HMSR

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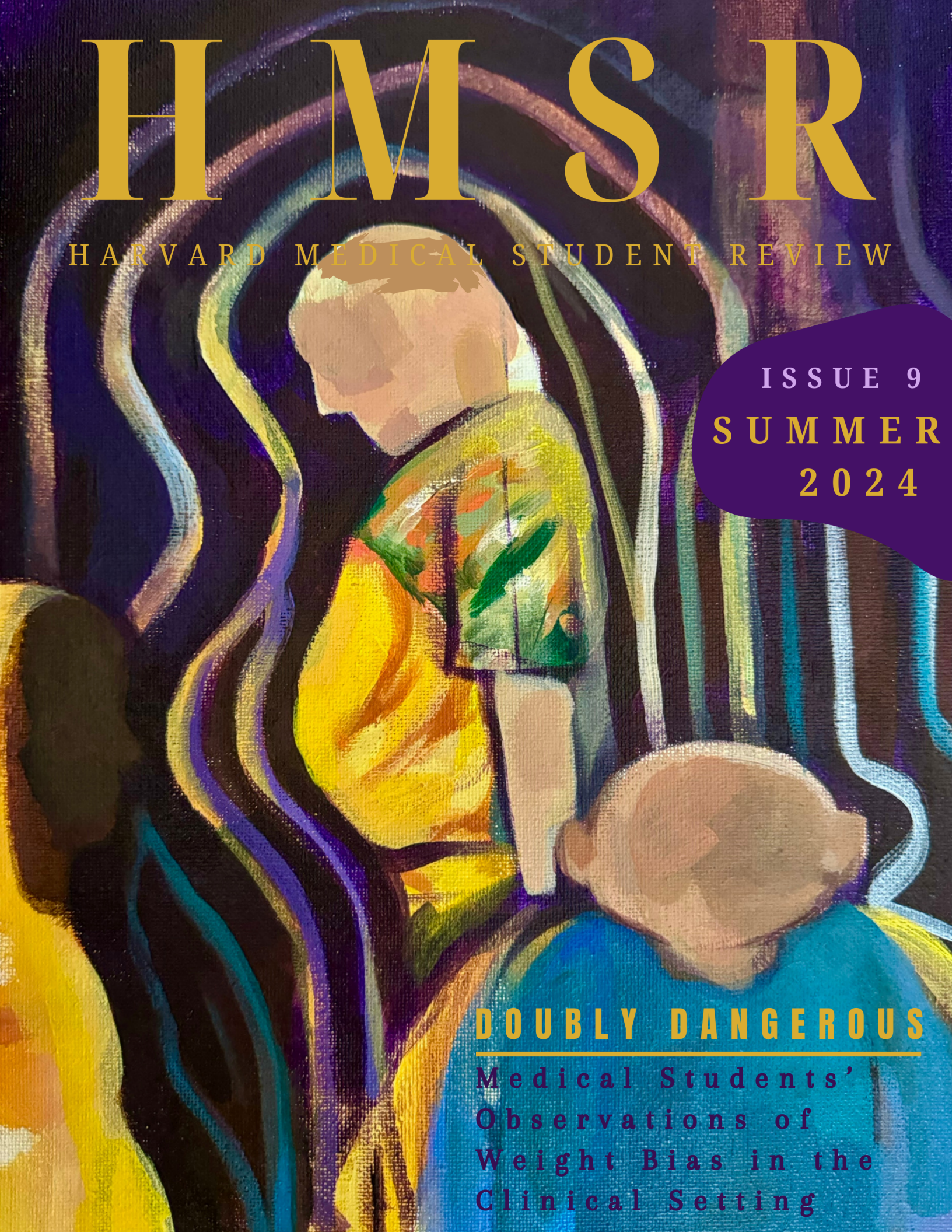

DOUBLY DANGEROUS

Medical Students' Observations of Weight Bias in the Clinical Setting

HMSR

Summer 2024

Dear Readers,

It is with great pleasure that I present the 9th edition of the *Harvard Medical Student Review*. The summer holds important milestones for medical trainees starting new phases of their careers; in this spirit, we at *HMSR* are delighted to continue to amplify the voices of medical students and resident physicians.

In this issue, we explore a wide range of topics that are shaping the future of medicine. Articles on the current state of solid organ xenotransplantation, the molecular mechanisms of familial hypertrophic cardiomyopathy, and neuro-immune crosstalk in cardiovascular disease highlight the importance of basic science advances in contributing to our understanding of disease mechanisms. Our authors also contribute to critical discourse on the social determinants of health via a comprehensive survey of oral health in unhoused populations and an insightful commentary on how provider bias can affect patient care. As always, *HMSR* endeavors to link art with medicine; visual art is showcased throughout this issue.

I would like to thank the editorial board, composed entirely of Harvard Medical School students, for their continued efforts to uphold our standard of excellence at *HMSR.* I would also like to thank our Faculty Advisory Board for their continued support. Most importantly, we extend our gratitude to the authors for their valuable contributions and to all our readers for your enthusiasm and engagement.

We hope you find this issue informative, inspiring, and thought-provoking. Whether you are a student, educator, or practitioner, we invite you to engage with the content, reflect on the ideas presented, and contribute to the ongoing dialogue that shapes the future of medicine.

Sincerely,

Arya Rao
Editor in Chief
Harvard Medical Student Review

HMSR

Executive Board

Cover Artwork

Danielle Li

Associate Editors

About HMSR

The Harvard Medical Student Review (HMSR) is student-founded, student-managed, and student-administered under the guidance of faculty and staff. Its mission is to provide a platform for students to contribute to important issues facing health and medicine through a variety of formats, including scholarly articles, editorials, and original artwork. Contributions are invited from the Harvard medical, dental, and public health schools, the rest of Harvard University, and other medical schools.

The works herein represent the views and opinions of the original authors and do not necessarily represent the views or opinions of the Harvard Medical Student Review or Harvard Medical School.

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ARTS: PAINTING

A calligram in ancient Greek of the Hippocratic Oath taking the shape of the Rod of Asclepius, *oil on canvas*

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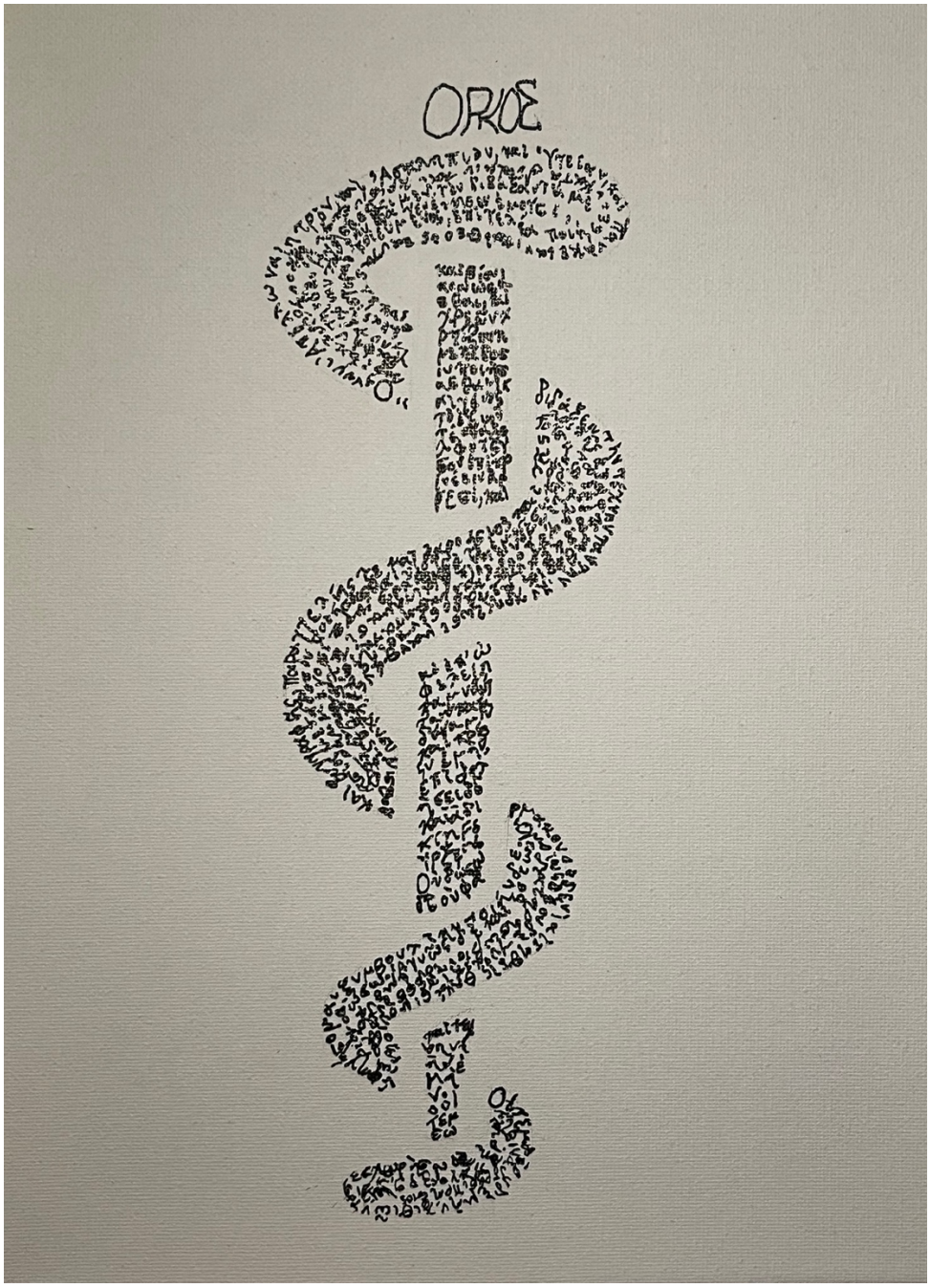

"Transplanting of Teeth" by Thomas Rowlandson. Courtesy Metropolitan Museum of Art, New York.

Assessing the Oral Health of the Homeless Population in Central Massachusetts

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Objectives: Oral health conditions are common yet evitable, and disproportionately plague underserved communities. This study aimed to survey the homeless and housing insecure in central Massachusetts to better understand their oral health disease burden and resource needs. **Methods**: Data was collected in 2021 from 144 individuals at four sites frequented by this population. The 26-item anonymous questionnaire was available in written, electronic, and verbal formats in English and Spanish, including questions on sociodemographic factors, access to and use of dental and primary care, self-reported oral and overall health statuses, and resources needed to improve oral health. **Results**: The study sample was 65% male, 58% White,

and 90% Medicaid insured. Nearly three-quarters of study subjects reported homelessness (27% unsheltered; 44% sheltered); the remaining were housing insecure. The percentage of participants with a PCP (79%) was more than three times that with a dentist (25%). Unsheltered homeless respondents were significantly less likely to have a PCP than sheltered homeless or housing insecure, and poor oral health was correlated with poor overall health. The most common resources needed were dentists accepting public insurance, dental supplies, and transportation. About two-thirds of respondents (66%) were amenable to receiving dental advice from a case manager or social worker. **Conclusions**: Our results demonstrate a need in the homeless and housing insecure community for dental supplies, more insurance-eligible providers and assistance to patients for finding them, education about oral hygiene, and transportation options, possibly with case management and primary care involvement.

INTRODUCTION

Despite being highly preventable, oral health conditions are among the most prevalent diseases in the U.S. and globally (1). In fact, the most common health condition worldwide in 2017 was untreated tooth decay in permanent teeth (2). Tooth decay and gum disease, the two most common dental pathologies (3), can be prevented by personal dental hygiene, fluoride application, routine dental visits, and reducing sugar consumption (4).

However, such prevention strategies for maintaining good oral hygiene can be particularly challenging in low-resource communities. As such, oral health issues disproportionately affect socioeconomically disadvantaged populations (3). According to the 2000 Report on Oral Health by the Surgeon General, the "silent epidemic" of dental and oral disease particularly affects the poor, members of racial and ethnic minority groups, the medically compromised, and individuals with disabilities.[5] Decades later, this still holds true. The association between low socioeconomic status and poor oral health has been extensively established in the literature (6-11). Racial disparities in oral health persist (12, 13). As examples, the number of Black and Mexican American adults with untreated cavities is almost double that of non-Hispanic White adults, and the five-year survival rate for oral pharyngeal cancer is a staggering 34% lower for Black men (41%) than for White men (62%) (14).

For the more than half a million individuals in the U.S. suffering from homelessness (15), these oral health disparities are particularly stark. Homeless individuals, defined as living on the street or in shelters, are 12 times more likely to face dental issues than their stably housed counterparts. For individuals who are housing insecure who live in hotels and motels or with relatives or friends, dental problems are six times more likely than for those in reliable residences (16). In a national study of homeless adults, 60% of those with a dental issue in the preceding year reported an unmet need for dental care (17).

Of all vulnerable populations, the homeless community may have the least access to health resources due to a lack of money, no permanent residence, and the unwillingness of providers to treat them (18, 19). The stigma of homelessness and visibly unhealthy mouths has also been identified as a barrier to seeking dental care (20). A lack of access to affordable health care is even attributed as a cause of homelessness (21). In the past decade, the U.S. Interagency Council of Homelessness credited Worcester, Massachusetts, with having "effectively ended chronic homelessness" (22). But as of January 2020, Worcester, centrally located in the state and the second most populous city in New England (23), had over 1400

homeless individuals, about 40% of whom were Hispanic and 21% Black or African American (24).

The main goals of our study were to: determine some of the unique aspects of the oral health disease burden on the homeless and housing-insecure population in central Massachusetts; identify their self-reported needs for improvement and resource availability; and compare this to their primary care utilization and barriers. This information could guide initiatives for improving the oral health of the homeless and housing insecure.

METHODS

This study was approved by the UMass Chan Medical School's Institutional Review Board (Protocol #H00023291) and granted an exemption waiver. It was also approved by the Family Health Center of Worcester Program & Policies Committee.

Study sample and recruitment: Inclusion criteria for the study were adults 18 years of age or older and able to speak or read in English or Spanish. Between June 1, 2021, and July 31, 2021, the survey was administered to individuals at the Homeless Outreach and Advocacy Program (HOAP) in Worcester, MA, a primary care clinic operated by the Family Health Center of Worcester, a federally qualified health center. Survey data were also collected at two local housing shelters and a food pantry. These sites where homeless and housing insecure persons visit for health care, temporary housing, and food were selected to reach those who both formally and informally access health care. The shelters and food pantry have a weekly on-site clinic staffed by a health care team to address health issues.

Survey development: The 26-item anonymous survey collected self-reported information about respondents' access to and use of dental care, barriers to dental care, dental hygiene status, and resources that would improve oral health. The survey also included questions about the use of primary and emergency medical care, overall health, and socio-demographic questions (e.g., gender, age, race/ethnicity, education level, preferred language, country of origin, insurance status, and current housing situation).

Data collection: An initial Fact Sheet was provided to all eligible participants which described the research project, data collection and storage, and any potential risks or inconveniences. Informed verbal consent was obtained from each respondent. The survey was voluntary, and its completion time was approximately ten minutes. Respondents could elect to complete the survey verbally, by paper, or via an anonymous survey link on a tablet, all available in both English and Spanish. At the completion of the survey, each respondent received a $15 gift card to a local retail store, a toothbrush, and toothpaste.

Statistical analysis: We used univariate analyses for the demographic characteristics of the survey respondents, as well as for independent and dependent variables. Bivariate analyses (i.e., chi-square tests and tests of proportions) were used to assess relationships between housing status (street, sheltered living, or renter/homeowner) and key independent variables (e.g., use of oral health care services, resource needs, primary care integration, and barriers to service use). A test of proportions was used to assess the difference in the homeless population comparing those without a dentist to those without a primary care provider (PCP). A p-value of $\leq .05$ was used to denote statistical significance. All analyses were conducted using SPSS statistical software (Version 27; IBM Corp) (25).

RESULTS

Study sample characteristics: We recruited 150 participants, of which 6 were excluded because they did not complete at least 50% of the survey. Fewer than five eligible individuals declined to participate in the study. Our sample predominantly was male (65%), between 40 and 64 years of age (65%), and received at least a high school education (83%) (**Table 1**). Most respondents were insured by Medicaid (90%). Over half of the study sample was White (58%), followed by Hispanic or Latino (24%), then Black or African American (16%). The U.S. was the most common country of origin (85%); the majority of the remaining countries were in Central and South America or Africa. Just over one-quarter (27%) of individuals indicated that they were living on the street, or unsheltered homeless, and two out of five (44%) individuals reported being sheltered homeless, meaning they described their housing situation as a shelter, safe haven, transitional housing, institution, hotel, motel, or living with friends or family.

Access to dental and medical care: Only one-quarter (25%) of respondents indicated they had a dentist or dental hygienist whom they saw regularly. For more than one-half (59%), it had been a year or more since they had visited a dental provider. These 83 respondents were asked about barriers that prevented them from seeing a dentist in the prior year (Figure 1). The most common barrier was a lack of knowledge of where to receive dental care (23%), followed by fear, requiring too much dental care, and insufficient time (each 19%). Sixteen percent of respondents noted "other" barriers for not receiving dental care, including: the COVID

**Within each variable, numbers may not total to 144 because of sporadic missing data. For some variables, the total may exceed 144 because respondents were able to select multiple options.*

Table 1: Characteristics of the Study Sample (n=144*), 2021

Characteristic	*N (%)*	
Gender		
Male	46	(31.9)
Female	93	(64.6)
Other	5	(3.5)
Age Group		
Under 21	64	(78)
21-39	18	(22)
40-64	94	(65.3)
65+	9	(6.3)
Race/Ethnicity		
White	83	(57.6)
Black/African American	23	(16.0)
Hispanic/Latino	34	(23.6)
Other	15	(10.5)
Multiracial	8	(5.6)
Education Level		
Less than high school	23	(16.1)
High school/GED	69	(48.3)
Some college/ associate degree	37	(25.9)
College/associate degree	8	(5.6)
Graduate school	6	(4.2)
Insurance Status		
No insurance	3	(2.1)
Medicaid	128	(90.1)
Medicare	24	(16.9)
Private insurance	10	(7.0)
Current housing status		
Unsheltered homeless	39	(27.7)
Sheltered homeless	63	(44.7)
Renter or homeowner	39	(27.7)
No insurance	3	(2.1)

pandemic, incarceration, depression/anxiety, forgetting appointments, not a high priority, having dentures, and "need new dentures, but heard that MassHealth doesn't pay for another set." One in five respondents (23%) reported having gone to the emergency department (ED) in the past year for a dental issue.

By comparison, the percentage of participants having a PCP was more than three times that of having a dentist (79% vs. 25%, respectively). The 30 participants who reported not having a PCP were asked which barriers they faced in seeing a PCP in the last year (**Figure 1**). The most frequently cited barriers to receiving medical care were time (30%) and transportation (20%). For 27% of respondents who listed "other" barriers for not seeing a PCP in the last year, they noted barriers such as: the COVID-19 pandemic, incarceration, access to a phone, can't get old records, depression/anxiety, keep changing PCP, laziness, losing appointment card, moving out of area where PCP was located, and not caring. Respondents living on the street were significantly less likely to have a PCP than respondents who either were sheltered homeless or were renters/homeowners (X^2=14.72, p<0.001). For the street homeless, the proportion of those who did not have a dentist (79%) vs. those who did not have a PCP (39%) was significantly higher (z=3.60; p<.001). Less than half (43%) of participants without a PCP had visited the ED for non-dental medical needs in the prior year. These respondents were significantly more likely to have sought care in the ED in the prior year (X^2=4.28, p=0.039).

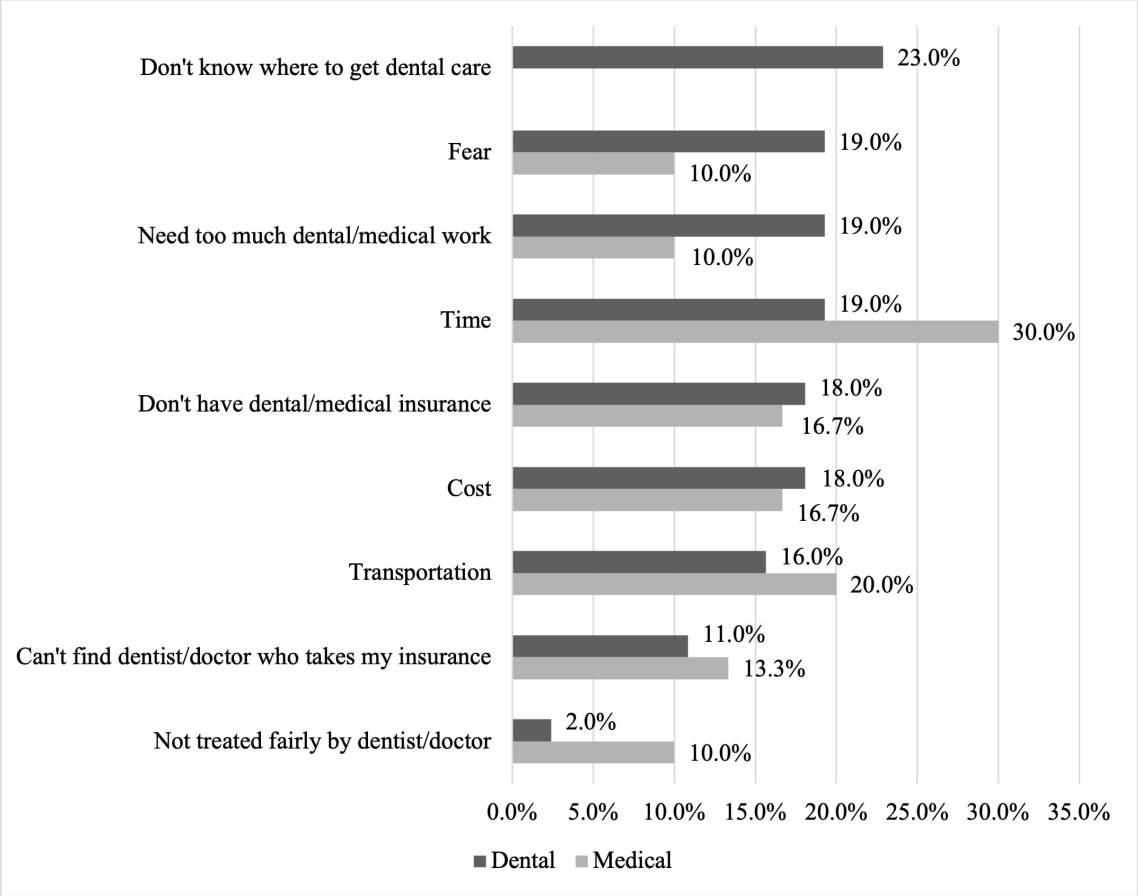

Figure 1: ***Reported Barriers to Receiving Dental (n=83) and Medical (n=30) Care, 2021***

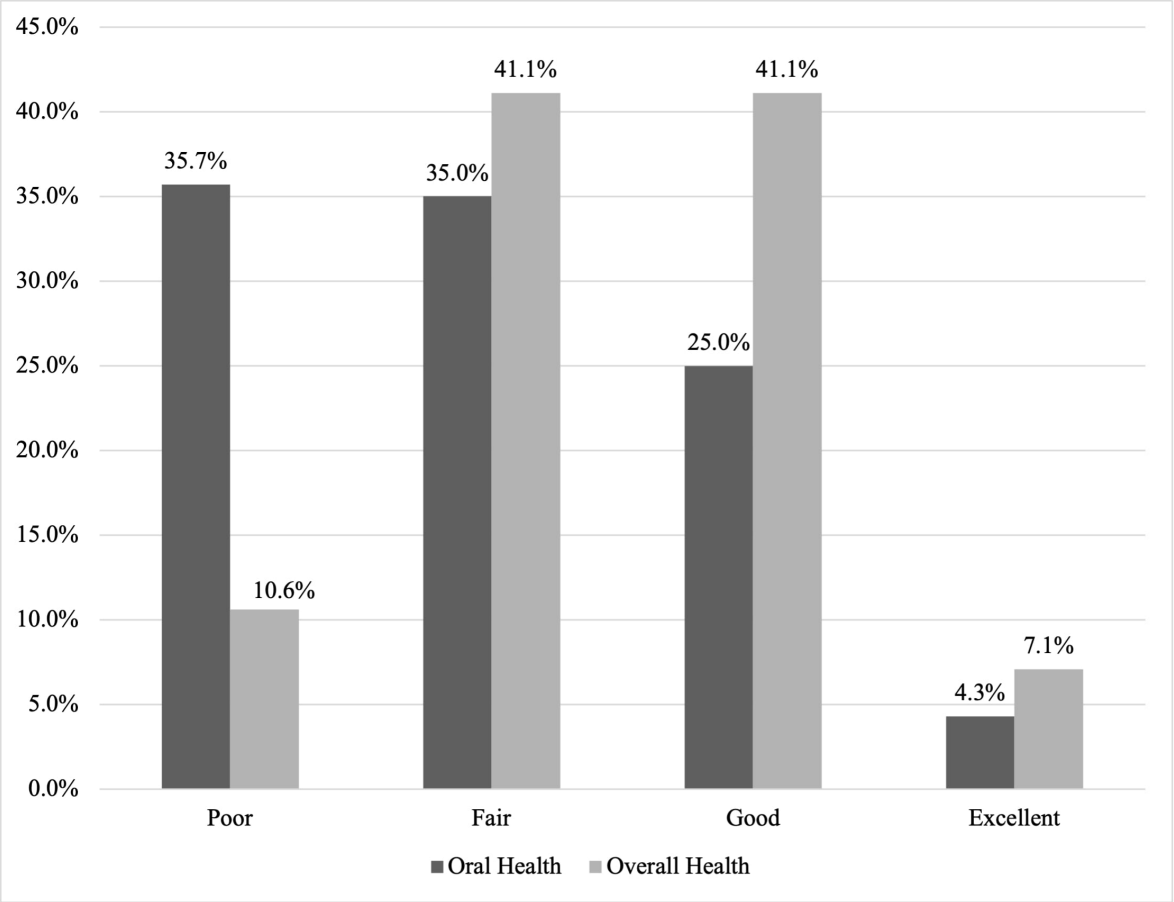

Figure 2: ***Reported Oral (n=140) and Overall (n=141) Health Status, 2021***

Self-reported oral and overall health status: Nearly three-quarters (71%) of respondents reported that the health of their teeth, gums, and mouth was 'poor' or 'fair', while only 29% endorsed 'good' or 'excellent' (**Figure 2**). More than half (57%) of respondents reported having lost teeth because they were unable to receive prompt dental care. For current overall health, 52% selected 'poor' or 'fair', and 48% said they were in 'good' or 'excellent' health. For individuals who noted that their dental health was poor, they were also more likely to rate their overall health as poor ($X2=60.04$; $p<0.001$).

Oral health needs: To improve their oral health, respondents most commonly expressed needs for: a dentist who would accept their insurance (33%), a toothbrush (30%), toothpaste (29%), transportation to the dentist (28%), and a protective case for dental supplies (28%) (**Figure 3**). One in five respondents (22%) also endorsed "other" resources that would help them better take care of their teeth, including: dentures, oral surgery, dental cleaning, denture adhesive, mouthwash, and a water pick.

Willingness to receive oral health support from other health care workers: When asked whether they would be willing to receive dental care advice from a nurse, 44% responded affirmatively, and 66% responded positively for willingness to receive dental advice from a case manager or social worker. Of interest, over half (54%) of respondents were amenable to getting dental hygiene advice from peer support teaching. Our study population also reported being more likely to

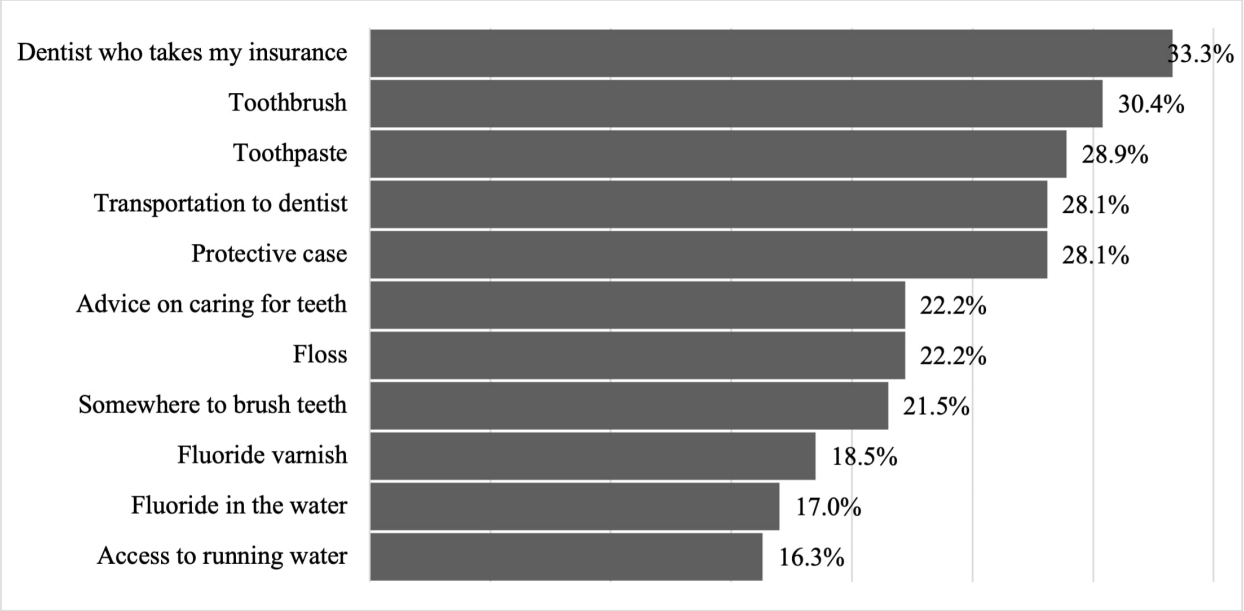

Figure 3: ***Reported Resources Needed to Improve Dental Health (n=135), 2021***

accept a referral to a dentist (62%) and receive an oral exam (59%) from a nurse in their doctor's office than the receipt of a toothbrush and toothpaste (39%) or fluoride varnish (32%) from a nurse.

DISCUSSION

There are a number of important findings from this study that have the potential to impact local and possibly national efforts to address the oral health issues of the homeless and housing insecure. There was no predominant barrier to receiving dental care; in fact, the barriers mirrored those that affect medical care, yet more people have a primary care provider and access to primary care. The focus, with a specific eye toward prevention, may be better placed on the resources that respondents identified as important to improving their dental health.

Currently, only a quarter of dentists in Massachusetts bill the state Medicaid program greater than $10,000, so it is not surprising that it is challenging for homeless populations to find a dentist that accepts their insurance, since 90% have Medicaid (26). A local survey of dental practices in central Massachusetts found that, of those reporting to accept MassHealth (Massachusetts Medicaid), 45% were not accepting new patients, and half of those accepting new patients spoke only English (27). This is not a barrier that can be easily addressed. However, several of the most prevalent barriers reported among our study sample can be attributed to a lack of dental supplies, which could more easily be addressed with small grants or working with local dentists to donate supplies.

A key component of the identified needs to improve dental care and prevent negative outcomes pertains to assistance and education. There are 18 dental offices in Worcester that accept Medicaid and are accepting new patients. The missing link here is assisting the homeless to find these practices. Anecdotally, the majority of the homeless in Worcester do have case management. Our survey indicates that respondents are very willing to receive dental information from case workers and social workers. Efforts to train these health extenders about dental access may be an important factor for accessing professional dental services. The same health care providers could be trained to teach about oral

hygiene and hand out self dental care supplies. Interestingly, one of the key barriers noted by respondents was the need for transportation, despite our state's Medicaid benefits paying for transportation to dental visits. So, again, case managers could serve as change agents helping to educate this population about transportation services.

The other solution that our data suggests is that primary care can be a key aspect of dental care improvement. More respondents identified a primary care provider whom they had seen within the last year. Two out of five respondents were willing to receive some interventions from nursing staff. It should be noted that this may not affect the street homeless as strongly, as they were less likely to have a regular primary care provider. With ED visits being relatively prevalent among the homeless for dental issues, efforts designed to improve dental follow-up directly from the ED could be useful. Currently, our state Medicaid program is working on a portal that EDs could use to notify Medicaid of the acute need and have their team help coordinate a dental appointment within 24 hours.

Our study has several limitations. First, the survey depended on self-reported responses, which poses a potential for information bias (including recall and social desirability). Second, our study was conducted in one locale (i.e., central Massachusetts) and may not be generalizable to all homeless and housing insecure communities; however, the study collected data from four different sites, and the demographics of our study population are similar to the demographics of our city's homeless population. Third, we were not able to assess for any potential non-response bias as we were unable to approach all individuals coming to each of the four sites where data was collected; however, of those approached, fewer than five individuals declined to participate. Lastly, while the study was carried out for two months across four different sites, the potential existed for some overlap of the populations coming to more than one site. Since our anonymous survey did not collect any identifiable information among respondents, there was a very small possibility of the same person completing the survey more than once, especially given that most respondents were wearing masks due to the pandemic. However, the survey was conducted by only two members of the research team.

In conclusion, this study highlights several factors that can contribute to improving the oral health of individuals facing homelessness and housing insecurity: improving access to dental supplies, training case managers and social workers to assist and educate about accessing dental providers and improving oral hygiene, increasing the number of local dental providers who accept Medicaid, and integrating oral health care into primary care.

DISCLOSURES

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Code availability: Not applicable.
Authors' contributions: Authors listed in the manuscript have contributed per submission guidelines and standards for authorship.
Ethics approval: This study was approved by the UMass Chan Medical School's Institutional Review Board (Protocol #H00023291) and granted an exemption waiver. It was also approved by the Family Health Center of Worcester Program & Policies Committee.
Consent to participate: Informed verbal consent was obtained from each respondent.

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"Young Spanish Woman with a Guitar", by Auguste Renoir. Courtesy National Gallery of Art, Washington.

Teaching Medical Spanish Alongside the Medical History: Evaluation of a Decade-Old Peer-Led Medical Spanish Program

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Introduction: LatinX individuals comprise 18.3% of the United States population, of which 40% have limited English proficiency. Medical Spanish programs are emerging to bridge barriers with these patients, but data are still needed to determine the most effective teaching practices. In this paper, we evaluate the efficacy of a decade old Peer Led Medical Spanish Program (PLMSP) that reaches over 50% of first year medical students at Stritch School of Medicine. **Methods**: Students were placed into levels based on a pre-test that assessed comfort

with Spanish, cultural competency, and reading/audio comprehension. After the completion of twenty classes taught by fluent peers that aligned with components of the medical history students were learning in English at that time, students were re-evaluated using the same exam. Intermediate and above students also completed an Objective Standardized Clinical Examination (OSCE) in which their performance in medical history taking was evaluated by standardized patients. **Results**: There was significant improvement in Spanish comfort for novice, beginner, and advanced students. Cultural competency growth was noteworthy amongst the novice and intermediate students. Nearly all levels showed statistically significant improvements in Spanish comprehension. For all levels participating in the OSCE, >90% of the history was discussed with standardized patients either agreeing or strongly agreeing that students had appropriate pronunciation, medical vocabulary, conversational fluidity, and cultural awareness. **Conclusions**: PLMSP offers promising results with regards to medical Spanish level of comfort, comprehension, and clinical performance. Further development of the program should focus on incorporating culture more effectively into the curriculum.

INTRODUCTION

LatinX Americans comprise the second largest ethnic group in the United States, consisting of nearly 60 million individuals or 18.3% of the population according to the U.S. Census Bureau (2018). Nearly 40% of LatinX individuals in the United States have limited English proficiency (1). It has been reported that language discordance in a healthcare setting is associated with increased health disparities, and consequently, more negative health outcomes (2) These include lower patient satisfaction, less access to preventative health care, increased risk of medical errors/adverse events, longer hospital stays, and increased cost of care (3, 4). Since language concordant care is associated with enhanced patient care, there is a growing necessity for effective Medical Spanish education efforts.

In 2012, The National Latino Medical Student Association (LMSA) assessed Medical Spanish Curricula in 132 US Medical Schools in a nationwide survey (1). Eighty-three percent of the schools completed the survey, of which sixty-six percent reported offering a Medical Spanish curriculum. Furthermore, 32% of schools reported an intention to initiate a Medical Spanish curriculum in the near future. These data show that medical schools are aware of the importance of Medical Spanish education in the training of future physicians and are acting to provide it. The increased interest in establishing Medical Spanish curricula in medical schools raises the question of best practices when it comes to curricula learner standards, efficacy, and evaluations.

To date, there are no guidelines on how to structure a Medical Spanish curriculum or how to evaluate programs. One of the reasons for this is that Medical Spanish education efforts are not consistently linked to learner assessments, and when they are, there is much variability in design without reliable outcome measures (5). For example, one longitudinal Medical Spanish program at a southeastern United States medical school evaluated its program utilizing a speaking proficiency phone interview test (6), yet other schools utilize standardized patient structured clinical examinations or oral proficiency interviews (2, 7). Lack of uniformity when it comes to evaluating Medical Spanish programs makes it challenging to compare program outcomes and determine best practices for curriculum establishment. In 2018, the University of Illinois College of Medicine and National Hispanic Health Foundation hosted a multidisciplinary expert panel to establish curricular guidelines for medical school

Medical Spanish courses. This panel established goals to standardize Medical Spanish learner competencies and move to assessments utilizing evidence-based methods (8). Despite this important step forward, more research is needed on effective teaching practices in Medical Spanish curricula, which prompted our own evaluation of the efficacy of the Loyola University Chicago Stritch School of Medicine (SSOM) Peer Led Medical Spanish Program (PLMSP), a renowned program that is unique in its fully peer-taught and led structure, its expansiveness, and the manner in which the history oriented curriculum parallels the Stritch Patient Centered Medicine course throughout the academic year.

SSOM's PLMSP began in 2009 and provides elective educational credit to medical students during their first two years of medical school. SSOM is one of only six medical schools to maintain the peer led method of teaching out of 62 total medical schools participating in the national LMSA study (1). Not only do medical students teach the course to their peers, but they also develop and update the curriculum, gather data on effectiveness, find and train student teachers, advertise and place students into classes, and oversee student growth over the course of the program. We have found that this model promotes acquisition of knowledge and skills across multiple competencies for student leaders, including the domains of professionalism and practice-based learning and improvement. For student participants, the model promotes flexibility and responsiveness to students' curricular needs and pedagogy. Student participants and leaders alike are fully immersed in their roles as students, teachers, or program leaders. The program is also unique in that the curriculum is entirely focused on gathering medical history and is taught concurrently with the English medical interview for first year medical students. Beyond the distinctive structure of Stritch's PLMSP, this program is wide-reaching, with greater than 50% of Stritch's first year medical students successfully completing all coursework for credit.

In response to the increased need for research on effective medical Spanish teaching practices, we evaluated the efficacy of SSOM's expansive, sustaining, and distinguished PLMSP by assessing student comfort, cultural competency, and comprehension skills before and after the elective and speaking skills following the elective. We hypothesized that the PLMSP improves student performance in the outcomes mentioned above, preparing intermediate, advanced, and proficient students to effectively obtain and comprehend medical histories upon completion of the course.

METHODS

Students interested in taking the Medical Spanish elective at SSOM during the 2020-2021 academic year took a placement exam administered electronically to demonstrate comfort with Spanish, cultural competency, and written and auditory Spanish comprehension (**Appendix A**, available online). This placement exam served as the pre-test and was used to place students into one of the following course levels: novice, beginner, intermediate, advanced, or proficient. Rather than having hard cut-off values for student placement, students were grouped with others who scored similarly to them on the pretest while simultaneously trying to optimize student:teacher ratios to <12:1. Valuing smaller teacher to student ratios rather than making sure students had strict level cutoff scores reflected the course's efforts to provide students with ample speaking opportunities with access to direct feedback/learning. Furthermore, teachers were encouraged to pull material from higher

or lower class levels as needed to assure they were addressing their students' individualized needs. After students were initially placed into levels, they were able to request to be moved up or down a level during their first three classes if they felt that a different level would better support their personal growth. The data collected was based on the level that the student ultimately chose by the end of the third class and which they remained at for the remainder of the course.

Teachers for the course were selected after an interview process based on language capability, cultural awareness, and teaching experience. There were 19 teachers total, including 14 first year medical students, three nursing students, and two graduate students. There were two teachers assigned per class for any class size over 12 students. Teachers received dedicated training time in which they learned about teaching theories and strategies from the Chair of world languages at a local college. They also had access to standardized materials (**Appendix B**, available upon request) and received continuous support and guidance from past peer mentors throughout the elective.

Medical Spanish classes were adjusted by teachers such that they could be administered online over Zoom. Students attended 20 classes from September to May. During each class, teachers delivered presentations with standardized daily objectives aligned to components of the medical history (**Appendix B**). These presentations incorporated interactive learning experiences including listening activities, reading activities, patient-doctor role-play, and games. Much time was spent in breakout rooms to give students the opportunity to practice speaking. Towards the end of the year, more time was dedicated to practicing full patient encounters to prepare for the upcoming Objective Structured Clinical Assessment (OSCE). Beyond the classroom requirements, Medical Spanish students were required to attend four cultural competency events, one practical experience in which students had to actively use Spanish or engage with the LatinX community, and an encounter with a standardized patient (intermediate, advanced, and proficient students only). The cultural competency events included, but were not limited to, monthly online seminars hosted by the National Hispanic Medical Association covering a wide range of topics such as health disparities, film screenings portraying immigrant experiences, and speaker panels of Deferred Action for Childhood Arrivals (DACA) recipients. Practical experiences included registering LatinX patients to vote, attending the LatinX health symposium, or participating in a language exchange buddy program.

During the last class of the elective, students completed the post-test, which was identical to their placement exam (pre-test) and measured comfort, cultural competency, and comprehension changes throughout the curriculum. The pre- and post-tests included the following tools:

Student comfort with speaking and comprehension was self-measured using the Interagency Language Roundtable (ILR) scale (9). This was developed by the U.S. State Department's Foreign Service Institute (FSI) and has been adopted as the standard measure for language proficiency in U.S. government agencies. The ILR is a scale from 0 to 5 with the following designations: 0 - No proficiency;

1 - Elementary Proficiency;
2 - Limited Working Proficiency;
3 - General Professional Proficiency;
4 - Advanced Professional Proficiency;
5 - Functionally Native Proficiency.

Cultural competency was determined using a five-point Likert scale associated with the statements "I am aware of the manner in which culture influences health

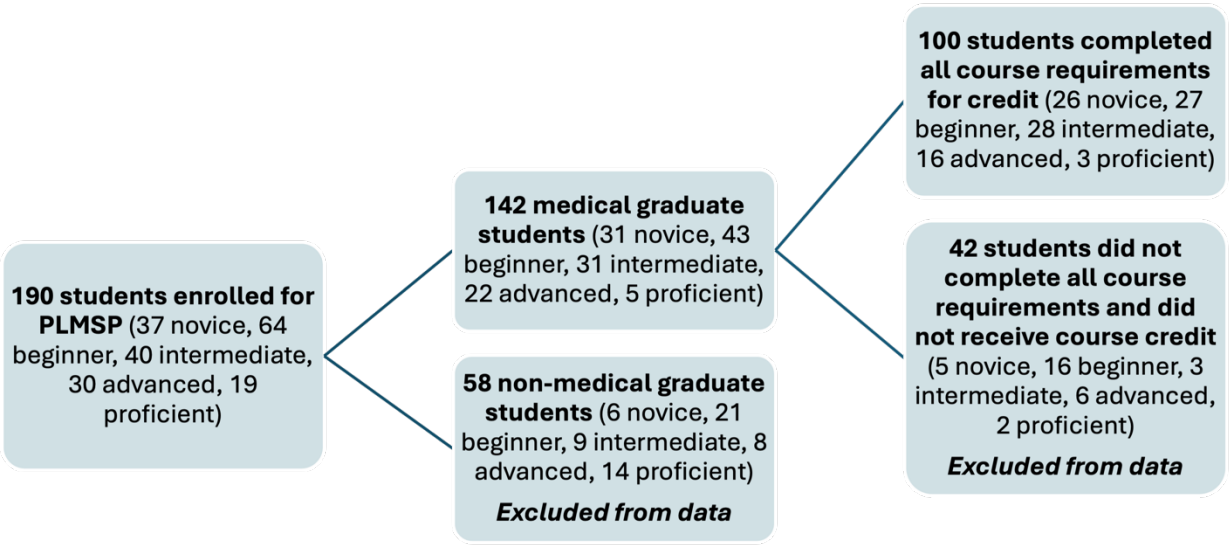

Figure 1: ***Study Participant Inclusion and Exclusion Data.*** *In order to be included in our study, participants had to be a medical student at Stritch School of Medicine and had to complete all course requirements including pre-test (placement exam), four cultural competency events, one practical experience, a post-test, and an OSCE for intermediate, advanced, and proficient students. Those who were not medical students (our program is open to nursing students and preliminary medical students as well) and who did not complete requirements necessary to receive credit for the course were excluded.*

care needs and outcomes in the LatinX community" and "I am prepared to engage with LatinX patients in a culturally competent manner." These statements were written to align with the program's objectives. While this measure is subjective in nature, this was utilized rather than asking specific cultural questions due to the fact that the cultural components of the class fluctuate per the teachers and course leaders each year in order to adapt to topics most relevant to the current political and cultural environment.

To measure comprehension, students answered ten multiple choice questions based on two audio selections of mock doctor-patient interactions. The other ten questions were based on written medical interactions between a doctor and patient. Both portions of the pre-test were created by the Medical Spanish leaders to align with objectives covered in the elective.

After completion of the final class, intermediate, advanced, and proficient students participated in an OSCE modeled off the SSOM clinical skills course. The OSCE included standardized patients who utilized one of two scripts correlating with responses to a complete history checklist (**Appendix C**, available online). This checklist consisted of 43 items (**Appendix D**, available online) and was utilized to ensure students elicited a complete history. The standardized patient was a Spanish speaking individual not involved with the research. Following this exercise, students completed a ten-question online quiz in English to gauge student comprehension of the clinical encounter (**Appendix E,** available online). Finally, the standardized patient assessed students on pronunciation, vocabulary, conversational ability, and cultural knowledge using a nine-point Likert scale (**Appendix F**, available online).

Excel was predominately used for data analysis purposes. Mean scores were established for the measures above (comfort, cultural competency, audio/written comprehension, OSCE history completion, OSCE quizzes, OSCE pronunciation/ vocabulary, conversational ability/cultural

Table 1. Student Comfort Interagency Language Roundtable Scale

Level	Pre-Test Mean Score (SD/IQR)	Post-Test Mean Score (SD/IQR)	Cohen's d	P-value
Novice	0.15 (0.37/0.00)	1.03 (0.60/0.00)	0.37	<0.001
Beginner	1.26 (0.59/1.00)	1.81 (0.62/1.00)	0.90	0.001
Intermediate	2.14 (0.71/1.00)	2.33 (0.68/1.00)	0.27	0.06
Advanced	2.60 (0.51/1.00)	3.13 (0.52/0.00)	1.04	0.001
Proficient	4.33 (0.58)	4.33 (0.58)	0.00	1.00

ILR scale is a device developed by the U.S. State Department's Foreign Service Institute (FSI) that has been adopted as the standard measure for language proficiency in U.S. government agencies. The scale ranges from 0 to 5 with the following designations: 0 - No proficiency; 1 - Elementary Proficiency; 2 - Limited Working Proficiency; 3 - General Professional Proficiency; 4 - Advanced Professional Proficiency; 5 - Functionally Native Proficiency. Standard deviation (SD) and interquartile range (IQR) values are included for each mean in the table above. IQR is not available for the proficient level due to limited participant number (n=3).

knowledge) and paired t tests were completed to analyze data and establish statistical significance with a predetermined cut off value of .05. Standard deviations and interquartile ranges were also established from each data set to better comprehend the range of values included in each data set. Finally, effect size was calculated from the mean values used for the paired t tests using Cohen's d.

RESULTS

The 2020-2021 PLMSP at SSOM graduated a total of 100 medical students with varying levels of Spanish proficiencies, including 26 novice, 27 beginner, 28 intermediate, 16 advanced, and three proficient students. These 100 students who were included in our data analysis were enrolled at SSOM as first or second year medical students, completed all course requirements, and completed both the pretest placement exam and the post-test at the end of the course. **Figure 1** demonstrates the total starting number of participants specifying reasoning for those who were excluded from our data.

All class levels except for proficient students, increased their comfort with Spanish by the end of the elective, with statistically significant improvement ($p<.05$) noted for novice, beginner, and advanced students (**Table 1**).

When it comes to cultural competency, while novice, intermediate, and proficient students felt they had improved in this measure, this difference was only significant ($p<.05$) for the novice and intermediate students with beginner students actually decreasing in their mean cultural competency scores (**Table 2**).

The Medical Spanish comprehension assessment demonstrated significant improvement amongst all levels except for proficient students (**Table 3**). Upon dividing up the Medical Spanish Comprehension exam into the listening and reading components, novice ($p<.001$), beginner ($p<.001$), and advanced students ($p=.02$) demonstrated statistically significant improvement in terms of listening beginner, intermediate ($p<.001$), and advanced ($p = .001$) students demonstrated statistically

Table 2. Cultural Competency

Level	Pre-Test Mean Score (SD/IQR)	Post-Test Mean Score (SD/IQR)	Cohen's d	P-value
Novice	3.40 (1.13/1.00)	3.92 (1.24/2.00)	0.44	0.006
Beginner	4.30 (0.81/1.00)	3.96 (0.88/1.00)	0.40	0.01
Intermediate	3.89 (0.86/1.00)	4.13 (1.05/2.00)	0.25	0.045
Advanced	4.27 (0.73/1.00)	4.27 (0.70/1.00)	0.00	0.50
Proficient	4.67 (0.41)	5.00 (0.41)	0.80	0.09

To measure cultural competency, students used a 5-point Likert scale to demonstrate agreement with the statements "I am aware of the manner in which culture influences health care needs and outcomes in the LatinX community" and "I am prepared to engage with LatinX patients in a culturally competent manner". Standard deviation (SD) and interquartile range (IQR) values are included for each mean in the table above. IQR is not available for the proficient level due to limited participant number (n=3).

Table 3. Average Medical Spanish Comprehension Exam Improvement

Level	Pre-Test Mean Score (SD/IQR)	Post-Test Mean Score (SD/IQR)	Cohen's d	P-value
Novice	3.81 (3.78/5.00) Audio: 2.23 (2.30/3.00) Reading: 1.50 (1.70/2.00)	14.88 (2.64/4.00) Audio: 8.42 (1.36/3.00) Reading: 6.54 (1.88/3.00)	3.40 Audio: 3.28 Reading: 2.81	<0.001 Audio: <.0001 Reading: <0.001
Beginner	12.74 (2.64/3.00) Audio: 7.19 (1.30/2.00) Reading: 5.56 (1.83/3.00)	17.56 (1.85/2.00) Audio: 8.93 (0.92/2.00) Reading: 8.63 (1.42/2.00)	2.12 Audio: 1.55 Reading: 1.87	<0.001 Audio: <0.001 Reading: <0.001
Intermediate	16.61 (0.92/1.00) Audio: 8.56 (0.74/1.00) Reading: 8.07 (0.96/1.50)	18.59 (1.76/2.00) Audio: 9.15 (1.75/1.00) Reading: 9.44 (0.64/1.00)	1.41 Audio: 0.44 Reading: 1.68	<0.001 Audio: .06 Reading: <0.001
Advanced	18.33 (0.82/1.00) Audio: 9.4 (0.51/1.00) Reading: 8.93 (0.80/2.00)	19.40 (0.51/1.00) Audio: 9.67 (0.48/1.00) Reading: 9.73 (0.46/1.00)	1.57 Audio: 0.55 Reading: 1.23	<0.001 Audio: 0.02 Reading: 0.001
Proficient	19.66 (0.58) Audio: 10.00 (0.00) Reading: 9.66 (0.58)	19.66 (0.58) Audio: 10.00 (0.00) Reading: 9.66 (0.58)	0.00 Audio: 0.00 Reading: 0.00	1.00 Audio: 1.00 Reading: 1.00

To measure comprehension, students answered 20 multiple choice questions, 10 of which were based on an audio selection of a mock doctor-patient interaction. The other 10 questions were based on a written medical interaction between a doctor and patient. Standard deviation (SD) and interquartile range (IQR) values are included for each mean in the table above. IQR is not available for the proficient level due to limited participant number (n=3). Furthermore, the total mean scores are subsequently subdivided into audio and reading comprehension in each section of the table such that they can be analyzed separately.

Table 4. Mean Scores on Objective Structured Clinical Examination (OSCE)

Level	Interview Questions Discussed (SD/IQR)	Quiz Score (SD/IQR)	Pronunciation Score (SD/IQR)	Vocabulary Score (SD/IQR)	Conversational Fluidity Score (SD/IQR)	Cultural Competency Score (SD/IQR)
Intermediate	38.96 (4.61/4.50)	9.14 (1.43/1.50)	7.79 (1.13/2.00)	8.18 (0.94/1.00)	7.89 (1.20/2.00)	8.61 (0.88/0.50)
Advanced	40.33 (3.52/4.00)	9.40 (0.83/1.00)	8.60 (0.63/1.00)	8.73 (0.46/1.00)	8.53 (0.74/1.00)	8.87 (0.35/0.00)
Proficient	40.33 (1.53/na)	9.66 (0.58/na)	9.00 (0)	9.00 (0)	9.00 (0)	9.00 (0)

In the OSCE, standardized patients had one of two different scripts correlating with responses to a complete history checklist as the student progressed with the interview. Student points were designated based on their ability to elicit up to 43 different points of the patient history (Appendix D). Quiz scores were based on a 10-point English quiz designed to gauge student comprehension of the history completed with the standardized patient. Pronunciation, vocabulary, conversational fluidity, and cultural competency assessments were assigned scores based on standardized patients' agreement with statements regarding student ideal capacity with each of these regards (0 = strongly disagree; 9 = strongly agree) as demonstrated in Appendix F. Standard deviation (SD) and interquartile range (IQR) values are included for each mean in the table above. IQR is not available for the proficient level due to limited participant number (n=3).

significant improvement in terms of reading comprehension.

In the OSCE, students across all levels assessed (intermediate, advanced, and proficient) successfully asked the majority of the 43 questions associated with the history taught in the clinical skills course at SSOM: intermediate students covered 91%, and advanced and proficient students covered 94% of the topics in the history. In the 10-point comprehension quiz in English to gauge understanding of the encounter, intermediates scored an average of 91%, advanced 94%, and proficient 97%. The standardized patients assessed students on pronunciation, vocabulary, conversational fluidity, and cultural competency with averages of 7.79, 8.60, and 9.00 for pronunciation for intermediate, advanced, and proficient students, respectively. In terms of vocabulary 8.18, 8.73, and 9.00 were the assessments for intermediate, advanced, and proficient students, respectively. When it came to conversational fluidity, intermediates averaged 7.89, advanced 8.53, and proficient 9.00. In terms of cultural competency, intermediate, advanced, and proficient students averaged at 8.61, 8.87, and 9.00, respectively. These numbers indicate that the standardized patients either agreed (8) or strongly agreed (9) that the students had good pronunciation, appropriate use of medical vocabulary, conversed fluidly with full sentences, and demonstrated sufficient cultural awareness (**Table 4**).

DISCUSSION

The results indicate a promising effect of the Peer Led Medical Spanish Program across a wide range of competencies including student comfort, reading and listening comprehension, and clinical performance. Particular areas of success include the notable improvement in comprehension exam scores across all levels of proficiency (with the exception of proficient students), which are statistically significant ($p<0.05$) and performance on the OSCE, with >90%

history completion and quiz scores for all levels of proficiency assessed (intermediate, advanced, proficient). Of note, results from the self-reported cultural competency surveys did not display similar improvement. This represents an area of study that can be analyzed and revised in subsequent Medical Spanish curricula to further enhance the cultural experience for future student cohorts. Furthermore, proficient students did not demonstrate statistically significant improvement across any measures taken. While this is likely due to their competent performance in the pre-test, further study of the proficient student cohort is needed.

This study sets an important precedent of measuring outcomes of Medical Spanish programs to determine efficacy, and ultimately, guide best teaching practices. The inclusion of measurements for cultural competency, objective evaluation methods in the form of the audio/reading comprehension exam, and an OSCE with its associated comprehension quiz allowed a more comprehensive understanding of strengths and weaknesses of the program that can be used to guide curricular improvement at SSOM and to provide direction for other medical schools working to create a Medical Spanish program. Furthermore, the peer-led model, at both a teaching and administrative level, provides increased student leadership development and academic skills (e.g., Curricular development, assessment creation, setting of learner goals and objectives), readily allows for course adaptation based on current student interests and community needs, and broadens opportunities for learning Medical Spanish in schools that may not have sufficient faculty or financial resources to meet student demand.

While this study provides insight into the efficacy of the PLMSP, there were a lot of students who did drop out of the course and who were excluded from our analysis. Gathering data on reasons for students to drop out of the course would be beneficial to curricular improvement if it were collected in the future. It also must be acknowledged that many of the metrics used are non-validated, and results may be open to biases. The Medical Spanish comprehension test was created by the Medical Spanish leaders and is based on important aspects of the curriculum as determined and agreed upon by them. The cultural competency and degree of comfort tests are subjective in nature, and while this encourages students to assess these characteristics of themselves, the interpretation of these results must take the subjectivity into account when evaluating improvement. The OSCE performances were judged by the standardized patients themselves, and while they had a checklist to assess percent completion of the history, the evaluation on vocabulary, fluidity, cultural competency, and pronunciation were evaluated on a 9-point Likert scale and were subjective to biases of the standardized patients. This allows for variability in interpretation of performance in these areas. Furthermore, the OSCE was conducted after the completion of the course only, so there was no way to judge if clinical performance, itself, was impacted by the PLMSP.

Beyond limitations with the metrics, some bias may be involved in terms of the curriculum. While the curriculum of the PLMSP is based on standardized interactive presentations, individual student experiences are certainly dependent on the content delivery, which is likely to differ based on each teacher's style of instruction. Of note, instruction for the 2020-2021 Medical Spanish Program was conducted fully online via Zoom due to the COVID-19 Pandemic. This mode of educational delivery, while convenient and necessary, creates significant difficulties in fostering the cultural competency component of the curriculum, as previously students were able to engage in-

person with activities that satisfy this requirement, including educational cultural lectures and shadowing Spanish-speaking physicians.

CONCLUSIONS

Overall, the SSOM PLMSP improved Spanish language competency in medical students. Further program development should focus on incorporating culture more effectively into the curriculum and developing measurement tools for more advanced students. This student-led program serves as a model that can accommodate students of various levels, be far-reaching in terms of student enrollment, reinforce the medical history taught in English, and be sustained over time. It is a helpful example to other schools aiming to establish a Medical Spanish curriculum that promotes student leadership and academic skills, while contributing to the development of more standardized guidelines in the effective teaching of Medical Spanish.

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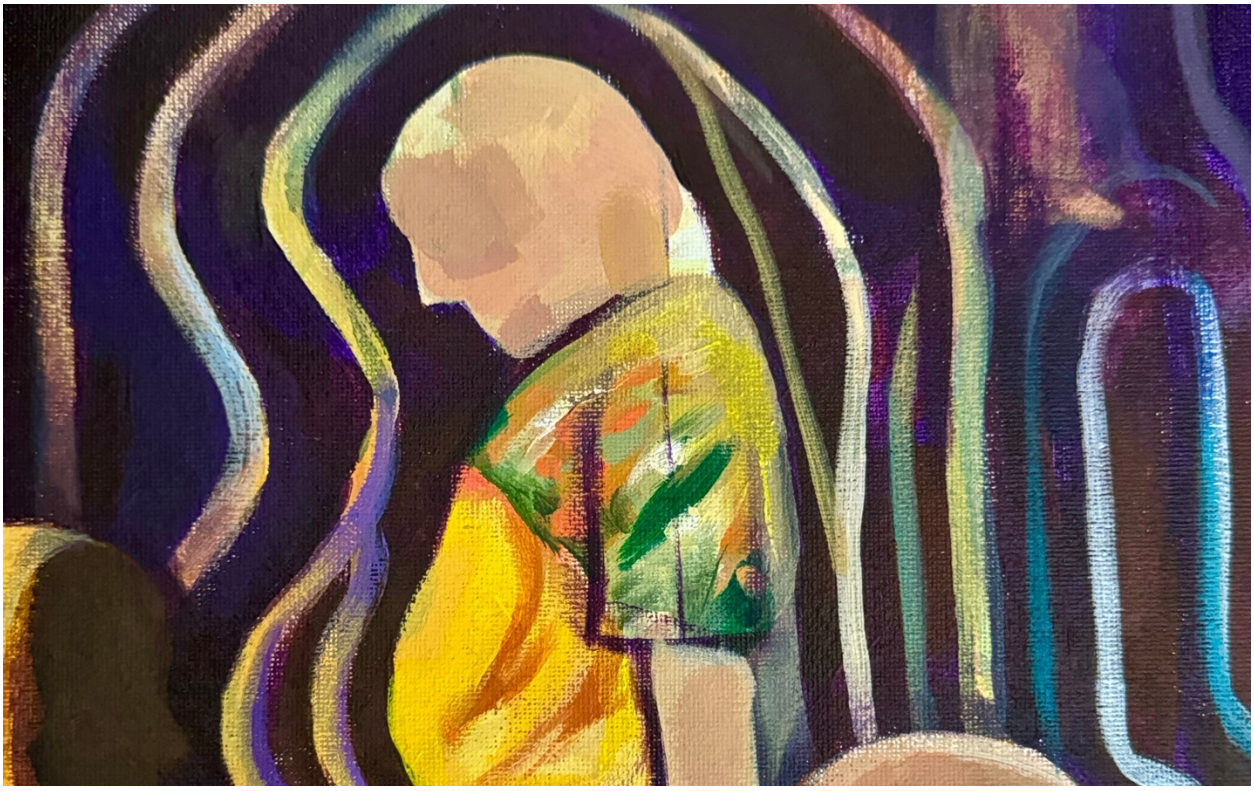

Cover artwork by Danielle Li.

Doubly Dangerous: Medical Students' Observations of Weight Bias in the Clinical Setting

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Weight bias is a form of discrimination that is pervasive in medical encounters yet often unacknowledged in medical education. In this essay, we reflect on the instances of weight bias witnessed during our clerkship year. Using vignettes gleaned from clinical encounters – an IVF patient with a Body Mass Index (BMI) of 44 accused of "doctor shopping"; a transgender man whose changing body size is emblematic of his transition; and a child receiving a striking visual lesson about fatty foods – we outline how weight bias violates the three fundamental principles of justice, autonomy, and non-maleficence. We propose a beneficent approach to BMI and weight bias that upholds these ethical principles in the clinic and medical classroom.

INTRODUCTION

Medical education emphasizes Body Mass Index (BMI) as a key indicator for health risks. Electronic medical records alert us to BMIs greater than 25 with bolded, red exclamation points. As medical students, we

learn that a BMI over 30 defines obesity, a disease we can treat with a menu of diets, medications, and surgeries. Yet, medical school curricula often overlook the link between BMI and weight bias, a pervasive form of discrimination in healthcare contexts. Countless patient narratives and international expert consensus state that weight bias exhibited by physicians damages health and undercuts human rights (1). This reality inspired the three of us (AMM, BB, MKV) to start a working group to address weight bias at our school.

As third year medical students at the end of our clerkship year, we reflect on how weight bias in clinical teaching spaces is doubly dangerous: it both undermines patient care and condones ongoing bias in future physicians. We present three cases in which the indiscriminate use of BMI came into direct conflict with the physician's responsibility to uphold three fundamental principles of medical ethics: justice, non-maleficence, and autonomy (2). These principles form an ethical framework emphasizing patient self-determination, welfare, harm prevention, and equitable healthcare access. By reimagining what a beneficent approach to addressing weight with patients and learners could look like, we argue that centering key ethical principles when caring for patients of diverse body sizes can help to reduce weight bias and promote patient-centered care.

JUSTICE

Case 1: "Kara is a 35-year old new patient, hoping to undergo a second egg retrieval for in vitro fertilization. When I asked why she transferred care from a local private practice, she cited dissatisfaction with her prior care, saying 'before I could ask what the lab work or hormone levels meant, the doctor was hurrying out of my room'". Dr. C, the attending, interrupted my presentation, scoffing, "That's not why she left the old practice. Her BMI is 44. Patients like her are always shopping for a new doc."

I (MKV) was on a reproductive endocrinology service, presenting Kara's case. My initial reaction to Dr. C's correction was to worry about my own performance. Did Dr. C think I was unable to elicit an accurate history? But another question worried me as well -- why were we assuming that Kara was being untruthful?

Several studies have shown that physicians spend less time in appointments with patients with elevated BMIs compared to patients with normal-range BMIs, raising concerns about inequitable allocation of healthcare resources on the basis of BMI (3-6). Patients whose BMI falls into the overweight or obese categories are also less likely to experience the same respect or emotional rapport with their physicians as their thinner peers (7, 8). A recent scoping review also suggested that when patients with elevated BMIs change doctors they usually do so because of differences in treatment, such as shorter visit times and stigmatization, rather than impulsiveness (9).

As medical students, we often adopt our instructors' heuristics and habits to enhance our clinical skills. As Dr. C corrected my history-taking, I was at risk of incorporating his weight bias (often an implicit bias) into my own practice. The incident illuminated that curbing the transmission of physician weight bias and addressing the resulting healthcare inequities necessitates raising awareness among both learners and educators. An awareness of weight bias in trainees, when integrated with a self-awareness of our own manner with patients, helps us to recognize and replace bias with empathy for patients' past healthcare experiences and curiosity about their goals while in our care.

AUTONOMY

Case 2: Taylor is a transgender man who

recently started gender-affirming treatment. At his annual visit, his primary care physician expressed concern about the increase in his BMI from 26 to 32 since starting testosterone. He counseled Taylor to reduce his caloric intake, prescribed phentermine-topiramate, and quickly moved on to see his next patient. As the door closed, Taylor's body language became tense. Sensing his frustration, I asked him how he had been feeling about his new body size. He shared that he sometimes worried about the health implications of his weight gain, while also feeling that it helped to align his appearance with his gender identity.

I (AMM) reflected that if we had elicited Taylor's experience at the outset, we could have seen past his BMI, validated his resilience, and helped him identify alternative ways to increase his body size. For example, working with a trainer could have centered his gender affirmation journey, helping him to build muscle mass while simultaneously optimizing his metabolism.

Taylor's narrative highlights that by reflexively defining BMI as a fixable problem, clinicians may default to paternalistic management rather than shared decision-making. This approach curtails autonomy, which hinges on patients having the chance to voice their preferences and make informed decisions. Concerningly, some medical ethics scholars believe that it is acceptable to limit the autonomy of patients with elevated BMIs, arguing that it is ethically justified insofar as it helps them lose weight (10). However, not only is there no evidence to show that paternalistic counseling helps patients lose weight,11–13 but an extensive body of research demonstrates that patients who feel disrespected (7), dehumanized (14,15), and stigmatized (6) by their physicians are less likely to adhere to their physician's advice (16,17), more likely to be lost to follow-up (18), and experience poorer long-term health outcomes (19).

The intersection of weight bias with other prejudices like sexism, racism, homophobia, transphobia, xenophobia and others amplifies the stigma experienced by marginalized patients once they enter a doctor-patient relationship (20,21). Taylor's experience shows how even subtle forms of weight bias encourage premature closure of the medical encounter, denying patients the opportunity to contextualize their attitudes towards food, exercise, and body image.

NON-MALEFICENCE

Case 3: A medical student, Rick (fictitious name to protect anonymity), shares excitedly about a "creative" intervention he learned for tackling obesity in the pediatrics population while seeing a 10-year-old boy with a BMI consistently at the 99th percentile. At the suggestion of his attending, Rick showed the child how many grams of fat are contained in potato chips, pizza, and cookies by measuring equivalent portions of lard into clear baggies. The patient was shocked to see his favorite foods transformed into fatty lumps and related this to his own body. Months later, the patient's mother joyfully updated the pediatrician that her son had lost 5 pounds. Rick expressed satisfaction that the intervention made a positive impact on the boy's health.

This story made me (BB) reflect on my own experiences at the pediatrician's office as an "obese" patient, which were punctuated by admonishments about my position on the growth chart and terse directives to eat less and exercise more. My repeated attempts to implement my doctor's guidance fueled patterns of obsessive food restriction, culminating in a diagnosis of anorexia which consumed my teenage years.

While such graphic efforts might seem fruitful in bringing about the desired goal of weight loss, clinicians often do not scrutinize the potential long-term harms of

this approach. There is a wide body of literature disproving the once-popular notion that shame is ethically justified if it motivates weight loss (10). Furthermore, creating associations between food and shame in children puts them at increased long-term risk for developing eating disorders (22, 23). Beyond eating disorders, weight bias in healthcare settings impacts patients' willingness to engage with care to avoid the discomfort of feeling stigmatized (9, 24), leading to withdrawal from care, delayed diagnoses and worse disease progression (8, 15).

The contrast between Rick's genuine belief that he had helped his patient and my own personal experience with my pediatrician emphasizes the insidious nature of weight bias. Without learning how to identify and combat weight bias, clinicians can unknowingly cause iatrogenic harm.

REFLECTIONS: A Note on Beneficence

As we reflect on the pervasive weight bias throughout our clerkship year, we hope to identify ways to provide more beneficent care for patients of all body sizes. The assumptions made about Kara's motivations for "doctor-shopping," and the ways biases can be transmitted to trainees motivate us to advocate for addressing weight bias in medical education. Taylor's story inspires us to learn about our patients' relationships with their bodies, irrespective of BMI. Rick's enthusiasm for the lard intervention reveals how even well-intentioned healthcare agents can cause harm by centering the benefit of weight loss without exploring the long-term consequences of inflicting shame on a patient.

We believe that teaching medical students about weight bias is essential to reduce its unforeseen ethical, emotional, and physical consequences. Medical trainees should learn evidence-based, weight-neutral clinical skills that empower patients to optimize their health, such as teaching mindful eating, promoting increased fruit consumption, and encouraging enjoyable physical activity (24-26). As future physicians, we will work with patients to understand the complexities of their health; in doing so, we have valuable opportunities to help patients cultivate healthier relationships with their bodies. Unchecked weight bias undercuts the healing potential of this privilege – and that is truly a shame.

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"The Hospital", series "The Small Miseries of War" by Jacques Callot. Courtesy National Gallery of Art, Washington.

Patient Discharge Decision Flowchart: Streamlining Disposition Management after Acute Hospital Stays

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Throughout medical school, you begin to acquire a more comprehensive understanding of the appropriate approaches to patient care in various settings. While you gain expertise in the intricacies of both inpatient care and outpatient care, there is little guidance on bridging the gap between the two for patients who have recently undergone hospitalization. Since every patient has unique functional capacity and rehabilitation requirements, this process can be entirely distinct for each person. Although Case Management and Social Work teams are often responsible for making decisions on rehabilitation needs, it is our responsibility as future physicians to be knowledgeable about the available options and assist in decision-making. To this end, my colleagues and I collaborated with a team of case management specialists to design an educational handout for students, physicians, and patients that outlines and simplifies post-discharge care and rehabilitation options. This project aims to improve awareness of rehabilitation options to ensure a more seamless transition for patients who require continued care of therapies outside of the inpatient settings. We believe that this will enable providers to assist in making better informed decisions on long-term planning and encourage more personalized care.

DISCHARGED FROM HOSPITAL STAY: WHAT COMES NEXT?

A guide to Case Management

01 LONGTERM ACUTE CARE HOSPITAL

For those patients who require complex management but do not require intensive care.

- Physician must supervise care and perform daily examination.[1]
- 24/7 nursing services available.[2]
- Coverage[1,3]
  - Medicare
  - Private insurance
  - Long-term care plans
  - Out-of-pocket payment

Average length of stay: 26.6 days[1]

02 ACUTE REHAB FACILITY

For those patients who require short-term intensive therapies to aid in recovery.[1,2]

- Patient requires therapy from 2 or more disciplines (PT, OT, ST, etc.).[4]
- Patient must be able to tolerate minimum of 3hrs/day 5day/wk of PT/OT/ST.[1,2]
- Nursing care available 24/7 with at least 3 in-person physician visits per week.[1,2]
- Coverage[1,3]
  - Medicare
  - Private insurance

Average length of stay: 13.1 days[1]

03 SKILLED NURSING FACILITY

For those patients who require direct skilled-nursing supervision or therapies that are too complex for the home setting.[5]

- Physician provides initial assessment within 30 days of arrival and is only required to see patient once every 30 days.[2]
- Ongoing nursing care.[2]
- Patient receives 1-2 hours of therapy per day.[2]
- Coverage[1,5]
  - Medicare
  - Private insurance

Average length of stay: 27 days[1]

04 ASSISTED LIVING FACILITY

For those patients who do not require therapy but need help with ADLs.[6]

- Patient must be ambulatory but may need assistance of cane or walker.[6]
- Patient must not require 24/7 skilled nursing care.[6]
- Facilities provide personal care (bathing/dressing), meals, transportation, social/recreational activities.[6]
- Coverage[1,6]
  - Majority out-of-pocket

05 HOME HEALTH CARE

For those patients who are able to maintain some independence but require minimal assistance for ADLs or intermittent nursing care.[7]

- Patient must be home-bound[7]
- May have home health aides, nurse visits, and/or skilled therapist visits[7]
- Coverage[7]
  - Skilled services paid for by Medicare and private insurance
  - Aides typically paid for out-of-pocket

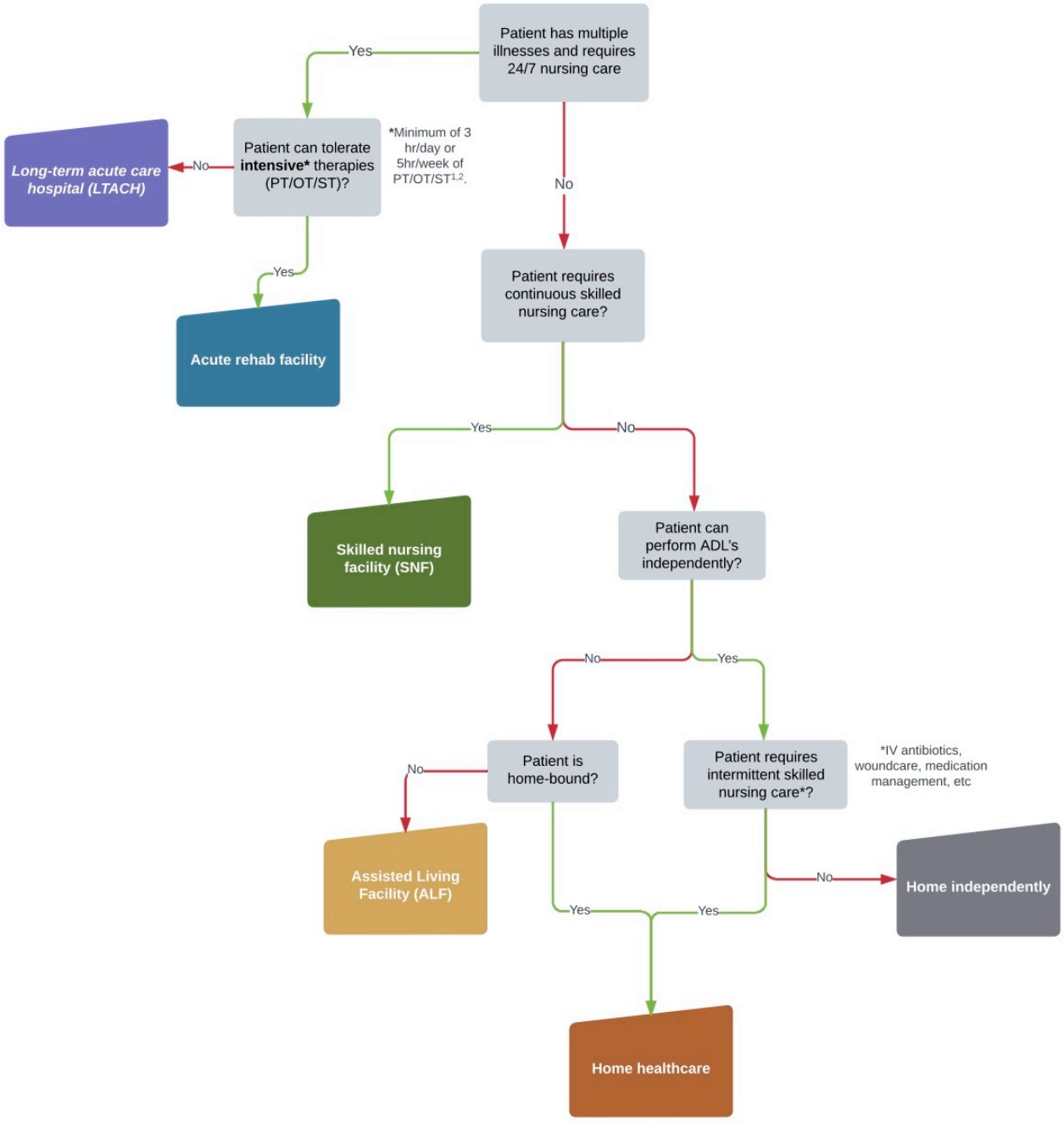
Average length of service: 45-69 days[8]

06 HOME SWEET HOME

If none of the above applies

- Independently returning home to self or with family support
- Managing self-care without additional formal nursing assistance

Rehabilitation Decision Aid: A Simplified Guide for Post-Discharge Care



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"My family" by George Bellows. Courtesy National Gallery of Art, Washington.

Emerging Relationships of Sarcomeric Mutations and the Cardiomyocyte Transcriptome in the setting of Familial Hypertrophic Cardiomyopathy

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Familial hypertrophic cardiomyopathy (FHC) is thought to be the most common genetically inheritable cardiac disease with a prevalence of 1 in 500 individuals. A classic sign of FHC is inappropriate asymmetrical thickening of the septum with the potential for heart failure and sudden cardiac death, in the absence of mechanical stress, pressure overload, or pathogenic infiltration. Molecular analysis of the thickened septum in the past has revealed that these cardiomyocytes are enlarged and disorganized with interstitial fibrosis, thus causing restricted blood flow out of the left ventricle. Significant causes of idiopathic FHC disease pathogenicity have been linked to sarcomere dysfunction in 8 key genes. Research over the years has identified two major sarcomere mutations such as myosin-binding protein C (*MYBPC3*) and

β-myosin heavy chain (*MYH7*). Together these gene mutations account for over 80% of causes for FHC phenotype presentation. The focus of this review will be to analyze current knowledge regarding the *MYBPC3* and *MYH7* gene mutations in the sarcomere, as well as take look at how they directly and indirectly affect the transcriptome associated with cardiomyocyte hypertrophy and fibrosis. Finally, we will identify current and future potential targets for disease-modifying diagnostics and therapy.

INTRODUCTION

Familial hypertrophic cardiomyopathy (FHC) is the most common monogenic cardiac disease in which the left ventricle (LV) and septum experience hypertrophy generally in the absence of any other cardiac or systemic disease. Clinically this is defined as unexplained LV hypertrophy with a maximum wall thickness greater than 15 mm in adults or a z-score > 3 in children (1). This asymmetric hypertrophy of the left ventricular wall and septum places individuals with FHC at an increased risk for sudden cardiac death (1). With a prevalence of approximately 1 in 500 amongst the general population (1). Familial hypertrophic cardiomyopathy is inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is sufficient to cause the disorder. While the clinical presentation and course display a large degree of heterogeneity, the disease is an important cause of disability and death among patients of diverse age. The estimated penetrance of hypertrophic cardiomyopathy (HCM) after a 15-year follow-up period was found to be 46% (2, 3).

A sarcomeric gene refers to a gene that encodes proteins involved in the structure and function of the sarcomere, which is the basic contractile unit of muscle cells, including cardiomyocytes in the heart. Since the majority of FHC cases have been traced to sarcomeric protein mutations, the focus of this review will be exploring two of the most implicated sarcomeric genes in FHC: β-myosin heavy chain (*MYH7*) and myosin binding protein C (*MYBPC3*), responsible for approximately 80% or greater of FHC (1). As seen in **Table 1**, it should also be noted that while other mutations in the sarcomeric genes such as *TTN*, *MYH6*, *MYL2*, *MYL3*, *TNNT2*, *TNNI3*, *TPM1*, *ACTC*, and *TNNC1* do occur and contribute to FHC, they together only account for less than 10% of cases and will therefore not be explored in this review. The protein produced from the *MYH7* gene is a major component of the thick filament in sarcomeres and is involved with the ATPase function used to generate force (4). The protein produced from the *MYBPC3* gene provides structural support and regulates muscle contractions by associating with the thick filament (4).

Patients with FHC who harbor sarcomeric gene mutations often exhibit a diverse array of clinical manifestations. Common clinical symptoms may include shortness of breath, chest pain, fatigue, and palpitations. It is noteworthy that the disease presentation can vary widely, with some individuals remaining asymptomatic for extended periods, while others may experience severe symptoms, such as heart failure or arrhythmias. Additionally, there is a notable risk of sudden cardiac death, particularly among younger patients, underscoring the importance of early diagnosis and comprehensive clinical management (5). Histological examination shows that FHC causes myofibrillar disarray as well as considerable degrees of tissue and interstitial fibrosis. Activation of the hypertrophic signaling pathways and profibrotic signals in the nonmyocyte cells produce the disease remodeling in FHC. Cytokines, microRNA's, and other cell cycle proteins have all been implicated in

Table 1. Genes Associated with Familial Hypertrophic Cardiomyopathy

Gene Name (full)	Gene Symbol	Chromosomal Location	Number of Mutations Associated with FHC
Myosin-binding protein C	*MYBPC3*	11p11.2	Over 500 mutations (Approximate)
β-myosin heavy chain	*MYH7*	14q11.2	Over 300 mutations (Approximate)
Titin	*TTN*	2q31.2	Varied mutations (Less common)
Myosin light chain 2	*MYL2*	12q24.11	Less common mutations
Myosin light chain 3	*MYL3*	3p21.31	Less common mutations
Troponin T	*TNNT2*	1q32.1	Less common mutations
Troponin I	*TNNI3*	19q13.4	Less common mutations
Tropomyosin	*TPM1*	15q22.1	Less common mutations
Cardiac alpha actin	*ACTC*	15q14	Less common mutations
Troponin C	*TNNC1*	3p21.31	Less common mutations

This table provides an overview of genes associated with Familial Hypertrophic Cardiomyopathy (FHC), including their full names, gene symbols, chromosomal locations, and an approximate number of mutations linked to FHC for each gene. The table highlights two major genes, MYBPC3 and MYH7, which are the primary contributors to FHC, along with other genes that also play a role in the disease, albeit less commonly (1, 5).

myocardial necrosis and fibrosis in patients with FHC. Transforming growth factor-β (TGF-β) is one such cell cycle protein that has been implicated as a major pro-fibrotic protein in the pathologic remodeling in FHC (1). MicroRNA's, such as miRNA29, have also been noted to be markedly elevated in FHC and are theorized to serve as markers for cardiomyocyte hypertrophy as well as a potential role in the development of interstitial fibrosis. Pharmacologic inhibition of these targets warrants further exploration as potential therapies for FHC.

This review aims to summarize some of the current knowledge about the pathogenesis of Familial Hypertrophic Cardiomyopathy, analyze new research studying the transcriptome changes underlying the hypertrophic phenotype seen in cardiomyocytes with this disease, and explore a few directions of the new therapeutics.

DISCUSSION

Sarcomeric Mutations

After the discoveries were made in the 1980s regarding the locations of the genes associated with FHC, more studies have been done to pinpoint the specific mutations. The study done in August 2010 by Tanjore et al. showed that there have been 186 mutations identified within the *MYH7* gene to date, mainly within the exons 7, 12, 19, and 20 that have been implicated heavily with the disease progression of FHC (6). While numerous studies are detailing the diverse array of disease severity associated with specific mutations, we will focus on the molecular mechanisms in a broader sense. The majority of genotyped sarcomeric FHC related mutations of *MYH7*, implicated only a single missense nucleotide substitution that results in a mutated protein. As seen in **Figure 1**, normally this protein is involved in interacting with the actin filament and using its ATPase activity required to produce a power stroke necessary in contraction. In an investigation into the *R723Q* mutation of *MYH7* by Kraft et al. in triton-permeabilized cardiomyocytes, it was found that maximum force was significantly lowered, even though the calcium sensitivity remained unchanged

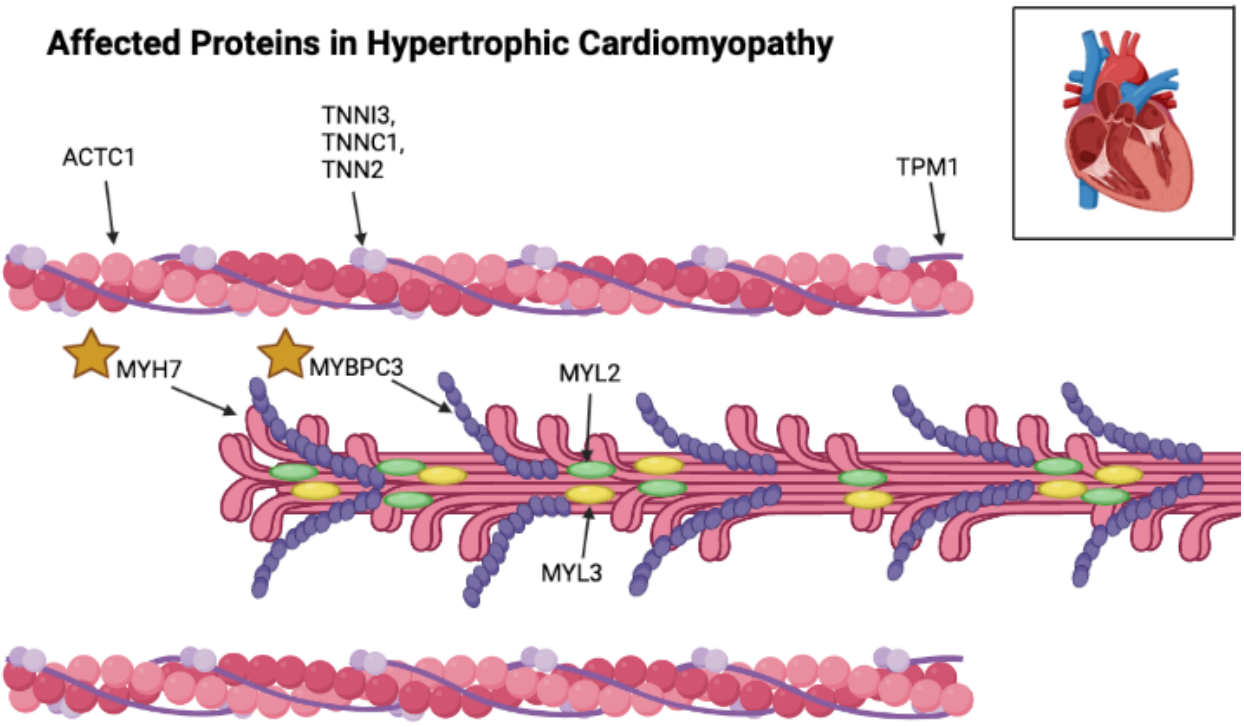

Figure 1. ***Causal Genes for FHC.*** *This schematic shows the structure of the sarcomere and the specific proteins of the thin and thick filament. Each of the labeled proteins are currently established as causal genes for FHC. The starred proteins,* MYH7 *and* MYBPC3*, both represent the elements of the thick filament that are most often implicated in the development of FHC.*

from the normal baseline. Further analysis revealed that protein phosphorylation was decreased in the other proteins in the sarcomere, such as troponin I and T, myosin-binding protein C, and myosin light chain 2 in the *R723Q* cardiomyocytes (7). This is interesting to note since it may provide evidence of secondary cellular effects that a mutation in the *MYH7* gene may cause. Histological sections of the tissue were taken and analyzed which showed that the myofibrillar density was greatly reduced along with irregular Z-discs and variable axes of the sarcomeres within the cardiomyocytes (7). This shows that the low cardiomyocyte force generation capacity in FHC patients can be explained by the reduced myofibril density and myofibrillar disarray. Furthermore, the hypo-contractile sarcomeres may present the primary cause for hypertrophy in patients with *MYH7* mutations. These findings were further corroborated by Witjas-Paalberend's research which studied whether cellular dysfunction is due to intrinsic sarcomere defect or cardiomyocyte remodeling by measuring maximal force-generating capacity (Fmax) in various mutations within the filaments (8). This study showed that *MYH7* mutations reduced force-generating capacity at all Ca2+ concentrations and is explained by hypertrophy and reduced myofibril density.

While missense mutations explain mutations in *MYH7*, *MYBPC3* is primarily affected by frameshift mutations (9). Frameshift mutations of the *MYBPC3* gene result in a truncated cardiac myosin-binding C protein (*cMyBP-C*) in the myocardium from patients with FHC. Normally *cMyBP-C*

interacts with both the myosin and actin via phosphorylation of its head to promote ATPase activity and cross-bridge formation. A study was done by Toepfer et al. investigated how mutations in *MYBPC3* alter cardiac muscle contraction and relaxation by using both mouse models and human fibers. They showed that *MYBPC3* mutations cause FHC by haploinsufficiency, and further demonstrated that cardiomyocyte phenotypes are dependent on *cMyBP-C* quantities by manipulating the levels of the protein present in the cardiomyocyte (9). By testing contractility of the cardiomyocyte sarcomere at varying levels of *cMyBP-C*, confirmed the dose-dependent relationship in disease presentation. *cMyBP-C* truncation and lower overall levels of functional *cMyBP-C* in the cell correlated with the hypercontractility of the sarcomere (9). This is interesting because this is a different pathogenesis than an *MYH7* mutation. Lack of *cMyBP-C* also altered the myosin confirmations during relaxation and encouraged more ATP hydrolysis leading to more thin filament interactions while discouraging the relaxed state of the myosin head. This information posits the theory that myosin dysregulation is the main pathology behind *MYBPC3* mutations. This hypercontractility, failure to properly relax, and increased energy consumption lead to hyperdynamic contraction, diastolic dysfunction, and energy inefficiency observed in FHC cardiomyocytes.

Mutant Cardiomyocyte Transcriptome

While the mechanisms are likely extremely complicated, it is helpful to see what the cardiomyocyte itself may be doing in terms of gene expression and production of transcripts by looking at the transcriptome of the cell. In a study by Farrell et al., they aimed to use a murine model to identify the early genetic mediators in the development of cardiomegaly seen in *cMyBP-C* mutations by studying the mutant cardiomyocyte transcriptome. By performing microarray analysis on left ventricles of wild type and *cMyBP-C* mutant mice at varying post-natal days, they were able to identify genes that were dysregulated in the mutant mice even prior to the hypertrophy phenotype (10). Some of these genes included genes in mechano-sensing pathways and potassium channels linked to arrhythmias (10). One of the genes, *Xirp2*, and its protein are normally regulated during normal growth but show significant upregulation in pre-hypertrophic mutant hearts (10). The researchers also found that transcription factor *Zbtb16* also shows upregulation in pre-hypertrophic mutant hearts (10). The dysregulation of both genes and their protein products even before the hypertrophic phenotype in *MYBPC3* mutant mice hearts may indicate that these are important stress sensing genes early in the development of FHC. It may also provide the door to genetic diagnostics that shed light on the stage of disease presentation. This study also underlines the importance of the extracellular matrix in the hypertrophic phenotype.

The pathologic mechanisms behind the development of FHC are certainly complex. Currently little is known about the upstream regulators that may be affected by sarcomeric mutations and thus cause the disease phenotype. One such protein involved in the metabolic stress response in FHC is p53. A study done by Cohn et al. applied RNA sequencing to the cardiomyocyte samples with *MYH7* and *MYBPC3* mutations. The results from this study implicated p53 signaling as a common molecular consequence of the thick filament mutations (11). RNA sequencing data for p53 dependent gene expression revealed an increased concentration of BBC3, BAX, and FAS transcripts within mutant cardiomyocytes (11). These transcripts all play a role in cytotoxicity and function in the regulation of cell death. Given the previously

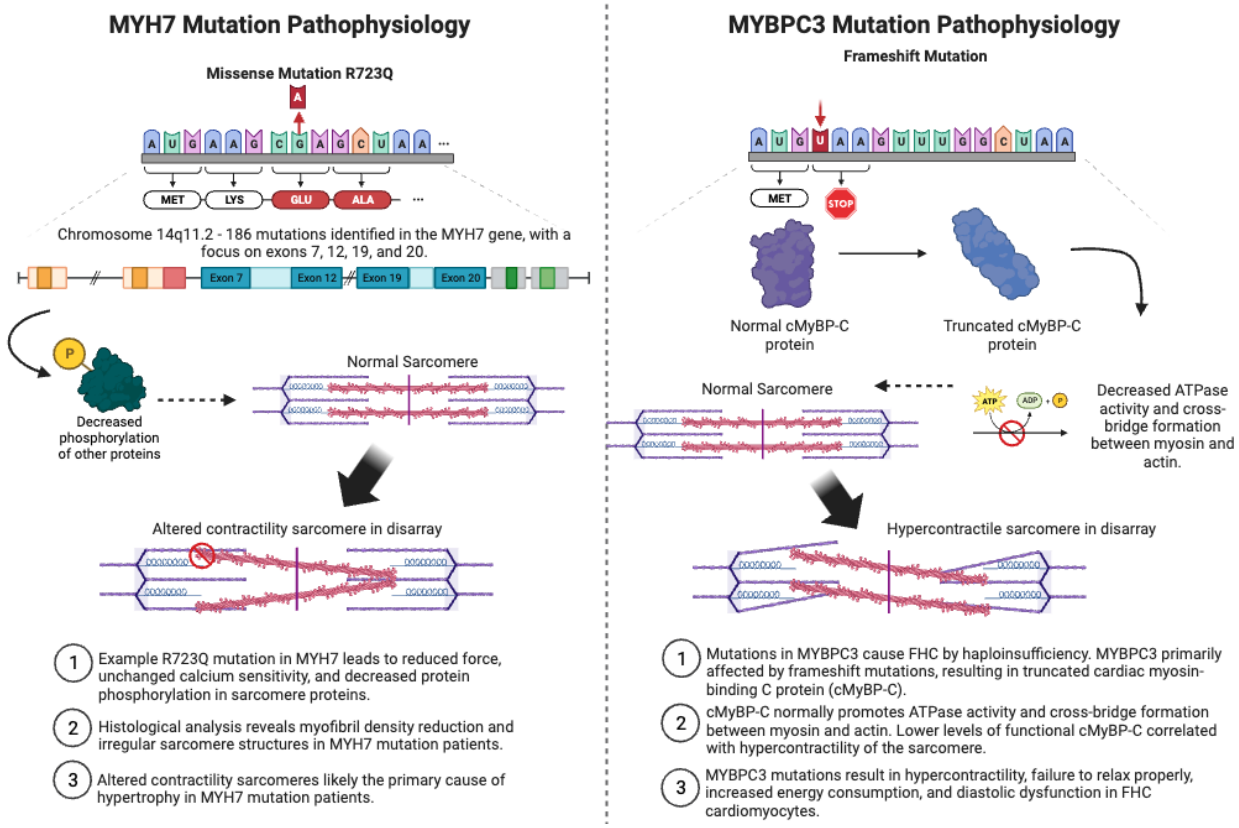

Figure 2. ***Key findings related to MYH7 and MYBPC3 mutations in Familial Hypertrophic Cardiomyopathy (FHC).*** *MYH7 mutations in FHC reduce maximum force generation despite normal calcium sensitivity, leading to hypo-contractile sarcomeres and hypertrophy. MYBPC3 mutations cause cMyBP-C haploinsufficiency, resulting in hypercontractility, impaired relaxation, and diastolic dysfunction in cardiomyocytes. These insights are vital for developing FHC therapies.*

established energy inefficiency problem of FHC cardiomyocytes, this is in line with p53 become activated as a problem solver of metabolic stress. It was also found that due to the increased energy usage and higher ADP:ATP ratio, mutant cardiomyocytes also contain higher mitochondrial-derived ROS (11). This also provides background on why p53 may be provoked in FHC cardiomyocytes.

Another important part of any cell transcriptome is microRNAs. Myocardial miRNA's may modulate the processes of cardiomyocyte hypertrophy, excitation-contraction coupling, and apoptosis. A study done by Roncarati et al. showed that 12 miRNAs were significantly increased in HCM plasma, however, only 3 of those miRNAs were found to be correlated with hypertrophy (12). Of those, it was significant that miRNA-29a was the only one correlated with fibrosis (12). Another study around the same time done by Kuster et al. studied the microRNA expression profile of FHC patients carrying *MYBPC3* mutations. The interesting thing here is that the 13 miRNA's that were found to be correlated with FHC hypertrophy originated from an intron in the *TRPM3* gene (13). RT-PCT analysis showed that the *TRPM3* gene was upregulated in FHC compared to the normal myocardium (13). These studies indicate that *MYBPC3*

mutations produce a specific miRNA expression profile which could be useful in understanding signaling pathways and designing therapeutics that target these specific miRNAs.

Current Recommendations and Novel Therapies

Since the characterization of FHC almost 60 years ago, the diagnosis and management of patients have moved forward with cardiac imaging and previous serious arrhythmias, and interventional cardiology measures. Concurrent with Landstrom et al. it's understood that it is not possible yet to determine prognosis based on the mutation (14). Given the sheer number of mutations that can lead to FHC with so many different modifying factors, it is difficult to establish a genotype to the phenotype endpoint with precision. Given the same mutation in two patients, it is almost certain that the phenotype will differ. The current pharmacotherapeutic recommendations for the management of FHC are aimed at alleviating symptoms, preventing complications, and enhancing cardiac function. Individualized treatment plans are essential, and specialized healthcare teams, including cardiologists and genetic counselors, play a pivotal role in providing comprehensive care.

Commonly used pharmacological interventions include beta-blockers like metoprolol and atenolol to reduce heart rate, relieve chest pain, shortness of breath, and palpitations, as well as to prevent arrhythmias. Calcium channel blockers such as verapamil or diltiazem may be employed, either alone or in conjunction with beta-blockers, to enhance heart muscle relaxation and reduce stiffness. In certain cases, anti-arrhythmic medications like disopyramide are used to manage abnormal heart rhythms. Patients at risk of atrial fibrillation or blood clots may be prescribed anticoagulants like warfarin or newer oral anticoagulants. Diuretics, such as furosemide, may help alleviate fluid retention and congestion in heart failure. ACE inhibitors or ARBs may be considered to manage blood pressure and reduce cardiac workload. Symptomatic relief for angina can be achieved using nitrates. Genetic testing and counseling are often recommended to identify specific gene mutations associated with FHC and assess familial risk. In severe cases with a high risk of sudden cardiac death due to arrhythmias, implantable cardioverter defibrillators (ICDs) may be implanted for continuous monitoring and intervention. For refractory symptoms and severe obstruction, septal reduction therapies like septal myectomy or alcohol septal ablation may be considered (5). Collaboration with healthcare providers specializing in FHC management and a multidisciplinary approach are essential for optimal care.

A new interesting approach has identified a small molecule MYK-461 (15). This small molecule was studied by Green et al. showed that it reduces the contractility by decreasing the ATPase activity of the cardiac myosin heavy chain. This study also shows that chronic heavy chain administration of MYK-461 suppresses the development of ventricular hypertrophy, cardiomyocyte disarray and prevents myocardial fibrosis by blocking fibrotic gene expression (15). Given that the hyperdynamic contraction and induction of profibrotic genes are a central tenet for the development of FHC, this new molecule presents an extremely promising therapeutic approach. Further research was done by Toepfer et al also showed that this molecule had the ability to attenuate myosin activity in cardiomyocytes with *MYBPC3* mutations. Mavacamten, a synthetic version of MYK-461, is the first in its selective allosteric inhibitor of cardiac myosin ATPase which serves to reduce actin-myosin cross-bridge formation and reduce cardiomyocyte

energy usage (16). This new drug was approved for use in the US in April 2022.

Mavacamten achieves its therapeutic effects by inhibiting the ATPase rate of beta myosin, shifting its equilibrium away from its activated state towards a super relaxed state. This reduction in beta myosin activity results in the inhibition of contractility and a decrease in excitotoxic calcium handling. Preclinical studies in rodent models demonstrated several beneficial effects, including the reduction of myocardial contractility, prevention of left ventricular hypertrophy, reduction of myocardial fibrosis, and suppression of pro-fibrotic signaling pathways. These effects translated into improved functional capacity and the prevention of hypertrophic remodeling in animal models. Positive results from the Phase II PIONEER-HCM (Hypertrophic Cardiomyopathy) trial paved the way for the Phase III EXPLORER-HCM trial, which assessed mavacamten's efficacy and safety in patients with obstructive HCM. The trial met its primary endpoint, with a significant improvement in New York Heart Association (NYHA) functional class and peak oxygen consumption. Subsequent studies, such as VALOR-HCM, explored mavacamten's benefits in patients eligible for septal reduction therapy, demonstrating a significant decrease in LV outflow tract gradients and NYHA class. Additionally, ongoing open-label extension studies suggest the potential for long-term benefits, including the reduction of LV wall thickness and myocardial fibrosis. Other cardiac myosin inhibitors, like aficamten, are under development and have shown promise in preliminary trials, offering additional therapeutic options for FHC (5).

As of now, the genetics aspect of this disease has remained largely diagnostic, rather than be wielded as a therapeutic tool, but it is now emerging as a promising strategy to target the genetic origins of this disease. Several approaches, including gene replacement using adeno-associated viral vectors, gene editing, allele-specific silencing, trans-splicing, and exon skipping, are being explored. Recent advancements, such as base editing to correct specific HCM-causing variants, have demonstrated potential in rescuing the disease phenotype in preclinical models. Gene therapy methods, like gene replacement, have shown potential in studies using special cells that lacked *MYBPC31718*. More recently, a technique called base editing was able to correct a common disease-causing variant in HCM, known as *MYH7* p.R403Q, and reverse the HCM symptoms in both lab-grown heart cells and a mouse model (19). However, early-phase human trials face ethical challenges, patient selection issues, outcome identification, and the management of off-target effects. Transcriptomic studies such as the ones above provide us incredible opportunities to control or prevent the disease progression in with the help of small molecule therapeutics like siRNAs to silence pathologic phenotypes.

CONCLUSIONS

FHC is just one subtype of an incredibly complex pathology collectively known as hypertrophic cardiomyopathy. The clinical phenotypes, histological presentation, and genetic causes of FHC are extremely diverse. They are the consequences of a large of mediating factors, ranging from causal genetic mutation to lifestyle and other genetic predeterminants. Progress in understanding the genetic basis for the disease has led to the identification of important causative mutations. Greater knowledge of the pathogenic pathways incriminated in sarcomeric mutations, cell cycle and regulatory proteins, and miRNAs will elucidate the way to treat the causes of the disease rather than symptoms. Ideally, this will also present solutions to shift from

treating myocyte hypertrophy, fibrosis, and obstruction to using genetic and phenotypic analysis to provide individual solutions for each patient. Insights into these processes from culture studies, murine models, and human clinical trials will advance the field of cardiology.

DISCLOSURES

Conflicts of interest: None.
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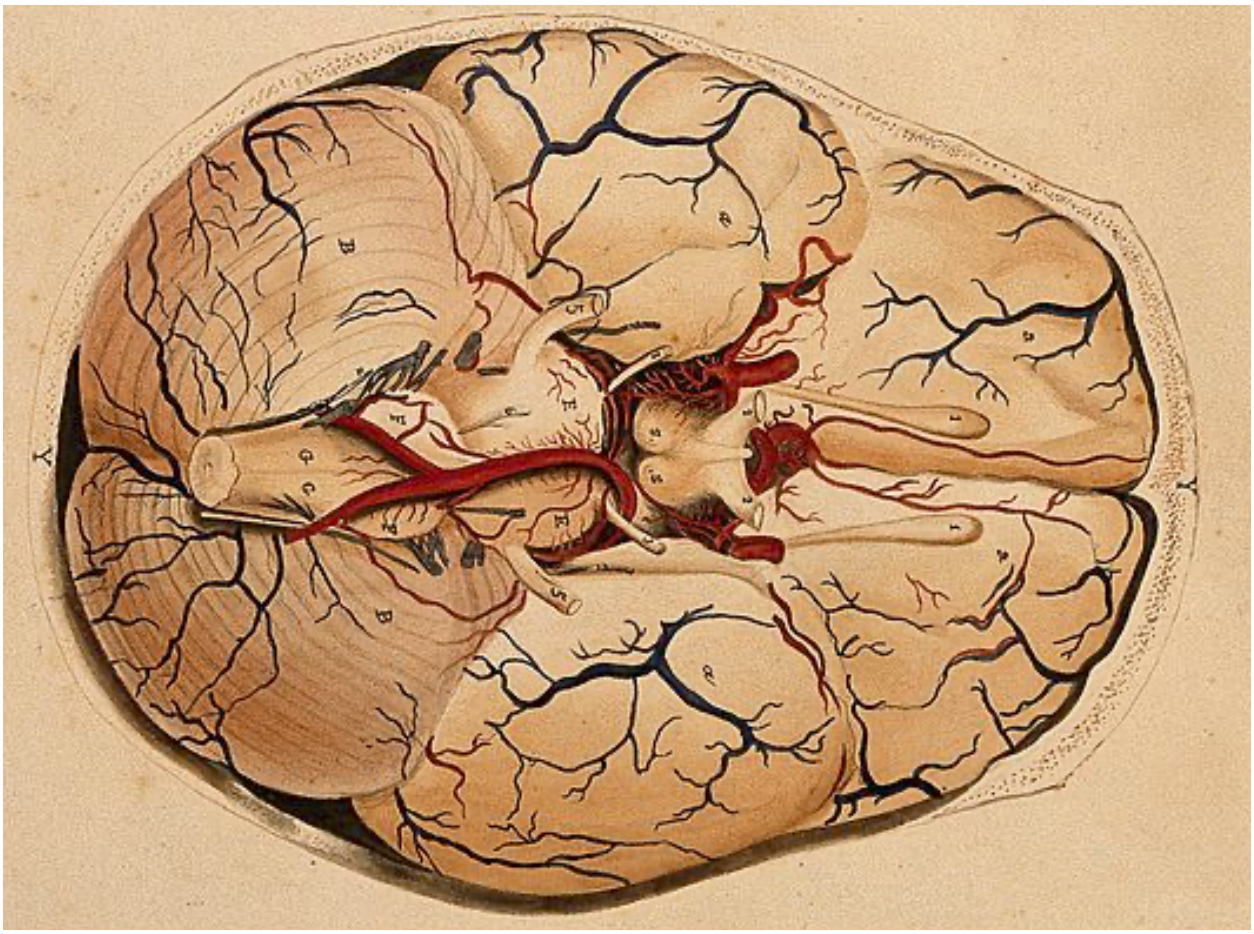

"Dissection Showing the Base of the Brain", artist unknown. Courtesy Wellcome Collection.

Neuro-Immune Crosstalk: The Relationship Between Adrenergic Stimulation and Macrophages in Developing Upstream Risk Factors for Cardiovascular Disease

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The development of cardiovascular disease is largely attributed to upstream endothelial dysfunction in the vasculature. While the exact mechanisms at the cellular level are not fully understood and always evolving, recent research has shown support for an interaction between macrophages and the sympathetic nervous system that amplifies endothelial damage in disease

states. Specifically, studies have focused on neuro-immune modulation in the context of obesity and hypertension. It appears that the innate immune system responds to adrenergic stimulation through various receptors and agonists, such as norepinephrine, to increase endothelial damage and further the risk for heart disease.

INTRODUCTION

Cardiovascular disease (CVD) currently stands as the leading cause of death in the United States. While there are a multitude of risk factors that leave individuals vulnerable to heart disease, obesity and hypertension (HTN) have proven to play significant roles. The effects of obesity and HTN at a cellular level leading to endothelial dysfunction may explain their role as risk factors for heart disease (1).

Endothelial dysfunction in obesity is correlated with an increase in macrophage proinflammatory expression (2,3). In an experiment where researchers indicated macrophages with F3/80+, they demonstrated that there is an increase in this cell type in areas dense with adipose tissue in obese mice compared to lean mice (4). Conversely, the role of adrenergic stimulation has also been associated with endothelial changes in obesity. Sympathetic stimulation in the vasculature has simply been shown to be higher at baseline for obese patients when compared to non-obese patients (5). Researchers have even shown that adrenergic stimulation may have differing consequences in the vasculature when comparing obese to non-obese patients. After administration of a β_2 adrenergic receptor (AR) agonist, one study shows that the augmentation index - a measure of vascular endothelial function - decreased significantly for only obese patients when compared to non-obese patients (6).

These trends are further mirrored in the pathogenesis of HTN. Using microneurography to measure efferent postganglionic muscle sympathetic nerve activity, Grassi et al. demonstrated that there is a significant and proportional climb in peripheral nerve activity when comparing normotensive individuals to individuals with severe essential HTN (7). Furthermore, studies with mice show a significant increase in the number of macrophages within the vasculature of hypertensive mice with left ventricular hypertrophy when compared to healthy mice (8). Similar results have been found by other researchers (9), and this concept was also recently reviewed by Drummond et al (10). Thus, it is well documented that the innate and sympathetic nervous system (SNS) independently contribute to changes in human vasculature.

What we aim to investigate in this review is how macrophages from the innate immune system are interacting with the SNS within the vasculature to affect the structure of the endothelial cells and contribute to the pathogenesis of upstream diseases that increase the risk for developing CVD. Macrophages populate arterial walls and recent studies have shown that immune cells share anatomical localization with peripheral neurons in the vasculature, hinting towards a potential neuro-immune interaction (11, 12). Certain macrophages have been found to express both α and β ARs, and are able to respond to various concentrations of norepinephrine (NE) (13). Many studies we will cite in this review suggest that there is a physiological connection between NE and macrophages that we are failing to consider when looking at endothelial homeostasis. While the exact mechanisms remain unclear, there is strong support for neuro-immunomodulation that may play an important role in various disease states. It is important to analyze these two systems within the vasculature and find roles in which they may communicate so that we gain a

better understanding of the influence they have on one another in the development of heart disease. We will begin by introducing molecular findings on the interactions between macrophages and adrenergic stimulation and then discuss these effects on the development of obesity and HTN.

DRIVING VASCULAR CHANGES

Macrophages

Macrophages and the SNS are both known to interact with vasculature (14, 15). Independently, macrophages play a key role in driving vascular dysfunction (14). In the vessel wall, there are resident macrophages that populate the vascular layers that arise in the vessels shortly after birth as well as originate from bone marrow derived monocytes (11). Macrophage differentiation, proliferation, and survival in the vessel is regulated by macrophage colony stimulating factor (m-CSF), and when m-CSF was depleted in deoxycorticosterone acetate (DOCA)-salt hypertensive mice, there was an associated reduction in vascular remodeling, endothelial dysfunction, NADPH oxidase (NOX) activation, and vascular inflammation in the mesenteric artery (16). This reduction in vascular inflammation and NOX activation is expected, as macrophages are recruited to the vasculature during inflammation and express NOX to produce superoxide, a reactive oxidative species which helps drive endothelial dysfunction in HTN (17). In a study by Nuki et al., blood flow was augmented in mice by ligating the left common carotid artery which increased the luminal diameter of the right common carotid artery. In this augmented group, they found an increase in the number of macrophages in the vessel wall. When they depleted macrophages, there was no alteration in the luminal diameter and a reduction in vascular remodeling through matrix metalloproteinases (MMPs) (14). Extra-cellular matrix metabolism is regulated by MMPs and their respective inhibitors, tissue inhibitors of metalloproteinases (TIMPs). Galis et al. showed that human atherosclerotic plaques had an increased amount of immunoreactive macrophages and activated MMPs and decreased TIMPs compared to normal vessel walls (18). Other studies have shown a similar imbalance of circulating MMPs and TIMPs in patients with premature coronary atherosclerosis (19), as well as a protective effect against atherosclerosis with the loss of MMP-9 expression (20), implicating an increased level of active MMPs and decreased TIMPs in the development of atherosclerotic disease. There is also strong evidence that macrophages do decrease luminal diameter in obesity by reducing the levels of gas transmitters in the vessels (21).

Sympathetic Nervous System

Independently, the SNS also plays a key role in driving endothelial dysfunction. It produces the catecholamine NE, which can bind α_1 ARs in smooth muscle cells located in the vasculature, causing vasoconstriction (22). Vascular endothelial cells have also been found to express various subtypes of α_1 ARs, regulating vasoconstriction or dilation (15). In inflammatory or diseased states, there is an increased activation of the SNS, leading to higher amounts of NE in the plasma. Under this increased activation of perivascular nerves, structural changes occur in endothelial cells along the arterial wall (23).

Anatomical Location

Given their individual interactions, it is likely that macrophages and the SNS together play a role in the upstream contributions to CVD. However, the mechanisms are complex and widespread. When plasma NE is elevated, CD14+ monocytes demonstrate increased adhesion to endothelial cells, identifying one of the initial steps in how SNS and

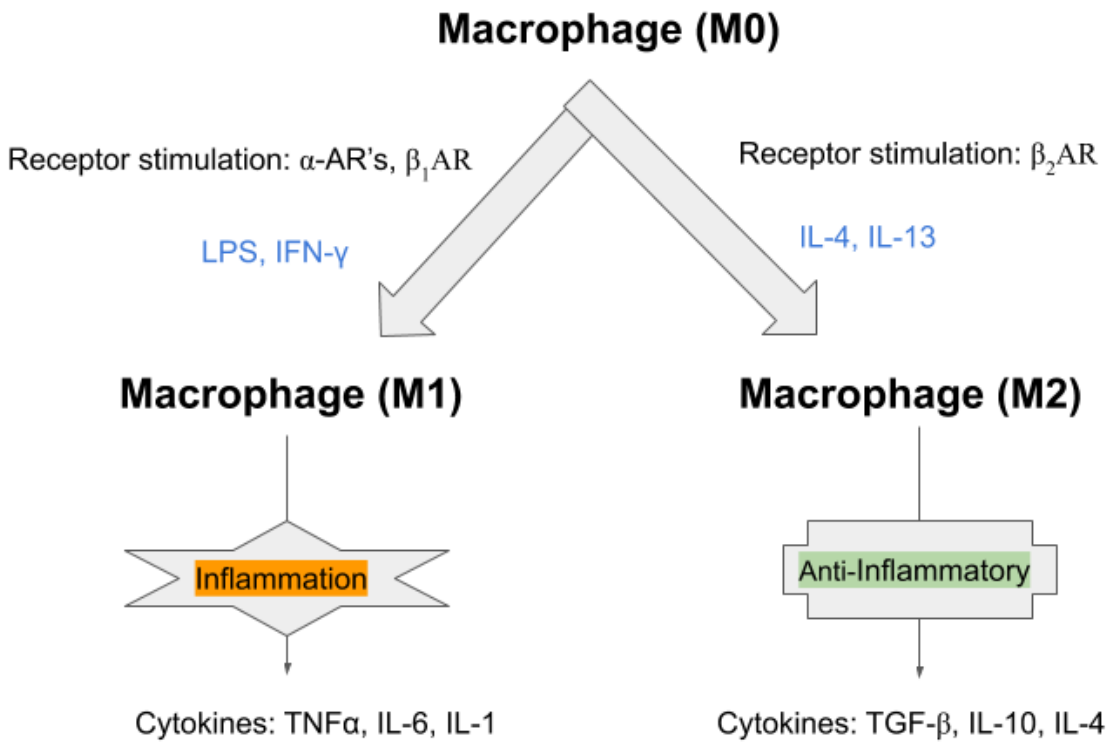

Figure 1: ***Adrenergic regulation of immune profile in macrophages****. Macrophages are known to express M0 (non-activated), M1 (pro-inflammatory), or M2 (anti-inflammatory) phenotypes; the latter two contribute different cytokines to their environment. Cytokines such as TNFα, IL-6, and IL-1 are released from M1 macrophages and promote inflammation and damage in the vasculature and tissues. Cytokines such as transforming growth factor β (TGFβ), IL-10, and IL-4 are released from M2 macrophages and act to suppress the inflammatory response and promote healing. There are different environmental factors that help macrophages polarize towards either an M1 or M2 identity, but one recently investigated driver is stimulation of their ARs. While it has been defined for a long time now that LPS and interferon γ (IFN-γ) promote the M1 phenotype and IL-4 and IL-13 promote the M2 phenotype, studies are beginning to support the notion that stimulation through the α_1, α_2, and β_1 ARs on macrophages promotes the M1 phenotype, while stimulation of the β_2 AR promotes the M2 phenotype.*

macrophage crosstalk contributes to endothelial dysfunction leading to CVD (17). Many immune cells are known to share anatomical localization with peripheral neurons, indicating local neuro-immune interactions may have an effect on tissue homeostasis and inflammation (12). For example, certain studies show that NE released in nerve terminals contains chemoattractant properties that help guide macrophages and monocytes towards them (24).

This local neuro-immune interaction is well defined in the gut. The gastrointestinal (GI) tract is innervated by the enteric nervous system (ENS), a division of the autonomic nervous system that helps regulate the function of the GI tract. Macrophages located in the muscularis mucosa were found to preferentially express β_2 ARs and reside near the myenteric plexus. Upon stimulation of the β_2 AR with NE, these macrophages upregulated anti-inflammatory genes and became tissue protective (**Figure 1**) (25). The ENS and macrophage have a reciprocal relationship to maintain survival. Macrophages in the muscularis externa release bone morphogenic protein-2, a protein which acts on neurons in the gut to maintain peristalsis, and the myenteric plexus

release m-CSF, a growth colony stimulating factor contributing to the survival of macrophages (26). We speculate that a similar relationship could be occurring in the vasculature, however it may lead to endothelial dysfunction instead.

CROSSTALK ON OBESITY

Obesity is a disease that is characterized by an excess amount of adipose tissue which leads to a higher amount of pro-inflammatory gene expression and a reduced expression of anti-inflammatory genes (27). In this state of inflammation, patients are at increased risk of developing complications, such as CVD. The macrophage plays a large role in the development of inflammation in obesity. In areas dense with adipose tissue there is an increase in pro-inflammatory macrophages, which were a significant source of tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and inducible nitric oxide synthase, all of which are pro-inflammatory cytokines (4).

SNS overactivity is also implicated in obesity and a known driver of obesity induced HTN. In normotensive obese individuals there is marked increase in sympathetic nerve firing and this increased sympathetic output increases blood pressure and cardiac output (5). As we can see, both sympathetic overactivation and macrophages are playing a role in facilitating downstream effects in obesity, but is there an interaction between macrophages and the SNS, furthering the dysfunction?

Sympathetic neuron-associated macrophages (SAMs) in adipose tissue are known to be upregulated during obesity and can directly alter adipocyte access to NE (28). They uptake NE through the solute carrier family 6 member 2 (SLC6A2) transporter protein and possess the ability to metabolize NE through monoamine oxidase A (MAO-A). When mice were treated with SLC6A2 ablation, they demonstrated an anti-obesity effect as there was now more NE in the adipocytes, leading to increased lipolysis and weight loss through thermogenesis (27). Furthermore, it was found that MAO-A expression is regulated by the NOD-like receptor protein 3 (NLRP3) inflammasome, and when this is inhibited, there is improved lipolysis due to increased NE availability (28). When the SNS is stimulated with optogenetics to upregulate NE uptake by SAMs, these SAMs increase expression of TNFα and IL-1α, cytokines that contribute to proinflammatory state and overall endothelial dysfunction, linking to the development of CVD (27).

Obesity can be induced in mice through a high fat diet Dahl salt-sensitive (HFD Dahl SS) method, and it also serves as an excellent model in studying HTN in obese mice. In a recent study by Mui et al., the researchers looked to see if dysfunction exists in the interaction between the mesenteric arteries and the α_2 ARs on the superior mesenteric and celiac ganglia (SMCG) of the SNS in driving HTN in this model. They found that systolic blood pressure was raised and there was an increase in macrophage accumulation in the mesenteric artery. However, there was a decrease in monocyte chemoattractant protein-1 and TNFα compared to normal fat diet mice, both proinflammatory cytokines. Unlike the DOCA-salt hypertensive model, prejunctional α_2 AR dysfunction was not detected in SMCG neurons and is not a likely contributor to obesity related HTN in this model. The authors point out that although this dysfunction was not seen in the mesenteric vascular bed, it is possible the α_2 AR dysfunction may occur in other organs important in blood pressure regulation such as the kidneys (29). Another study using HFD Dahl SS mice reports that in males, but not females, development of HTN may be driven by a transient and mild increase in neurovascular transmission driving vasoconstriction in mesenteric arteries, but it

is not likely implicated in maintenance of HTN (30). These studies show that the pathogenesis of obesity driven HTN is multifactorial and that alterations in the vascular sympathetic neurotransmission and macrophages are not entirely responsible for the development of this complication.

Leptin seems to be a key intermediate in propagating the connection between pro-inflammatory macrophages and the SNS. Adipocytes secrete the adipokine leptin, which is a hormone increased in obesity that plays numerous roles in the disease. When adipose tissue macrophages were treated with leptin, they paradoxically expressed the M2 surface markers (IL-4r) but were able to secrete proinflammatory cytokines such as TNFα, IL-6, and IL-1β (31). Hyperleptinemia is also a potent stimulator of the SNS. Carlyle et al. studied rats with increased leptin and recorded an overall increase in mean arterial pressure (MAP) and heart rate over seven days. When treated with an α AR antagonist, chronic leptin infusion did not cause an SNS-induced increase in MAP (32).

CROSSTALK ON HYPERTENSION RELATED TO DEVELOPING CARDIOVASCULAR DISEASE

HTN increases the risk for CVD. It has long been understood that overactivation of the SNS and the response of baroreceptors plays a significant role in this development (7), but it is also well studied that the innate immune response through the function of macrophages contributes to vessel wall thickening and hypertensive heart disease (8). Recent research has focused more on the interaction between these two drivers of HTN and atherosclerosis in the development heart disease. In a study focusing on IL-6 messenger RNA (mRNA) expression in cell cultures, a team of researchers found a time and concentration dependent rise in IL-6 from U937 resident macrophages after administration of NE. They attributed the increased production of IL-6 to an interaction between NE and macrophages involving the β adrenoreceptor-reactive oxygen species-NF-kB signal pathway (33).

IL-6 may be a strong link between the effects of adrenergic stimulation and macrophages on promoting vascular inflammation leading to heart disease. A three-year prospective case control study published in the New England Journal of Medicine looked at various inflammatory markers as a predictor of CVD in post-menopausal women with no reported underlying health conditions. They found elevated levels of IL-6 in the plasma to be strongly correlated with the risk of future cardiovascular events in this population (34). This is further supported by recent research that discovered phagocytic cells from the innate immune system, such as macrophages, synthesize and release catecholamines under inflammatory conditions. When macrophages face insult such as with lipopolysaccharide (LPS), they release NE to act in an autocrine fashion in order to promote the release of cytokines such as IL-1β and TNFα (13). These cytokines play crucial roles in vascular remodeling during inflammatory states, promoting smooth muscle migration and leading to increased likelihood of CVD secondary to HTN (**Figure 2**).

Macrophages and adrenergic receptors interact through free radicals, and this has been well studied in the mesenteric arteries in DOCA-salt hypertensive rats. The mesenteric arteries are major resistance arteries and large contributors to the development of HTN in this model. These arteries are innervated by the superior mesenteric and celiac ganglia of the SNS. Under normal conditions, the prejunctional α_2 AR inhibits NE release from sympathetic nerves through G_i proteins which inhibit voltage gated N-type Ca^{2+} channels that control NE release. This negative feedback

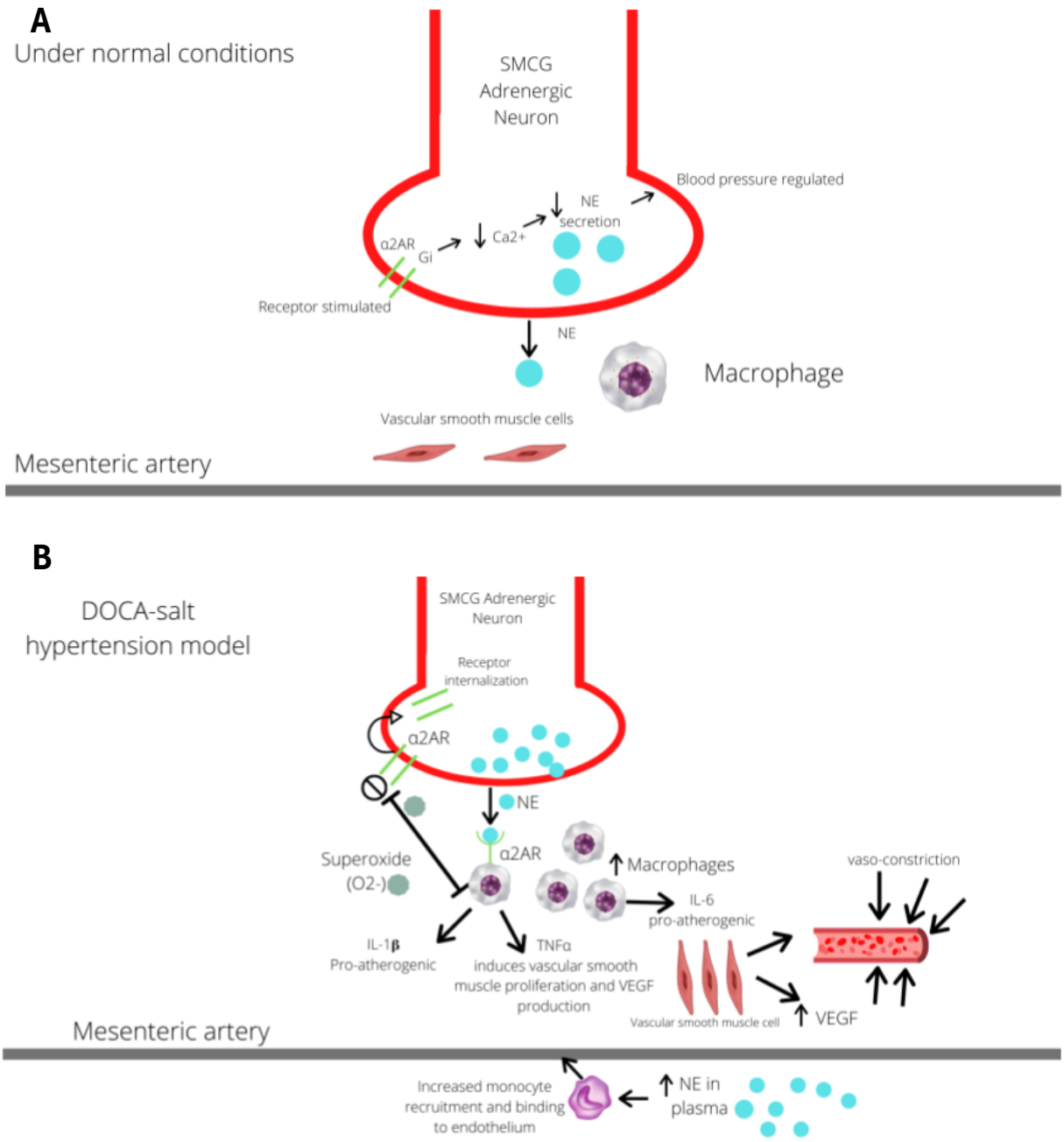

Figure 2*: **SMCG of the SNS located near the mesenteric artery**. **A)** Under normal conditions, the prejunctional α_2 AR inhibits NE release through G_i proteins which inhibit voltage gated N-type Ca^{2+} channels that control NE release, this negative feedback allows regulation of sympathetic tone and increases in blood pressure (28). **B)** In DOCA-salt hypertensive mice, macrophages invade the synapse and can produce superoxide, which in turn cause prejunctional internalization of the α_2 AR. When the α_2 AR is internalized, this inhibits the regulation of NE production and secretion, so now NE is secreted unopposed, worsening the inflammation and vasoactive effects on the mesenteric artery. When macrophages are stimulated by α_2 AR, they produce pro-inflammatory cytokines IL-1β, TNFα, and IL-6 which go on to further exacerbate the inflammatory effects and vascular remodeling. This vascular remodeling is the initial step in the development of CVD.*

allows for regulation of sympathetic tone and increase in blood pressure (29). A study conducted by Thang et al. found that macrophage NOX produces superoxide which interacts with the α_2 ARs in the vasculature to raise blood pressure. Analyzing the mesenteric artery in DOCA-salt hypertensive rats, macrophages recruited to the vasculature led to an increase in levels of superoxide in the vascular adventitia compared to rats who were not treated with mineralocorticoid-salt excess, and thus had no significant increase in their SNS activation. Superoxide acts to impair prejunctional α_2 AR receptors by causing receptor internalization, which leads to an increase in production of NE due to disruption in feedback inhibition (**Figure 2**) (29). Therefore, macrophages that were recruited because of HTN in the DOCA-salt rats were ultimately leading to greater sympathetic activation via decreased inhibition of NE release (35). This becomes a positive feedback loop as studies also show that NE increases superoxide production through stimulation of α_2 AR on peripheral blood monocytes (17). This was measured with p22phox mRNA expression and stimulated a similar physiological response as when macrophages contact a pro-inflammatory marker such as LPS. However, peripheral blood monocytes were not able to be isolated and instead the CD14 marker was obtained as an indicator of macrophages. In addition to raising blood pressure, these interactions ultimately lead to greater vascular remodeling and further increase the risk of CVD (16).

It is important to mention that in other studies with similar models, they found that adenosine is produced by the perivascular SNS in the mesenteric arteries and can bind to the adenosine 1 receptor in the periarterial nerves. This disrupts NE regulation, resulting in a greater increase and providing an alternative pathway to explain the rise of NE levels (36).

CONCLUSIONS

Potential Therapeutic Strategies

As we move forward, we must not only consider the independent interactions of macrophages and SNS in driving endothelial dysfunction, but also how their intimate interaction is contributing to a pathologic state. The general trend we have seen is that the SNS interacting with macrophages through physiological NE stimulation has supported a pro-inflammatory response leading to endothelial damage and increased likelihood of CVD (**Figures 1, 2**). We have also seen macrophages interacting directly with sympathetic neurons in the mesenteric artery, furthering the development of hypertensive disease (29). However, the pro-inflammatory profile of circulating monocytes and macrophages seems to be altered under β_2 AR stimulation. In a study done by Galvez et al., high fat diet mice were used as a model of obesity and compared to standard diet in control lean mice. As expected, the circulating monocytes in the obese group expressed inflammatory cytokines (TNFα, IL-1, IL-6). When stimulated with β_2 AR agonist terbutaline, the pro-inflammatory monocytes in obese mice shifted to an anti-inflammatory gene expression, with an increase in anti-inflammatory cytokines (IL-10, IL-4, and IL-5) (37). Another laboratory found results supporting these findings after treating Zucker diabetic fatty rats with a β_2 AR agonist for 12 weeks. They concluded that this mediated the inhibition of inflammatory cytokine production and lowered monocyte activation, speculating that the β_2 AR agonists may have protective effects against diabetic cardiovascular complications (38). These revelations provide therapeutic thought to work upstream and prevent the development and progression of CVD in obese individuals.

Limitations and Future Directions

We still face many limitations and topics that need to be further studied. As previously noted, the mechanisms of neuro-immune crosstalk in developing and maintaining HTN in high-fat diet mice is different from the DOCA-salt model at the mesenteric arteries (29). This notion is important as it furthers the evidence that the molecular mechanisms contributing to the pathogenesis of HTN and CVD is quite complex, and there is still a lot of work to be done under the topic of neuro-immune interaction.

Furthermore, the SNS is multi-level depending on the initial stimulus and power of inflammation. The effects of the SNS with macrophages in perivascular adipose tissue can vary depending on the initial driver of stimulation, making potential pharmacological treatment complicated. For instance, studies have shown that a healthy increase in the SNS, such as exercise, can promote the M2 phenotype for macrophages. Alternatively, a pathological increase, such as a high fat diet, can promote the M1 phenotype for macrophages (39). Thus, the neuro-immune crosstalk may differ for individuals depending on comorbidities and the effects on vascular disease may vary not only by the quality of their interaction, but also with proper timing.

We have highlighted various neuro-immune interactions that may contribute to upstream risk factors for CVD, but there are still more factors that need to be studied. For example, recent studies hint at the possibility of hydrogen sulfide acting as a liaison between macrophages and the SNS in vascular homeostasis and atherosclerosis (21, 40). Ultimately, with greater research in this subject we may be able to target and treat the upstream vascular pathology that aids in the development of CVD more effectively.

DISCLOSURES

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"The Organ Rehearsal", by Henry Lerolle. Courtesy Metropolitan Museum of Art, New York.

History, Recent Advances, and Ethical Controversies of Solid Organ Xenotransplantation: Review and Implications for Future Clinical Trials

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The recent transplantation of a genetically modified pig heart into a human, performed at the University of Maryland in early 2022, indicates that xenotransplantation research is at a key juncture. As a successful alternative to allotransplantation, xenotransplantation would offer an immediate solution to the current organ shortage. The Maryland transplantation is a culmination of approximately six decades of research, beginning with the earliest xenotransplantation attempts in the 1960s, concurrent with the first allotransplants. In recent studies, porcine renal and cardiac xenografts have been maintained in primate models for months to years. However, the possible initiation of xenotransplantation clinical trials involves multiple ethical quandaries, especially regarding the risk of infectious disease and the selection of patients for future clinical trials. This review traces the early history of xenotransplantation to the current state of the field and explores the myriad of associated ethical questions.

INTRODUCTION

In the United States, 17 people on the waiting list for an organ transplant die each day (1). In 2021, the number of kidney transplants performed (24,670) was approximately 27% of the total number of people on the waiting list (90,483) (1). As of 2020, 17.6% of people on the heart transplant list and 31.1% of

people on the kidney transplant list had been waiting for three years or more (2, 3). Therefore, there is a significant shortage of organs available for transplantation.

Xenotransplantation has been suggested to be the most feasible and promising answer to the organ shortage (4-6). Instead of relying on human donors, genetically engineered pigs can theoretically provide an unlimited source of organs, greatly reducing or even solving the organ shortage. It would also eliminate the morbidity and mortality associated with long stays on transplant waiting lists and decrease healthcare costs associated with sustaining patients waiting for transplant (6). However, xenotransplantation is still a technology in development, with great aspirations, but minimal clinical success thus far. Recent advances suggest that xenotransplantation is poised to make the jump into clinical trials, but controversies about the logistics and ethics of such trials abound. This review will survey the history, recent advances, and ethical controversies surrounding xenotransplantation, with a focus on cardiac and renal xenotransplantation.

HISTORY OF XENOTRANSPLANTS

Early xenotransplantation was intertwined with the initial development of allotransplantation. In the 1960s, when renal allotransplantation was in its infancy, Keith Reemstma and Thomas Starzl both attempted renal xenotransplantation using chimpanzee and baboon, respectively, as donors (7, 8). These transplants were largely unsuccessful, with most patients dying within days of the transplant. The limited methods of immunosuppression available – azathioprine, prednisone, actinomycin C, and local irradiation – were insufficient in some patients to prevent rejection and caused severe infections in others (7, 8). In 1964, three years before the first modern human heart allotransplant, James Hardy attempted the xenotransplantation of a chimpanzee heart, which survived for less than an hour[9]. In 1985, xenotransplantation came to increased public attention with the transplant of a baboon heart into neonate with hypoplastic left heart syndrome, "Baby Fae". Treated with cyclosporine, she survived for 20 days post-transplant, dying due to graft necrosis as well as lung and kidney failure (10).

By the 1990s, xenotransplantation research mainly focused on pigs as the optimal donors, because of their easy availability for breeding and reasonably concordant size and physiology (11, 12). While xenotransplants sourced from non-human primates posed a lower immunological risk of rejection, practicality concerns about breeding primates in large numbers, potentially discordant organ size, and public acceptance of breeding primates to harvest organs prevented their use (11, 12). Pigs were easily bred and appropriately sized alternatives that were already farmed in large numbers for human use.

When initially attempted, early pig xenotransplants in the 1990s led to immediate hyperacute rejection as a reaction to xenoantigens present on porcine cells (12). Around this time, Uri Galili discovered the α-galactosyl epitope (α-gal), which was determined to be the main xenoantigen responsible for hyperacute rejection (13, 14). Humans, apes, and Old World monkeys do not produce α-gal, while New World monkeys and non-primate mammals do (13). Approximately 1% of human B cells produce antibodies against α-gal (anti-Gal) and the IgG anti-Gal titer increases 100-fold in the two weeks following exposure to a xenograft (13). This strong immunological response made controlling hyperacute rejection by immunosuppression very difficult, and thus attention turned to the genetic modification of pig donors to minimize the issue. The first α-1,3-galactosyltransferase homozygous

knockout pigs (GTKO) were developed in 2003 using nuclear transfer cloning technology (15, 16). Early experiments using GTKO pigs as donors for xenotransplantation in a primate model showed significantly improved success, with xenografts surviving for a median time of 78 days (17). In comparison, xenografts from pigs engineered to express low levels of α-gal were universally rejected within 20 minutes (17). The development of GTKO pigs was a major advancement and ushered in the modern era of xenotransplantation research.

RECENT ADVANCES

Animal Models

In the two decades since the development of GTKO pigs, modern xenotransplantation research has focused on further genetic modification of porcine donors, as well as optimizing the immunosuppression regime necessary to maintain graft survival in primate models. The use of CRISPR/Cas9 allows researchers to insert a large number of modifications into the genome with much greater speed and precision (18). This has facilitated the proliferation of multiple genetic modifications tested in animal models of xenotransplantation. The wide variety of genetic modifications attempted has been reviewed elsewhere (19). In brief, while GTKO pigs greatly improved the risk of hyperacute rejection, complement activation and dysregulation of the coagulation cascade still impaired graft survival, even in the absence of antibody binding (19-22). Common genetic modifications to address these issues include the insertion of human complement regulatory transgenes, such as CD46 or CD55, and human coagulation regulatory transgenes, such as thrombomodulin (19-23). Two additional xenoantigens have been discovered to also play an important role in the immunological reaction to xenografts: N-glycolylneuraminic acid and the Sda blood group antigen (19).

Genetic modification of donor animals is only one aspect of efforts to sustain a xenograft. While the eventual goal would be sufficient genetic modification to eliminate the need for immunosuppression post-transplant, currently significant immunosuppression is necessary. Recent experiments in animal models have employed a combination of conventional immunosuppressants used in allotransplants, including anti-thymocyte globulin, rapamycin, corticosteroids, mycophenolate mofetil, and anti-CD20 antibody (21, 24-29). The addition of a costimulation blockade via anti-CD40 or anti-CD154 antibodies significantly improves graft survival along with the conventional regimen (21, 24-29).

These innovations have allowed for the prolonged survival of xenografts in primate models. Kim et al. regularly sustained renal xenografts for a year, with several surviving for up to 400 days using monoclonal antibody depletion of CD4+ T cells (30). In other experiments, renal xenografts repeatedly lasted over 120 days, with the longest survival times of 7, 8, and 10 months (32-34). Heterotopic cardiac xenografts have a median survival of 298 days, with the longest survival being 945 days (29). Orthotopic cardiac xenografts, which are a more challenging model to sustain, have repeatedly lasted up to three months, with the longest survival of 195 days (23). These results demonstrate that research has begun to reach the standards for clinical trial initiation set by the Xenotransplantation Advisory Committee of the International Society for Heart and Lung Transplantation (6).

Human Models

Given these promising results in animal models, initial attempts have been made at solid organ xenotransplantation in humans. In three instances, porcine kidneys were

transplanted into human recipients who were declared brain-dead and who were ineligible to serve as organ donors (31, 32). The model of a brain-dead human recipient is limited, because the environment created by brain death may affect xenograft function, and the nature of the experiment prevents long-term follow-up (31, 32). In particular, these experiments were criticized because of their time-limited nature and the difficulty of interpreting physiologic parameters post-transplant because the recipients' kidneys were not removed[33]. However, this work can still provide valuable initial data as a stepping stone to a clinical trial, without many of the risks and ethical quandaries of a clinical trial. In all three instances, the kidneys remained viable and produced urine throughout the 54- or 72-hour follow-up period, with no evidence of hyperacute rejection or antibody mediated injury (31, 32).

In early 2022, researchers at the University of Maryland performed the first transplant of a genetically modified pig heart into a living human with the possibility of recovery (33, 34). While the UMD team was denied authorization for a full clinical trial of cardiac xenotransplantation, the Food and Drug Administration granted an authorization for compassionate use in the case of a 57-year-old man who was ineligible for mechanical support devices or an allotransplant and had been dependent on venoarterial extracorporeal membrane oxygenation (ECMO) for two months (33, 34). The transplanted pig heart had 10 genetic modifications: knockout of the three main pig xenoantigens and 6 modifications to minimize the immune response. The patient received B- and T-cell depleting therapies, anti-CD40, and additional immune-suppressive therapies (34).

The xenograft showed normal cardiac function, and the patient demonstrated clinical improvement in the first seven weeks post-transplant. At the seven-week mark, the patient started to deteriorate significantly, and the graft showed diastolic failure and myocardial thickening, although the systolic function was preserved (34). Supportive care was withdrawn 60 days after transplantation. Throughout this process, no evidence of acute cellular or antibody-mediated graft rejection was observed. The mechanism for the pathologic changes observed in the graft are unexplained at this time. This is additionally complicated by the detection of porcine cytomegalovirus and human herpesvirus 6 in the patient's later tests, although the donor animal initially screened negative for cytomegalovirus (34). Overall, the patient's initial progress and recovery, as well as the life-sustaining nature of the porcine graft are promising results for the field of xenotransplantation. However, this experience also emphasizes that there are still important gaps in the knowledge about xenotransplantation.

ETHICAL CONTROVERSIES

Xenotransplantation sparks a multitude of ethical questions, including but not limited to the appropriate use of animals, acceptability from a religious perspective, the utility of investing in xenotransplant, the infectious disease risk, and the design of an eventual clinical trial. The choice of pigs as the source animals for xenografts effectively minimizes concerns regarding animal use and animal rights. Pigs are farmed by millions as a food source and are already used in medical settings as sources of heart valves and insulin (35, 36). Individuals or communities may object to the use of pigs in this manner. As this is not a widely held belief, it does not constitute a sufficiently strong objection to impede further progress in xeno-transplantation research (35, 36). Regarding religion, Christian, Jewish, and Muslim theologians have written about the acceptability of xenotransplant (37-40). While teachings of Judaism and Islam

prevent the consumption of pork, theologians have deemed porcine xenotransplant acceptable given the primacy of preserving human life in both religions (37-40). However, this does not exclude the possibility that individuals may decide against a xenotransplant on these grounds. Thus, in both the case of animal use and the question of religious acceptability, overarching systemic beliefs support xenotransplant. However, individual beliefs about these topics may affect the choices made by future patients regarding whether to accept a xenotransplant.

The significant resource investment necessary to develop any new technology such as xenotransplantation should be examined carefully to ensure its worth. The dedication of resources towards xenotransplant research compared to prevention, nonsurgical treatments, or other emerging technologies for organ replacement is a decision to be made by governments individually, based on societal and cultural beliefs and standards. However, as described earlier in this review, there is a clear and pressing need to address the shortage of organs, and xenotransplantation is one of the technologies closest to clinical application that could remedy this issue. In the ideal future, transplant surgery would be nearly obsolete, because prevention measures and medical treatments will have advanced to the point that very few patients end up in organ failure. However, this utopia is likely to be unobtainable for decades, if ever. Recent trends, such as the 243% increase in patients on transplant waiting lists from 1991 to 2001, suggest that the pressing organ shortage is more likely to worsen than improve (4). In addition, improvements in medical care that improve lifespan may also increase the need for organ transplants to address age-related decline in organ function (18). There may also always be cases that require transplants, such as congenital organ defects. The potential benefit of xenotransplantation in providing an unlimited source of organs in these cases should not be overlooked (41).

Infectious disease risk and the design of future xenotransplant clinical trials are more complicated ethical questions. In a world still reeling from the COVID-19 pandemic, the risk of spreading new zoonotic infections via xenotransplantation should not be underestimated. Potential culprits include porcine cytomegalovirus, porcine endogenous retroviruses (PERVs), and other porcine microbes. Much of the concern around zoonotic transmission centers on PERVs, because the risk of other infections can be minimized, but not eliminated, by raising donor animals in specific pathogen free environments, repeated testing, and other infection control measures (35, 42, 43). In addition, there are concerns that PERVs, like other retroviruses, could cause malignancies or immunodeficiency when introduced to human hosts. However, the evidence thus far in primate models as well as the monitoring of humans exposed to pig tissues suggests that the risk of PERV transmission is extremely low (44-47). This evidence does not entirely ameliorate concerns, as it is possible that immunosuppressed conditions of solid organ xenotransplantation in humans could increase the likelihood of PERV transmission and replication. One possibility is to use animals in which PERVs were inactivated in the genotype (48). However, it is not clear whether investment in developing these animals with the necessary genetic modifications is worthwhile given the seemingly low risk. There is also the additional concern of introducing genomic instability by inactivating all PERVs in the genotype, given that there are approximately 25 copies of PERV in genomic DNA (48).

Each government needs to make its own determination about the severity of the infection risk inherent in xenotransplantation. However, given the large stake that society

has in preventing new zoonotic infections, evaluation of this issue warrants special care. Citizen panels and public discussion after education on the topic should be considered so that a wide variety of opinions are weighed and to ensure that this decision is not made in an ivory tower (49). In addition, the COVID-19 pandemic has clearly demonstrated the inherent global interest in preventing the spread of new zoonotic infections. As such, even though it is reasonable that different nations may have varying levels of risk tolerance regarding the infection risk, it is critical that all nations pursuing xenotransplantation research do so while following accepted guidelines for minimizing infection risk. International bodies such as the World Health Organization can help encourage adherence to such practices, even if there is no way to mandate it.

One practice that has been proposed to minimize infection risk is to require that all participants in a future xenotransplant clinical trial be closely evaluated for infection for the rest of their lives (50, 51). This is a troubling requirement from an ethical perspective, as it violates a fundamental right of clinical trial participants outlined in the *Declaration of Helsinki* – to withdraw from the trial at any time (49, 52). The necessity of lifelong surveillance has been challenged recently, but remains a consensus guideline for future clinical trials (53). Ultimately, it is reasonable that early xenotransplantation clinical trials begin with the intention of lifetime surveillance, a requirement that could then be reduced or increased depending on the new data collected. Building this lifetime surveillance into future clinical trials means that the *Declaration of Helsinki* cannot be applied to its fullest extent (52). This should be clarified to any potential participant as part of the informed consent process. In addition, this may preclude early trials of pediatric xenotransplantation. Even though neonatal heart xenotransplantation may be one of the most promising early applications of clinical xenotransplantation, committing pediatric patients to lifetime monitoring would be overly problematic from an ethical perspective (41, 54, 55).

Much has been written about the design of a potential xenotransplant clinical trial, specifically on the appropriate patient population (35, 41, 52, 54-56). The specific indications favored for trial inclusion vary, but the consensus is that initial trial participation should be limited to those who are highly unlikely to receive an allotransplant and who are both medically and psychosocially healthy enough to maximize the chances of a successful transplant (35, 41, 52, 54-56). However, it remains a challenging task to balance the need for xenotransplantation research done in humans with the risk of taking advantage of vulnerable and desperate patient populations, especially given that there is no guarantee that initial trials of xenotransplantation will have significant clinical success.

DISCUSSION

Xenotransplantation research appears to be at an important crossroads, as it teeters from preclinical models into clinical trials. Recent advances in preclinical models have demonstrated significant success, and the potential benefits of clinical xeno-transplantation are tremendous. However, transitioning into clinical trials is an especially difficult proposition, given that so much remains unknown about this technology. Recent attempts at xeno-transplantation in humans highlight that there are still major gaps in our knowledge. Fully addressing these gaps will require clinical trials. Additionally, a clinical trial, rather than additional case studies, would be better poised to address ethical concerns and produce generalizable data on the genetic

modifications and immunosuppression regimen necessary to sustain a xenograft. Therefore, it is reasonable to proceed with small early clinical trials in the near future, and reports from the Food and Drug Administration in July 2022 suggest that these trials may soon be on the horizon in the United States[58]. However, the regulation of these trials may require modification of existing standards. For example, adapting the standards for approval of genetic modifications may be necessary, as many of these transgenes have only been tested in combination, which makes determining the individual benefit of each construct difficult[59]. In addition, requirements for lifetime monitoring for infectious disease risk threaten long-held ethical standards, but a shift in these standards may be necessary to pursue the incredible benefit offered by xenotransplantation in the clinical world.

DISCLOSURES

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