des études extensives ont démontré que la possession de deux copies de l'allèle long (L/L) du gène *SLC6A4* peut prédire de meilleures réponses à l'IRSR Escitalopram. Toutefois, ce résultat n'a été pertinent qu'avec la population caucasienne. En plus, plusieurs polymorphismes d'un seul nucléotide dans le gène *HTR2A* prédisent également le résultat clinique, bien que les mécanismes moléculaires restent incertains. Ainsi les résultats indiquent que même s'il y a beaucoup de potentiel pour prédire les réponses aux traitements associés avec ces derniers et d'autres traitements génétiques, il reste encore beaucoup du travail à faire afin d'établir l'évidence conclusive et la faisabilité de l'analyse pharmacogénétique.

Major depressive disorder (MDD) is a multifaceted, debilitating condition affecting over 300 million people worldwide (1). It often contributes significantly to social, psychological and economic burdens on individuals and society at large (2). Furthermore, the World Health Organization (WHO) has declared MDD to be the leading cause of disability worldwide (1). According to the Diagnostic and Statistical

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Manual of Mental Disorders, Fifth Edition (DSM-5), depression is characterized by the presence of at least five out of nine symptoms for a minimum of two weeks (3). Some of these symptoms may appear ambiguous and include fluctuations in weight, sleep and energy; however, there must be the presence of a depressed mood and/or anhedonia (loss of pleasure in formerly pleasurable activities), which are unexplainable by bereavement, drug use or other mental disorders for a diagnosis of MDD. Investigations into the etiology of MDD have unveiled several environmental risk factors such as childhood abuse, childhood neglect and stressful life events (4). The contributions of genetic risk factors to MDD are also significant, as estimates of inheritance based on twin studies suggest a heritability of 40-50% (4-10).

It is likely that genetic factors may mediate responses to psychotropic medications, giving rise to pharmacogenetics, defined as the study of the genetic variants associated with producing a drug response (11). Variance in the human genome arises from several genetic alterations including deletions, insertions and changes in the number of copies of base pairs. However, the most common and most studied variations are those that arise from single-nucleotide polymorphisms (SNPs) in candidate genes (12). SNPs are substitution mutations at a given location on a chromosome (11) that may alter the function of the encoded protein. Most pharmacogenetic studies in depression have focused on identifying SNPs in loci that encode proteins related to neurotransmission.

Currently, the most widely prescribed antidepressant medications are selective serotonin reuptake inhibitors (SSRIs), which act by blocking serotonin (5-HT) reuptake into presynaptic neurons, thereby increasing the extracellular 5-HT concentration in the brain. Response to SSRIs and other psychotropic medications used to treat depression is highly variable, with only about one third of patients responding to treatment with an SSRI (13). This may reflect, at least in part, the genetic heterogeneity of depressed individuals (12). Irrespective of the variability in response, SSRIs are considered first-line drugs in the treatment of MDD (14). Hence, this review will focus on two well-studied 5-HT related genes: *SLC6A4* and *HTR2A*. To date, several studies have provided a comprehensive overview of the genes involved in predicting an antidepressant response (12,15). In contrast, few studies have addressed how these genetic alterations may alter key molecular processes that are correlated with producing a therapeutic effect, and ultimately affect the prescriber's choice of medication (often referred to as personalized medicine). Furthermore, the review will address what types of studies are required to further the field of pharmacogenetics.

ARTICLE SELECTION CRITERIA

Research articles were selected by conducting a search for key words on "google scholar" and "PubMed" websites. Key words include: pharmacogenetics, major depressive disorder, SLC6A4, HTR2A, antidepressant response, serotonin transporter and serotonin-2A receptor. Peer-reviewed articles observing the effect of genetic alterations on the antidepressant response and/or treatment outcome were included. Furthermore, the antidepressant response and/or treatment outcome refers to the primary outcome measure; commonly reported as the difference between baseline and last-assessment scores calculated by the Hamilton Rating Scale for Depression (HRSD) or the Montgomery and Asberg Rating Scale (MADRS). All positive and/or negative results on the antidepressant response, as well as sample size, were reported.

RESULTS

SLC6A4 - candidate gene encoding the serotonin re-uptake transporter (5-HT transporter)

The 5-HT transporter has been the target of numerous studies since the discovery of SSRIs, which bind to and inhibit the 5-HT transporter to produce a therapeutic effect. Counterintuitively, mice lacking the 5-HT transporter demonstrate an increase in anxiety-like and depressive-like behaviours (16). Nevertheless, preclinical studies have demonstrated the importance of the 5-HT transporter in mediating depressive behaviours. Indeed, *SLC6A4* is perhaps the most studied gene with a potential for predicting an antidepressant response (17,18). In particular, the 5-HTTLPR (5-HT transporter linked polymorphic region) in the *SLC6A4* gene has been widely researched. There are two alleles in the 5-HTTLPR (L; long allele, or S; short allele), which can combine for three possible genotypic variations: L/L, L/S, and S/S (19). A meta-analysis of 15 studies from 1435 subjects sought to determine the effect of the 5-HTTLPR on the antidepressant response. Their results showed a significant correlation between the L allele of the 5-HTTLPR and clinical response in patients undergoing treatment with SSRIs such as citalopram, fluoxetine, sertraline and paroxetine (20). The effect of the 5-HTTLPR was most pronounced for response within 4 weeks of treatment in Caucasian patients receiving these medications (20). Furthermore, patients homozygous for the L/L allele showed a better response to these SSRIs than homozygous S/S or heterozygous (L/S) patients. More

recently, a meta-analysis by Porcelli et al (2012) of 33 studies from 5479 subjects confirmed that individuals carrying the L/L genotype were about one and a half times more likely to remit on SSRIs (21). Closer analysis revealed that only L carriers of Caucasian ethnicity predicted antidepressant response and remission (21). However, despite the successful identification of a genetic predictor, several studies failed to find any effect of the 5-HTTLPR on antidepressant response (22-24). Negative results could be due to several reasons such as strict inclusion criteria and failing to analyze results based on the class of antidepressant medication (21).

Epigenetic alterations can also influence the antidepressant response, specifically the methylation of the promoter region of *SLC6A4* encoded by 5-HTTLPR. A study by Zhao et al. (2013) involving 84 matched monozygotic twins found that variations in methylation levels of the promoter region of the 5-HT transporter gene were associated with variation in depressive symptoms (25). On average, a 10% increase in the methylation of the 5-HT transporter was associated with a 4.4x increase in the patient's Beck Depressive Inventory II (BDI-II) score, which suggests an increase in depressive symptom severity. In contrast to this, Domschke et al (2014) found that a lower average 5-HT transporter methylation across all nine DNA sites investigated in the 5-HTTLPR was associated with an impaired response to escitalopram after six weeks of treatment (26). As both rodent and human studies suggest that the 5-HT transporter is an important marker for pharmacogenetics, further research to understand if/how it can be targeted to improve treatment response in patients with MDD is warranted.

HTR2A - candidate gene encoding the serotonin-2A receptor (5-HT2AR)

Pre-clinical studies have suggested a role for the 5-HT2AR in depression onset as well as treatment outcome. For instance, in 5-HT2AR KO mice, corticosterone administration increased depressive-like symptom severity relative to wild-type controls (27). These mice also failed to respond to SSRIs as assessed by the forced swim test: a measure of despair, where rodents are placed in an inescapable container filled with water and escape-related movements are monitored. In this paradigm, antidepressant response is associated with increased escape-related movement over a longer period. In addition to poor performance in the forced swim test, 5-HT2AR KO mice did not show an SSRI-induced increase in neurogenesis; a common neurobiological effect of several antidepressants (28). Together, these studies indicate 5-HT2AR KO mice are resistant to antidepressant treatment, suggesting that the 5-HT2AR may prove useful in predicting treatment outcome. Human studies have also provided evidence for the involvement of the 5-HT2AR in mediating an antidepressant response. At least three studies examining post-mortem brain tissue have reported a decrease in 5-HT2AR expression in the hippocampus of individuals with MDD, while a positron emission tomography (PET) study reported no change (29-32). Furthermore, these changes were hypothesized to be due to hypersensitive 5-HT2AR (32). Additionally, in the frontal cortex of human participants, the 5-HT2A receptor has been shown to be downregulated with SSRI treatment and is correlated with the improvement of clinical symptoms (33). A study by Cutler et al also showed that medications that block the 5-HT2A receptor such as quetiapine are effective as a monotherapy in MDD (34). Furthermore, several medications used as adjuncts in MDD, namely aripiprazole, brexpiprazole and cariprazine block the 5-HT2A receptor as well (35-37). Together, these human studies demonstrate the importance of blocking the 5-HT2AR in improving clinical outcome.

Indeed, a number of studies have confirmed the predictive value of several SNPs within *HTR2A*. The most recent meta-analysis using pooled data from 11 studies (n = 1775) found that the rs6313 and rs7997012 were associated with a higher response rate to antidepressants in MDD patients (38). Although a third SNP, rs6311, was not predictive of treatment outcome (38), a meta-analysis consisting of seven studies (n = 590) found that it was predictive of adverse effects associated with SSRIs (39). Overall, there appear to be several SNPs within the 5-HT2AR that may predict treatment outcome.

DISCUSSION

Genetic variance produces changes in key molecular mechanisms underlying the antidepressant response. In the *SLC6A4* gene, the L allele is more efficient at transporting 5-HT while the S allele confers reduced re-uptake of 5-HT into the presynaptic neuron (19). As the L allele confers greater transporter efficacy, the administration of an SSRI may inhibit re-uptake to a greater degree relative to an individual with an S allele, resulting in better treatment outcome. Furthermore, mice homozygous for the transporter (analogous to L/L) exhibited a gradual recovery of 5-HT neuronal firing after 21 days of administration of the SSRI escitalopram, which was not the case in mice heterozygous at the serotonin transporter (analogous to L/S; 40). This is significant because recovery of 5-HT neuron activity has been proposed to be correlated with producing a therapeutic effect (41). Alternatively, SSRIs may not be effective in those carrying the S/S allele because 5-HT re-uptake is already

impaired in these individuals. As 5-HT re-uptake is a necessary process for the recycling of neurotransmitter, inhibiting re-uptake additionally may deplete 5-HT stores.

Overall, there appear to be several SNPs within *HTR2A* that may predict treatment outcome. Studies moving forward should assess the functional consequences of these SNPs to provide an optimized treatment regimen for individuals with depression. Functional consequences may include changes in receptor expression in the brain and altered signaling at the receptor once 5-HT is bound. For example, the rs6311 SNP has been demonstrated to possibly affect the expression of 5-HT2AR. Parsons et al. (2004) reported that the presence of rs6311 SNP (associated with a poorer antidepressant response) significantly increased promoter activity in cell-based assays (42). This implies that the 5-HT2AR is expressed to a greater extent in the brains of individuals with this receptor variant. However, others have noted that the rs6311 SNP is associated with greater methylation and consequently decreased 5-HT2AR mRNA translation efficacy in cell-based assays, implying that the 5-HT2AR is expressed to a lesser extent in the brains of individuals with this receptor variant (43). These contradictory findings highlight the need for assessing the effect of SNPs on receptor protein expression possibly through PET studies in humans. Through PET studies, one may be able to detect changes in brain receptor expression of the individuals who have inherited these SNPs. Taken together, the results suggest that while there is promise with the *SLC6A4* and *HTR2A* genes in relation to treatment response, more studies are necessary to clarify if and how they contribute to response to antidepressant medications.

Thus far, a major limitation to research focusing on SNPs associated with depression is cost. Previous research by Perlis et al. (2009) evaluated the cost-effectiveness of pharmacogenetic testing for a single SNP (rs799012) in the *HTR2A* gene. The study demonstrated that pharmacogenetic testing, (i.e genetic testing to ascertain the most efficacious antidepressant medication for a specific individual), was less cost-effective than using an SSRI or bupropion as first and second-line treatments, respectively (44). In other words, it may be less expensive for clinicians to proceed with a trial-and-error approach than to test for a single pharmacogenetic marker. Although several SNPs have been identified, single gene analysis is only able to explain a small amount of the variance observed in the antidepressant response. For pharmacogenetic testing to be effective, several genes must be analyzed at once. This is now possible due to the exponential decrease in the cost of human genome sequencing and has led to the feasibility of genome-wide association studies (GWAS) (12). GWAS allow for the simultaneous analysis of hundreds of SNPs, and may reveal complex interactions between candidate genes, which could account for a larger amount of variance in the antidepressant response. For example, it has been reported that the rs7333412 SNP within the *HTR2A* gene influences the degree of 5-HT transporter expression and/or the affinity of 5-HT for the 5-HT transporter (45). Therefore, simultaneous analysis of SNPs within these two candidate genes and others may provide greater predictive value and ultimately polygenic risk scores (PRS) for a single person (12). The development of PRS may lead to improvements in predicting the antidepressant response leading to more efficient clinical care. First, it may increase the efficiency and safety of clinical trials by identifying and including those who are more likely to respond to medication and excluding those who pose a higher risk for serious adverse events (11). Secondly, pharmacogenetic testing would reduce reliance on trial-and-error prescribing and maximize the cost-effectiveness of the first clinician visit (11).

CONCLUSION

In conclusion, several pharmacogenetic markers in the *SLC6A4* and the *HTR2A* genes have been identified. Despite these robust findings, individual studies continue to find negative results. This is in part explained by the complex nature of the disease. While some environmental risk factors for depression have been identified, there seems to be no clear consensus on genetic proclivities or a clear correlation between genetic factors and drug response. To acquire more robust pharmacogenetic markers, larger sample size studies must be conducted. This is especially true for more rare SNPs within the population. Also, SNPs must be analyzed in concert to reveal interactions that may prove to be more predictive of treatment outcome. Lastly, it is imperative that we understand the functional consequences of SNPs in order to tailor antidepressant treatments to the individual. SNPs that confer greater or less signaling and/or expression at individual receptors may be mitigated with adjunct treatment or the use of a different medication which might be more efficacious. This is a more cost-efficient future of pharmacotherapy with the potential to reduce suffering in individuals living with depression.

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Gene Therapy: A Promising Therapeutic Strategy for Malaria

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ABSTRACT

Malaria is a serious illness caused by the *Plasmodium* parasite, which places approximately 3.5 billion people at risk. Currently, preventative measures are key in combatting this disease. However, gene therapy is an emerging field that shows promising results for the treatment of malaria, by modifying cells through the delivery of genetic material. Most notable was the discovery of CRISPR-Cas9, which not only allows deleterious mutations to be repaired, but does so with specificity, speed, and simplicity. There are numerous ongoing trials focusing on gene therapy in malaria treatment and prevention. They involve different approaches such as the genetic modification of vector mosquitoes to interfere with malaria transmission, use of CRISPR-Cas9, maternal-effect dominant embryonic arrest, homing endonuclease gene drive systems, and the design of specific Morpholino oligomers to interfere with the expression of parasitic characteristics. Overall, this emerging field shows promising results to treat and prevent not just malaria, but other diseases such as cancer, diabetes, and obesity.

RÉSUMÉ

Le paludisme est une maladie sévère causée par la parasite *Plasmodium*, qui met en danger environ 3.5 milliards de personnes. Actuellement, des mesures préventives sont la clé pour combattre cette maladie. Toutefois, la thérapie génique est un domaine émergent qui montre des résultats prometteurs pour le traitement du paludisme, en modifiant des cellules par la livraison de matériel génique. En particulier, il faut reconnaitre la découverte de CRISPR-Cas9, qui permet non seulement la réparation de mutations délétère, mais la fait avec spécificité, rapidité et simplicité. Il y a plusieurs essais en cours concentrant sur la thérapie génique pour le traitement et la prévention du paludisme. Ils impliquent plusieurs approches telles que la modification génique de moustiques vecteurs pour intervenir dans la transmission du paludisme, l'utilisation de CRSIPR- Cas9, l'utilisation du gène Medea (arrêt embryonnaire dominant d'effet maternel), les systèmes de forçage génique par endonucléase de homing, et la conception d'oligomères morpholino spécifiques pour intervenir dans l'expression de caractéristiques parasitiques. En gros, ce domaine émergent montre de résultats prometteurs pour le traitement et la prévention de non seulement le paludisme, mais d'autres maladies telles que le cancer, le diabète et l'obésité.

Malaria is a severe disease that places almost 3.5 billion people at risk, resulting in approximately 500 million new infections and 473,000 deaths per year (1,2). It is caused by several species of parasitic protozoans of the genus *Plasmodium*, which are transmitted through the bite of the vector mosquito *Anopheles*. Malaria symptoms include fever, vomiting, fatigue, and headaches (3). However, in severe cases, jaundice, seizures, coma or even death have been observed. Symptoms usually arise within 10-15 days of being bitten, and if left untreated can reoccur months later. Malaria primarily affects children under the age of 5 and has a significant impact on pregnant women (2). Thus, it is imperative that an effective vaccine is developed, as nearly half the world's population is at risk. This article will serve as a review of the current gene therapy strategies that have been developed and tested for combatting malaria.

LIFE CYCLE

The development of a malaria vaccine is difficult, in large part due to the complex life cycle of the malaria parasite (2). Transmission of malaria begins when malaria-infected *Anopheles* mosquitoes bite and inject *Plasmodium* sporozoites into the human bloodstream. These sporozoites then travel to the liver and infect hepatocytes, thereby initiating the hepatic stage of the parasitic life cycle (4). Subsequently, the sporozoites grow and differentiate into schizonts, which contain a multitude of haploid merozoites. The merozoites leave the hepatocytes and enter the bloodstream, where they

Keywords: Gene therapy; Malaria; Gene editing; CRISPR

invade erythrocytes and begin the asexual erythrocytic stage of the parasitic life cycle (5). For certain species such as *P. vivax* and *P. ovale*, however, the hepatic stage of the life cycle does not involve the development of schizonts from the sporozoites (6).

Inside the erythrocytes, the merozoites mature into erythrocytic schizonts. At full maturity, the erythrocyte ruptures, releasing new merozoites that can then invade additional erythrocytes. This cycle repeats every few days, resulting in a multitude of malaria-infected erythrocytes (5). In some cases, the merozoites can leave the asexual replication cycle, and develop into sexual male and female gametocytes. Initially, gametocytes remain in the bone marrow and some other organs, but after maturation, gametocytes typically circulate within the bloodstream. Malaria is transmitted to the mosquito after it bites an infected human, ingests the gametocytes, and the erythrocytes within the midgut rupture and release gametocytes, which further develop into mature gametes and later fuse to form a diploid zygote. After further maturation, the zygote develops into a motile ookinete and burrows through the midgut to form an oocyst on the opposite side. After 8-15 days of maturation the oocyst ruptures, releasing thousands of sporozoites that may migrate to the mosquito's salivary glands. Some malaria parasites can form hypnozoites, which can remain dormant for extended periods of time, causing reoccurrences of symptoms weeks to months later (7).

CURRENT THERAPEUTIC OPTIONS

The treatment of malaria depends upon which *Plasmodium* species has caused the infection. Almost 75% of all reported malaria cases are caused by *P. falciparum*, which is responsible for the most severe cases. Most non-*P. falciparum* cases are caused by *P. vivax*, and a few cases are reported by the other two species *P. ovale* and *P. malariae*. They are typically treated with chloroquine (10mg/kg) for a period of 24-48h. The problem with treatment of *P. falciparum* is that the parasite is resistant to many antimalarial drugs available on the market, resulting in increased severity of *P. falciparum* infections. Its treatment consists of intravenous or intramuscular therapy of artesunate (4mg/kg) and amodiaquine (10mg/kg) for 3 days (8). Complete protection can be achieved in humans via immunization with live, irradiation-attenuated sporozoites; however, this is impractical for widespread use (9).

CURRENT APPROACHES TO GENE THERAPY

There are numerous ongoing trials focusing on gene therapy for malaria treatment and prevention. They involve different approaches, such as the development of a genetic vaccination using plasmid DNA (2); genetically modifying vector mosquitoes to interfere with malaria transmission (intended to prevent parasite development and lower transmission) (9), Cas9-mediated modification of the *Anopheles* mosquito to breed within a mosquito population that does not support the malaria parasite (10), genome editing of *Plasmodium sp.* to remove its parasitic effects in humans (11), and designing specific morpholino oligomers to interfere with the expression of parasitic characteristics (12). Malaria treatment will require vector-control strategies that are self-sustaining and unaffected by the migration of infected humans or mosquitoes, and must also be complemented by other therapeutic means due to the continuous evolution of resistant *Plasmodium* strains.

Plasmid DNA Vaccination

Developing a vaccination for malaria has proven difficult, given that the *Plasmodium* parasite transitions through various stages over the course of its life cycle. A successful malaria vaccination therefore requires multiple components, which can target each stage of the parasite's life cycle (2). A nucleic acid-based vaccination shows promise as it can be genetically modified to induce the expression of the required antigens for each stage in the life cycle. Researchers have shown that in a mouse model, intramuscular injection of a DNA-based vaccine increased serum levels of antibodies specific to the malaria parasite, thus providing protective immunity (13). However, another phase 1 clinical trial conducted in 2016 for the EP1300 DNA vaccine against *P. falciparum* was not deemed fit for further progression due to several safety issues, where subjects experienced spontaneous adverse effects and reported local reactogenicity (14).

Vectored Immunoprophylaxis

Immunoprophylaxis has been pursued in malaria prevention for over 50 years. Significant investments in this field have led to the development of the RTS,S vaccine, which contains a fusion protein derived from different domains of the circumsporozoite protein (CSP), an antigen present on the surface of the malaria parasite during its infective stages. In a phase 3 trial conducted in 2012 (15), this vaccine provided modest protection against the *P. falciparum* malaria parasite in infants when critical serum concentrations were reached. The World Health Organization (WHO) is presently coordinating a pilot program to evaluate the feasibility of routine administration of this vaccine in African countries (16).

Developments in the field of gene therapy have allowed for a new strategy involving vectored immunoprophylaxis (VIP), which involves the delivery of genes encoding targeted neutralizing antibodies to human cells (1). This approach utilizes adeno-associated virus-based vectors (AAV), which do not integrate into the human genome and allow for the expression of antibodies for months to years. Genetic components of mouse antibodies were inserted into the human IgG gene to develop AAV encoding antibodies targeted against the P. falciparum circumsporozoite protein (17). These tests were carried out in mice to validate the approach. The results show that intramuscular injection of these AAV can neutralize malaria sporozoites and can provide protection from mosquito-transmitted malaria. This approach is currently not validated for human use. However, after the approval an AAV vector to treat Familia Lipoprotein Lipases deficiency in Europe, further experiments are being carried out in non-human primates to determine VIP protocols for dosage and injection sites (18).

Genetic Manipulation of Mosquito Vector Competence

Plasmodium transmission is dependent upon the *Anopheles* mosquito. Reducing or eliminating malaria transmission can be accomplished by preventing the mosquito from supporting parasite development. A probable solution would be to genetically modify the expression of effector genes within the *Anopheles* mosquito midgut, in order to express products that inhibit parasite development. For example, *Anopheles stephensi* mosquitoes were genetically engineered to express the SM1 peptide (9), which binds to an ookinete receptor on the luminal surface of the midgut epithelium and prevents ookinete midgut invasion. These genetically modified mosquitoes were impaired in their ability to transmit the malaria parasite. This proof of concept shows that *Plasmodium* transmission can be reduced or eliminated via genetic modification of the vector mosquito. However, this method faces many challenges namely the translation of these effector gene modifications into wild mosquitoes as releasing transgenic mosquitoes into the wild is simply not enough. One would have to give these transgenic mosquitoes a competitive advantage over wild mosquitoes for gene transmission to be effective. Approaches such as MEDEA (maternal-effect dominant embryonic arrest) and HEG (homing endonuclease gene) are two proposed approaches utilizing transposable elements.

The MEDEA drive system has shown positive results in the *Drosophila* model system. It involves linking the effector gene to a toxin that inactivates an essential maternal gene required for embryonic development (9). Transferring this technique into mosquitoes is not yet feasible as the technology required does not exist. However, with time this approach may be seen in the practical field.

The HEG drive system encodes highly specific endonucleases that contain recognition sequences (19). Mosquitoes carrying the HEG can mate with wild mosquitoes and their resulting offspring will have an increased likelihood of inheriting the drive gene, eventually resulting in HEG expression within the entire population. HEGs have shown to induce chromosomal cleavage and gene conversion, and inhibit parasite development within the progeny of these transgenic mosquitoes (19).

RNA Interference

RNA interference (RNAi), involving the use of non-coding RNA fragments paired with specific molecular machinery to silence genes, has become a widespread mechanism for gene regulation. This type of epigenetic regulation is heritable and present in a variety of eukaryotes, but its existence in *Plasmodium* is debated (20). Initiation of this mechanism begins with the introduction of double stranded RNA (dsRNA) to target a specific gene product. The dsRNA is cleaved into short interfering RNAs (siRNAs) of ~21-23 nucleotides in length by the enzyme Dicer, a cytoplasmic dsRNA-specific RNaseIII endonuclease. The siRNAs are unwound into two single stranded siRNAs, where one strand (the passenger strand) is degraded, and the other (the guide strand) becomes associated with the RNA-induced Silencing Complex (RISC). Once associated, the guide strand helps RISC to locate a complementary mRNA strand. Once found, the mRNA is cleaved by the enzyme Slicer, a protein from the Argonaute family that resides within the RISC (21). RNAi is therefore a potent and extremely specific technique that allows for reverse genetic experimentation and provides a time-efficient means to determine gene function.

Application of RNAi to malaria is still in early stages of research. However, studies have shown successful growth inhibition via RNAi (22,23). In one study conducted by McRobert and McConkey (24), RNAi was used to target the gene encoding dihydroorotate dehydrogenase (DHODH), which is an enzyme involved in pyrimidine synthesis. Unlike most organisms, malaria parasites utilize *de novo* pyrimidine synthesis; therefore, the disruption of DHODH should inhibit parasite growth. In the same study, RNAi was applied to the gene for chorismate synthase (CS), which is involved in the synthesis of chorismate, a precursor in folate synthesis via the shikimate pathway. Parasite growth

was inhibited from 50 to 90% via RNAi, suggesting that DHODH and CS are valid chemotherapeutic targets for gene silencing to limit *Plasmodium* growth. This application of RNAi provides useful insight and lays a strong foundation for developing an effective vaccine and anti-malarial chemotherapeutic strategy.

Research indicates that the introduction of dsRNA into the asexual forms of the malaria parasite could downregulate gene expression (25). However, genomic analysis of *Plasmodium* confirmed that there are no genes involved with RNAi and no response to dsRNA could be documented (26).

Morpholino Oligomers

A morpholino oligomer (MO) is a molecular complex used to modify gene expression. MO have identical base pairing characteristics as DNA and RNA, and resist degradation by nucleases while maintaining a neutral charge. They are utilized in gene editing techniques due to their ability to block access of other molecules to small specific sequences of roughly 25 base pairs in size on RNA. In a 2015 study, Yale researchers utilized MO to directly affect gene expression within *P. falciparum* (12). Since this parasite lacks RNAi machinery, a MO-based, RNA-targeting approach is much more appropriate. The basis behind this technique is much like RNAi, without its respective machinery. These mechanisms take place within the cells of the parasite itself, thus utilizing an MO strategy that targets and block certain key genes. In this way, this strategy can potentially be much more effective than an anti-malarial drug. The research undertaken at Yale suggests that MO can be utilized to weaken the parasite, and can potentially cause resistant strains becoming susceptible to anti-malarial drugs.

CRISPR-Cas9 Gene Drive

The CRISPR-Cas9 system has allowed users to genetically engineer endonucleases which can target and cleave specific genomic sequences in a more simple fashion than the previous transcription activator-like effector nucleases (TALENs) system. A Cas9 endonuclease is guided to a target DNA site by a complementary guide RNA, where it can specifically edit the DNA sequence, all without the need for complex protein engineering and selection techniques (27). The CRISPR-Cas9 system offers promising opportunities to develop gene drive systems for the control of mosquito vectors of malaria, due to its specificity and flexibility. The use of the CRISPR-Cas9 system for vector control requires specific engineering to diminish the ability of the *Anopheles* mosquitoes to transmit disease. Developing an endonuclease that produces a mutation in a gene required for female *Anopheles* fertility or parasite development is promising in preventing the transmission of malaria from mosquito to humans. It is essential that endonuclease homing is confined to germline cells, either before or during gamete formation. This avoids disruption of the wild type allele and allows for the normal development of heterozygous mosquitoes. It also ensures their viability and fertility, which is critical for transmitting the endonuclease to their progeny.

A 2015 study utilized the CRISPR-Cas9 system as a gene drive mechanism in *A. gambiae,* which is the most common malaria vector mosquito (27). The researchers identified three genes that, when disrupted, expressed a recessive female-sterility phenotype. CRISPR-Cas9 gene drive constructs were inserted into each of these three loci, and the constructs were designed to target and edit each gene. Two classes of mosquitoes were found in the progeny; ones which were homozygous and ones which were heterozygous for the edited genes. The homozygous female mosquitoes were infertile, while the heterozygous female mosquitoes were fertile. Both homozygous and heterozygous males were fertile. A strong gene drive was observed at each of the loci, and the noted transmission rates to progeny were significantly high, ranging from 91.4-99.6%. The study showed that gene knockouts at the three loci were achieved with a high frequency, and the rates of inheritance that were observed provide a solid basis for the development of a gene drive system that shows promise in reducing mosquito populations. The study validates the CRISPR-Cas9 gene drive system as a valuable gene editing tool that will play a vital role in functional genetics in the malaria vector mosquito, due to its suitability for population-modification strategies that incorporate the use of transgenes with expressed phenotypes, such as parasite resistance and female fertility.

A study conducted at UCLA-San Diego (28) successfully engineered a gene drive in fruit flies using the CRISPR-Cas9 gene drive system, which provided beneficial information to the researchers, who then tested the Cas9 system with *A. stephensi* (10). The researchers used the mutagenic chain reaction (MCR) method with CRISPR-Cas9 to produce mutations in fruit flies, which would then be copied from one chromosome to another, with the intent of producing a homozygous mutation resulting in a loss of function (28). The principle was then applied to mosquitoes, where the genes targeted were involved with malaria resistance. The modified genes were present in the offspring of these mutant mosquitoes, which were also resistant

to malaria.

Gene drive is a profound technique that, when coupled with the CRISPR-Cas9 system, can modify not only a single organism, but an entire population. In the case of malaria, this technique can be used to produce mosquitoes that are incapable of carrying the malaria parasites, but it can also have unanticipated environmental costs that may be irreversible. As of now, the CRISPR-Cas9 system is limited by the currently available delivery methods, as well as the risk of possible off-target edits that may lead to deleterious mutations. Advancements and computational optimization of the guide RNAs has resulted in significantly more accurate gene editing with minimal off-target modifications. CRISPR gene drive technology is developing at an extremely fast pace with a wide variety of applications. However, the fact remains that it has the potential to alter ecosystems in unpredictable ways.

CONCLUSION

Malaria is one of the world's most severe diseases, killing nearly one million people every year. Since non-genetic techniques have slowly reached a plateau regarding the effective treatment of malaria, genetic techniques have begun to prevail. While VIP can be a viable treatment option, VIP protocols need to be modified and tested regarding dosage and injection site. HEG gene drive systems do show some success, although the CRISPR-Cas9 system prevails due to its specificity and flexibility regarding its complex engineering and selection techniques. The aforementioned techniques therefore provide a foundation for future research methodologies intended to combat malaria, whether it be through the development of an effective multicomponent vaccination, the introduction of malaria resistant mosquito populations, or by genetically modifying the pathogen itself.

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Agenesis of the Gallbladder: A Phantom Menace

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ABSTRACT

Objective: Gallbladder agenesis (GBA) is a rare congenital disorder with an estimated incidence of about 0.06%. Despite the absence of a gallbladder, these patients may present with symptoms mimicking biliary colic or cholecystitis. Ultrasound findings and liver function tests are often misleading. Some of these patients undergo laparoscopy without successful identification of gallbladder and paradoxically report symptom relief.

Case: We present a case of GBA in a 54 year-old female, who presented with right-sided abdominal pain. The clinical history and examination were consistent with biliary colic. Initial investigations, including liver function tests, upper endoscopy and ultrasound did not demonstrate upper gastrointestinal pathology and did not clearly identify a gallbladder. Subsequent HIDA scan and CT of the abdomen did not visualize a gallbladder. An MRCP confirmed gallbladder agenesis. The patient was managed conservatively and was symptom free on discharge and follow-up.

Discussion: We wish to highlight four learning points: 1. Patients with gallbladder agenesis often present with biliary symptoms. 2. Ultrasound and CT of the liver may not always identify this anomaly. 3. MRCP is the gold standard for making a diagnosis of gallbladder agenesis. 4. Surgeons must have a high index of suspicion of GBA when the gallbladder is poorly visualized or not identified on ultrasound.

RÉSUMÉ

Objective: Agénésie de la vésicule biliaire (AVB) est un désordre congénital rare avec une incidence d'environ 0.06 %. Malgré l'absence d'une vésicule biliaire, ces patients peuvent se présenter avec des symptômes qui imitent une colique biliaire ou une cholécystite. Les trouvailles à l'echographie et les tests de la fonction hépatique sont souvent trompeurs. Quelques patients subissent une laparoscopie, sans identification de la vésicule biliaire, mais rapportent une amélioration de symptômes paradoxalement.

Cas: Nous présentons un cas de AVB chez une femme de 54 ans, qui se présentait avec une douleur abdominale droite. L'historique et l'examen physique étaient concordants avec une colique biliaire. Les investigations initiales, y inclut les tests de la fonction hépatique, l'endoscopie haute et l'echographie, n'ont pas démontré une pathologie gastro-intestinale supérieure et n'ont pas identifié clairement une vésicule biliaire. Ni une scintigraphie HIDA ni une TDM abdominale n'ont visualisé une vésicule biliaire. Une CPRM a confirmé l'agénésie de la vésicule biliaire. La patiente a été gérée de façon conservatrice et a été asymptomatique lors de son congé et suivie.

Discussion: Nous voulons surligner quatre points d'enseignement : 1. Les patients avec l'agénésie de la vésicule biliaire se présentent souvent avec des symptômes biliaires. 2. L'echographie et la TDM du foie ne trouvent pas toujours cette anomalie. 3. CPRM est le test de référence pour le diagnostic de l'agénésie de la vésicule biliaire. 4. Les chirurgiens doivent avoir une haute suspicion de l'AVB quand la vésicule biliaire n'est pas facilement visualisée ou identifiée à l'ultrason.

A 54 year-old female presents to the Emergency Department with right-sided abdominal pain radiating to her back. The pain has been intermittent for several months, is aggravated by meals and is associated with nausea without vomiting or diarrhea. There has been no history of jaundice. Her past surgical history is significant for a Roux-en-Y gastric bypass surgery, during which there was a planned concurrent cholecystectomy but the gallbladder could not be identified. She is known to have mild dumping syndrome since her bypass.

On examination, her vital signs are normal. She is tender in the right abdomen and has a positive Murphy's sign. All laboratory tests, including complete blood count, liver function tests, bilirubin and lipase are normal. An abdominal ultrasound is performed but her gallbladder cannot be visualized (**Figure 1**). Upper endoscopy is performed to rule out peptic ulcer disease and is normal. Because of the clinical suspicion that her symptoms were biliary in nature, a HIDA scan is done (**Figure 2**) which demonstrates no filling of the gallbladder. A CT scan of the abdomen shows a wedge-shaped area of

Keywords: Gallbaldder agenesis; Biliary surgery; Laproscopy

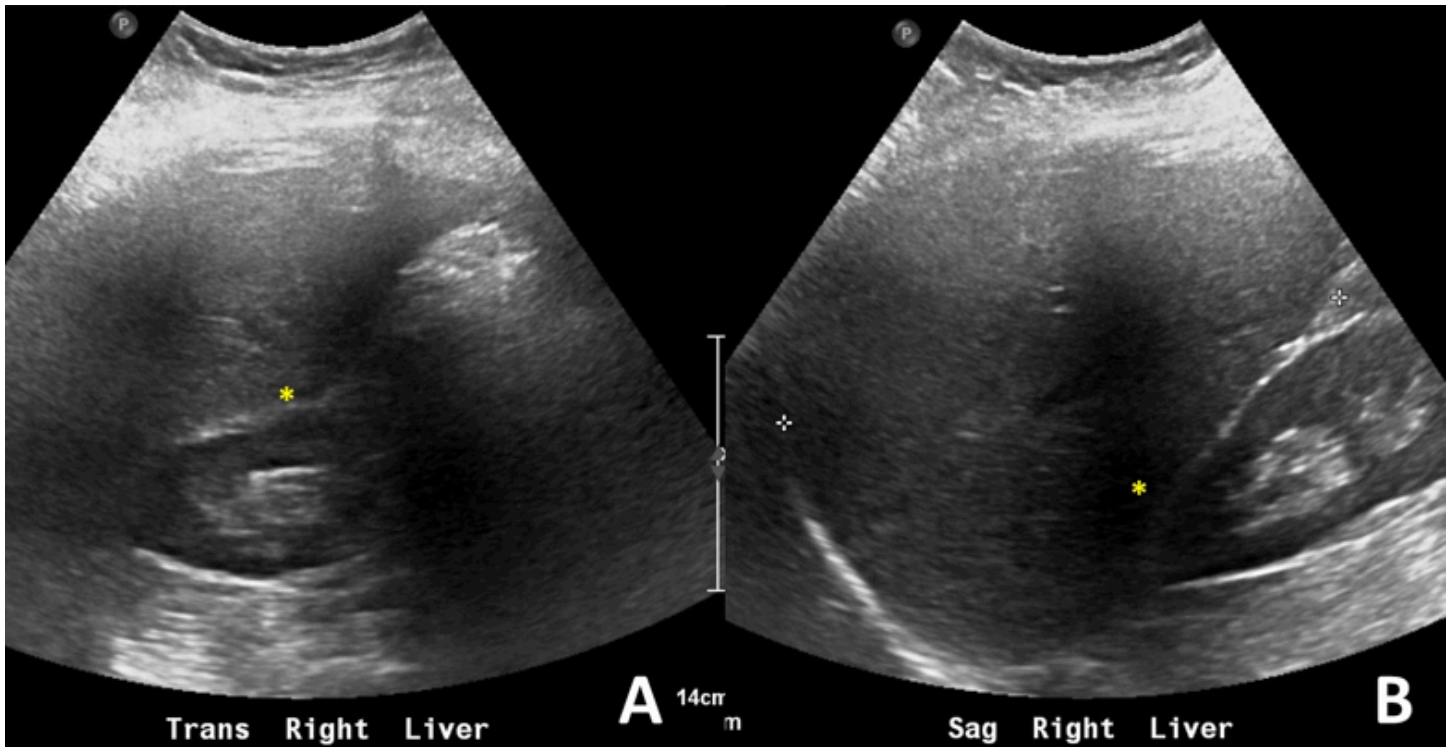

Figure 1. Ultrasound images from our patient with gallbladder agenesis. Stars (*) are located at the interface between the liver and right kidney, and the lack of a fluid-filled sac in the subhepatic space represents the absence of a gallbladder. (A) Sagittal view of the liver. (B) Transverse view of the liver.

hypoattenuation at the expected location of the gallbladder fossa without appreciation of a gallbladder (**Figure 3**). Having failed to obtain a definitive diagnosis, a MRCP is done which confirms absence of a gallbladder consistent with a diagnosis of gallbladder agenesis (**Figure 4**). The patient is managed nonoperatively and improves.

DISCUSSION

Epidemiology

Gallbladder agenesis is an anomaly that results from the failure of budding of the gallbladder and cystic duct from the common bile duct during the fourth and fifth weeks of embryogenesis (1). The first case reports were published in 1701 by Lemery and Bergman (2). It is exceedingly rare with an estimated incidence of about 10-65 per 100,000 in clinical practice, predominating in middle aged women (2). In autopsy series, the incidence is estimated to be higher at 90 per 100,000 with both sexes equally affected (2). It has been suggested that there may be a familial association since it has been noted to occur in familial clusters, but no specific genotypic predisposition has been identified (1).

Presentation

Based on clinical presentation and physical findings, GBA has been classified into three types: asymptomatic, symptomatic, and associated with other congenital defects. Asymptomatic GBA is identified incidentally on imaging studies or at autopsy. Patients with symptomatic GBA represent over 50% of cases. They often present, interestingly, with biliary colic, dyspepsia or jaundice (2,3). It is in this group that diagnosis becomes a challenge. Patients are often subjected to a surgical intervention with a presumed diagnosis of cholelithiasis. Hence, in a number of cases of symptomatic GBA, the diagnosis is made intraoperatively (4). Congenital defects often associated with GBA include cardiac, gastrointestinal, and genitourinary abnormalities such as duodenal atresia, intestinal malrotation, pancreas divisum, imperforate anus, hypoplasia of the right hepatic lobe, duplication cysts of the hepatic flexure, ventricular septal defect, renal agenesis, undescended testes, and syndactyly (1,3). It can also be associated with genetic syndromes such as trisomy 18 (1).

It is theorized that symptoms in symptomatic GBA may arise from sphincter of Oddi dysfunction, since sphincter spasms may mimic the symptoms of biliary colic (3,4). Interestingly, the

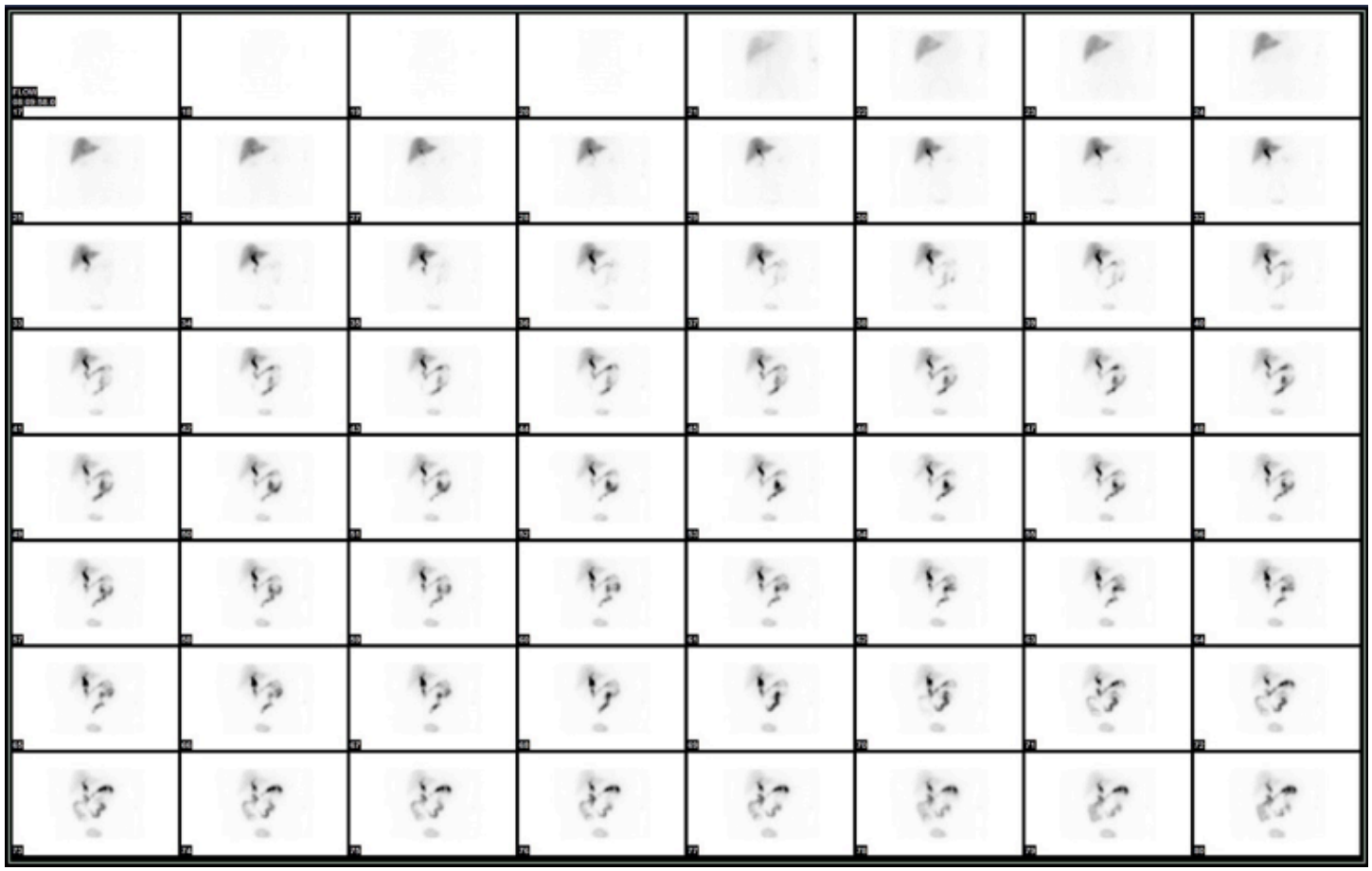

Figure 2. HIDA scan imaging of gallbladder agenesis

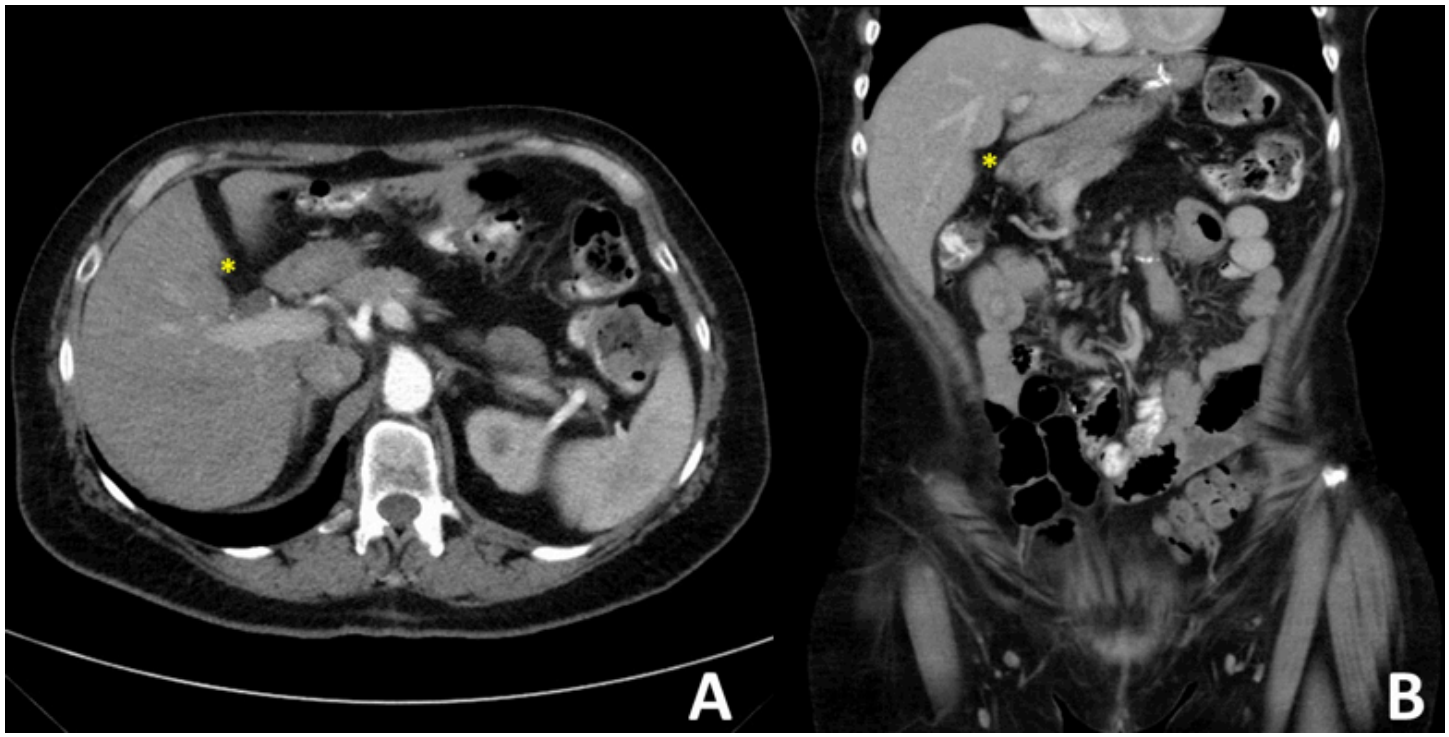

Figure 3. Imaging by CT scan in the patient with gallbladder agenesis. Stars (*) are found in the fossa on both views to highlight the absence of a gallbladder in that location. (A) Axial views. (B) Coronal views.

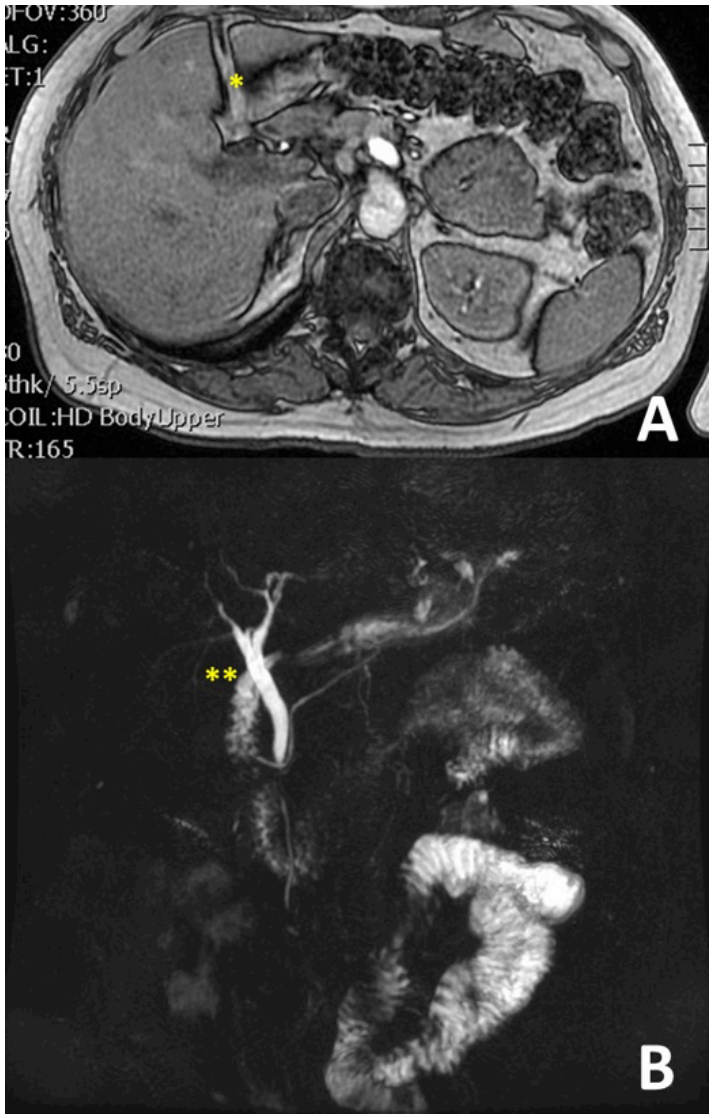

Figure 4. MRCP imaging demonstrating gallbladder agenesis. (A) T1 axial view. Star (*) represents the lack of gallbladder in the normal gallbladder fossa. (B) MRCP T2 reconstruction of the biliary tree, which consists of the common bile duct and left and right hepatic ducts. There is no evidence of a cystic duct or gallbladder. Stars (**) present at the expected location of the cystic duct and gallbladder.

frequency of primary stones in the common bile duct in patients with GBA (23%) is much higher than the post-cholecystectomy rate (5%). This finding may support the theory that a sphincter of Oddi dysfunction leads to both symptoms and stasis in patients with GBA, and thus to choledocholithiasis (3,4).

Although the morbidity and mortality associated with a nontherapeutic laparoscopic cholecystectomy is low, the risk of complications during an extensive search for an ectopic gallbladder is increased (5). The risks of iatrogenic injury are increased when normal anatomical landmarks are missing and traction on the gallbladder to dissect the triangle of Callot is not feasible (5). There are cases of undiagnosed GBA that have undergone extensive laparoscopic exploration searching for an ectopic gallbladder, with occasional conversion to laparotomy, addition of intraoperative cholangiography through the common bile duct, intraoperative ultrasound, and eventual abandonment of the procedure for further post-operative imaging (5). The option of terminating the procedure and pursuing additional imaging is favored in the literature in order to prevent iatrogenic injury when accurate imaging modalities are now widely available (6). Surgeons need to be aware of this anomaly and maintain a high index of suspicion for GBA when initial investigations do not clearly demonstrate the presence of a gallbladder or biliary disease in order to avoid these intraoperative dilemmas.

In 1967, Frey et al. published that "The preoperative diagnosis of agenesis of the gallbladder is impossible" and thus only a thorough dissection of the entire biliary tract during laparotomy and intraoperative cholangiograhy could confirm the diagnosis (7). However, in the 21st century, imaging modalities have significantly improved and can provide an accurate diagnosis when there is a high index of suspicion for GBA (7). Malde proposes an algorithm (**Figure 5**) in which radiological investigations are used preoperatively based on available modalities, including MRCP, CT, ERCP, and EUS (6).

Investigations

Laboratory findings for symptomatic GBA are often unremarkable. In patients with choledocholithiasis, there may be corresponding transient elevations in liver function tests and lipase.

Ultrasound often provides the first clues to GBA. When the gallbladder is not clearly visualized on ultrasound, additional investigations should be directed at excluding this diagnosis. In many cases, GBA is diagnosed only intraoperatively, often after the US reports the presence of a contracted or fibrotic gallbladder (8). Many factors, such as body habitus or presence of bowel gas, can obscure visualization of the gallbladder and may lead to confusion (2,3). In cases where the WES triad (visualized gallbladder wall, echo from gallstone, and acoustic shadow) or the double-arc shadow are not clearly seen, both clinicians and radiologists must consider GBA in their differential diagnosis (4,8).

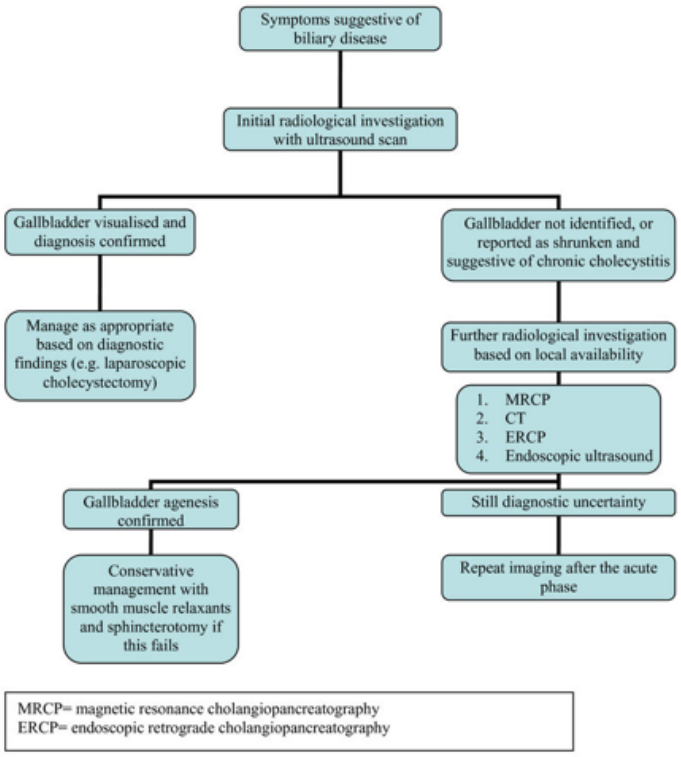

Figure 5. Decisional algorithm in suspected gallbladder agenesis proposed by Malde (6)

HIDA scan is not helpful in differentiating between a diagnosis of acute cholecystitis and GBA as the gallbladder is not filled in both situations. ERCP may also be misleading as non-filling of the gallbladder could be attributed to an obstructed cystic duct or technical errors (8). Most individuals consider GBA to be the least likely diagnosis (7).

MRCP is the modality of choice for diagnosing GBA. With T1 and T2 phases, the presence or absence of the gallbladder is easily identified. In addition, it also provides clinicians with information regarding any other abnormalities in the biliary tree, including choledocholithiasis, abnormal/variant anatomy, as well as ectopic gallbladder (3,5,8). In cases when MRCP is not readily available, CT scan may also be used (6,8).

Treatment

There is no consensus as to how to approach this rare condition. Based on case reports, 98% of symptomatic patients who undergo laparoscopy for possible cholecystectomy have symptom resolution with nontherapeutic laparoscopic cholecystectomy (2,3). One cannot help but ponder on the pathophysiological mystery of this disease, which presents with biliary colic when no gallbladder exists, and resolves when no gallbladder is removed. It is theorized that symptomatic GBA is due to sphincter of Oddi dysfunction, which explains the intermittent nature of the biliary colic in these patients, but not the resolution with nontherapeutic procedures. Some physicians have used hyoscyamine, a levoratory isomer of atropine that acts as a smooth muscle relaxant with success (2). In instances of choledocholithiasis in patients with GBA, ERCP with sphincterotomy and stone extraction is often successful (5).

In our case, after excluding peptic ulcer disease and choledocholithiasis, and confirming the diagnosis of GBA, the patient was managed conservatively with oral analgesia. Her symptoms improved and she was discharged home in stable condition, avoiding an unnecessary operation.

CONCLUSION

GBA is a rare disease that poses a diagnostic challenge for surgeons. GBA needs to be considered in the differential diagnosis of patients presenting with biliary colic and inconclusive initial ultrasound findings. Appropriate imaging studies such as MRCP are particularly helpful to confirm the diagnosis. Conservative management is often effective and avoids an unnecessary surgery with its associated potential morbidities.

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A Medical Student's Elective Experience in Pediatric Rheumatology through the Canadian Rheumatology Association

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ABSTRACT

This elective report provides an overview of the experience of a first-year medical student completing a pediatric rheumatology elective through the Canadian Rheumatology Association (CRA). Students apply to work with a rheumatologist and experience alternating schedules between inpatient and outpatient clinical medicine over the course of the summer. This elective is unique, as it exposes pre-clerkship medical students to learning experiences that will prepare them for clerkship and beyond. It provides practical experience as well as insight into research within the specialty at a world-renowned Canadian academic institution.

RÉSUMÉ

Ce rapport de stage donne un survol sur l'expérience d'un étudiant en médecine de première année complétant un stage en rhumatologie pédiatrique par l'entremise de la Société canadienne de rhumatologie (SCR). Les étudiants peuvent faire demande pour travailler avec un rhumatologiste afin d'avoir une opportunité de vivre un horaire alternant entre des soins externes et des soins hospitaliers, au cours de l'été. Ce stage est unique : il expose aux étudiants de médecine au préexternat des expériences d'apprentissage qui leur préparent pour l'externat et l'avenir. Il offre des opportunités pratiques ainsi qu'un aperçu de la recherche dans cette spécialité ; tout dans un établissement scolaire canadien mondialement connut.

On my first day of work, I set my alarm early to account for any Toronto subway delays, and inevitably arrived to work with ample time to spare. I would be spending the next six weeks working in the Rheumatology Division of the Hospital for Sick Children (SickKids), as part of a clinical studentship through the Canadian Rheumatology Association (CRA). Upon entering the doors of SickKids on my first day, I felt both out of place and in awe, but excited to learn in this new and foreign environment.

Every summer across Canada, the CRA offers pre-clerkship medical students the opportunity to work with an adult or pediatric rheumatologist in order to observe and practice their clinical skills. I was interested in this program because I enjoyed the rheumatology lectures given in my first semester of medical school, and knew that if I did not seize this opportunity I would not encounter this content again until clerkship. I specifically chose to apply to work with a pediatric rheumatologist for the opportunity to explore my interest in pediatrics, and meet and work alongside the physicians at SickKids. Therefore, this program is a useful opportunity to learn more about the subspecialty of rheumatology for students who are considering applying to Internal Medicine or Pediatrics residency programs due to the availability of both adult and pediatric rheumatologists. My learning objectives for the program were to explore the field of pediatric rheumatology, gain experience observing and performing histories, physical exams, and procedures such as joint injections on pediatric patients, as well as learn about the lifestyle of this subspecialty in pediatrics.

DISCUSSION

Over the course of six weeks I was made to feel like part of the team and truly experienced the day-to-day life of a pediatric rheumatology fellow by participating in journal clubs, teaching sessions, academic half-days, and grand rounds. The program consists of working alongside staff physicians, clinical fellows, pediatric residents, physical therapists, and nurses. I had the opportunity to alternate between spending weeks in outpatient clinics and working with the inpatient care team. There are various clinics scheduled throughout the week, including the General Rheumatology, Lupus, Juvenile Dermatomyositis, and Vasculitis clinics. When treating inpatients, I had many opportunities to work closely with other care teams and learn about other specialties as well, such as general pediatrics, nephrology, dermatology, neonatology, radiology, and pathology and gained an appreciation for the multi-systemic

Keywords: Pediatrics; Rheumatology; The Hospital of Sick Children; Canada

and interdisciplinary nature of this specialty. Additionally, I observed how rheumatologists and pediatric anesthesiologists collaborate, when every Friday, the week culminated in a fellow performing sedated joint injections on patients with joint effusions. I really enjoyed the collaborative atmosphere and am grateful to my seniors for welcoming me into the world of clinical medicine early on in my medical career. I appreciate their teaching and mentorship and had many opportunities to practice taking histories on patients, physical exams, and putting in notes after patient encounters. My gratitude also extends to all of the fellows who took time out of their busy schedules to work with me and provide informal teaching sessions and advice.

During this elective, I increased my knowledge and skills and grew as both a clinician and a person. For example, I am much more equipped with the knowledge necessary to approach the differential diagnosis of a monoarthritis, apply the classification criteria for Juvenile Idiopathic Arthritis (JIA) and Systemic Lupus Erythematous (SLE), and examine a child using the Pediatric Gait Arms Legs and Spine (pGALS) screening approach. I was also able to practice clinical exam skills such as performing a joint exam through inspection, palpation, range of motion, and special tests for fluid to correctly identify a joint effusion. I became more comfortable with my cardiorespiratory, abdominal, and skin examinations. At first it was difficult for me to examine a patient and competently assess and form an impression about all of these findings, however I am beginning to feel more confident with these skills. With increased patient exposure and continued practice of my history taking and physical exam skills I foresee myself becoming much more capable by the time I begin my clerkship. One highlight of this experience was following my own inpatient for the first time, presenting his case, building a relationship with the patient and his family, checking his chart every morning for new results, and updating the team about his status. I learned how important it is to know your patient and communicate well so that the care team can succeed.

One aspect of the subspecialty of rheumatology that I discovered I really enjoy is the longstanding impact we can make on the lives of patients living with a chronic disease. For example, many of the patients I saw in the clinic had been diagnosed JIA when they were young children. Seeing them now as fully functioning adolescents, with active lives and minimal functional impairments due to the great care they have received, was really motivating for me. I learned that I would be very satisfied treating patients with chronic diseases so that I can follow them throughout their lives and provide the best possible care to minimize the impacts of their disease. One particular patient interaction that stands out was a teenaged female who had been diagnosed with JIA at the age of two and was encouraged by her physician to become active at the time. Because her disease is currently so well controlled, she is able to actively participate in competitive dance, and was invited to perform at a parade at a popular amusement park. This would not be possible without her resilience as well as the hard work and efforts of her care team. I also enjoyed interacting with the SLE patient population, which mainly consisted of adolescent females. Many had minimal clinical findings or disease impairments and were able to fully participate in school and activities. Many were nearing the end of high school and making decisions regarding future education and career plans. I find these interactions to be the most meaningful and appreciate the continuity of care we are able to provide as patients transition through different stages of life. Additionally, I learned that even though we see patients from a rheumatologic perspective, it is also essential to approach the patient from a broad perspective and consider other causes of their disease, such as infection, malignancy, or other systems. These lessons are essential throughout the training of both internists and pediatricians, and this program therefore benefits students interested in both of these fields.

FINAL REFLECTIONS

Spending six weeks at one of the best academic centres in the world, I was also able to learn about myself and identify areas where I can improve in the future. For example, although I gained more confidence in clinical encounters, I think I could have been more assertive and taken more initiative by requesting more responsibility. I learned that even though it may seem difficult or daunting, the best way to make the most of my medical education is by being an active, motivated team member that comes ready to learn each and every day. I realized that if you do not take risks, you will not learn. I recommend this elective to any pre-clerkship student who wishes to gain experience and confidence prior to beginning clerkship. This is a unique opportunity that places the expectations of upper year medical students on pre-clerkship medical students. Additionally, this elective is located very conveniently because students can apply to rheumatologists from all across Canada, providing the chance to spend their summer at home or explore a new city. As the summer progressed I noticed myself becoming more confident charting notes and seeing patients on my own, and

this is a skill I will have to continue to build upon. This experience will help prepare me for my future medical career by instilling in me knowledge, skills, and a more positive attitude to facing challenges. These new insights will help me succeed in any specialty of medicine I decide to pursue in the future. I encourage everyone to seek out unfamiliar and exciting learning opportunities, internationally or locally, and step outside of their comfort zones in an effort to accomplish something new.

ACKNOWLEDGEMENTS

Thank you to all of the staff members, fellows, residents, and the entire team at SickKids for the warm welcome to the specialty of Pediatric Rheumatology, and to the CRA for organizing this unique elective opportunity.

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The University of Ottawa Journal of Medicine (UOJM) is a peer-reviewed journal published by graduate and medical students of the Faculty of Medicine. The UOJM is the only bilingual institutional medical journal in Canada, welcoming high-quality submissions in English or French. Accepted articles include original research, reviews and clinical practice, news and commentaries, case and elective reports, and interviews. The UOJM is currently accepting submissions for our upcoming **Spring 2019 Issue 9.1: Medical Innovations.**

Every year, ever-curious physicians and scientists are revolutionizing the study and practice of medicine: from breakthroughs in diagnostic and imaging software to multinational drug trials and transformative therapies that are fundamentally revolutionizing how diseases are managed. For instance, in the past decade alone, society has been witness to a paradigm shift in how medicine fights back against invading cancer cells, how statistics can predict the course of a disease prior to its first symptom, how artificial intelligence can be used for image-recognition in radiology, and how surgeons can use wireless networking to perform operations from across the Atlantic. This innovative spirit is what drives patient care and what will inspire future individuals to pursue a career in healthcare.

Issue 9.1 of the UOJM is therefore intended to explore all those innovative and passionate medical minds who constantly push boundaries and tirelessly strive to improve the lives of those around them. The submission deadline for our Spring issue is **March 1, 2019 at 11:59PM**. High-quality writing will be recognized with an honorarium award. Submissions can be made online and questions can be directed to contact@uojm.ca.

Call for Artwork

With the upcoming release of UOJM 9.1, we are looking for creative artwork to be featured on the cover of our issue! Submissions must fit with the theme of 'Medical Innovations' and may be drawn by hand or digitally. PDF files are preferred but not required. Artwork submissions can be emailed to contact@uojm.ca by March 1st, 2019!

Linda Fei & Phillip Staibano

Co-Editors-In-Chief

University of Ottawa Journal of Medicine

Appel de Soumissions

Le Journal médical de l'Université d'Ottawa (JMUO) est une revue évaluée par les pairs publiée par les étudiants de troisième cycle et les étudiants de médecine de la Faculté de médecine. Le JMUO est la seule revue médicale institutionnelle bilingue au Canada, accueillant des soumissions de haute qualité en anglais ou en français. Les articles acceptés comprennent la recherche originale, les revues et la pratique clinique, les nouvelles et les commentaires, les rapports de cas et de stage, et les entrevues. Le JMUO accepte actuellement des soumissions pour notre prochain **numéro 9.1 printemps 2019 : Les innovations médicales.**

Chaque année, des médecins et des scientifiques toujours curieux révolutionnent l'étude et la pratique de la médecine : dès les percées en logiciels diagnostiques et d'imagerie, aux essais de drogue multinationaux et thérapies importantes qui révolutionnent fondamentalement la gérance des maladies. Par exemple, dans la dernière décennie seule, la société a pu témoigner un changement de paradigme comment la médecine se bat contre des cellules cancéreuses envahissantes, comment les statistiques peuvent prédire l'évolution d'une maladie avant ses premiers symptômes, comment l'intelligence artificielle peut être utilisée pour la reconnaissance d'une image en radiologie, et comment les chirurgiens peuvent utiliser des réseaux sans fil pour opérer à travers de l'Atlantique. Cet esprit innovateur pousse les soins et sera l'inspiration aux individus futurs qui désirent poursuivre une carrière dans le domaine de la santé.

Ainsi, le numéro 9.1 du JMUO vise à explorer tous les esprits médicaux innovateurs et passionnés qui poussent constamment les frontières et s'efforcent sans relâche d'améliorer la vie de tous ceux qui les entourent. La date limite de soumission pour notre numéro de printemps est **le 1er mars 2019 à 23 h 59.** L'écriture de haute qualité sera récompensée avec un prix d'honneur. Les soumissions peuvent être faites en ligne et les questions peuvent être dirigées à contact@uojm.ca.

Appel d'œuvres

Avec la publication imminente du JMUO 9.1, nous cherchons des œuvres créatives pour être mises en exergue sur la couverture de notre numéro ! Les soumissions doivent s'aligner sur le thème 'Les innovations médicales' et pourront être dessinées à la main ou numériquement. Les fichiers PDF sont préférés, mais ne sont pas obligés. Les soumissions d'œuvre peuvent être envoyées par courriel à contact@uojm.ca par le 1er mars 2019 !

Linda Fei & Phillip Staibano

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