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Table of Contents

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Letter From the Editor

It is with great honor that we welcome you to the inaugural issue of Georgetown Scientific Research Journal. Since the journal's founding in the fall of 2020, our mission has been to provide a professional, open-access platform for student researchers to publish their work in order to celebrate the scientific accomplishments of the Georgetown student body. We aim to provide a space for researchers to learn from each other, collaborate with one another, and reach a broader audience. Our semesterly issues, along with weekly student highlights, biweekly faculty highlights, and research-oriented events, strive to encourage members of the Georgetown community to pursue and explore research both in and outside of the classroom.

We would like to acknowledge that the COVID-19 pandemic has been an incredibly difficult challenge for people all over the world. Scientists and non-scientists alike have had to adjust to the virtual environment, overcome potential changes in employment status and housing, and cope with the tragic death of many of our loved ones. The dedication and perseverance of our authors and editors have been a true testament to their passion, character, and resilience. These authors have shown that even during this unprecedented global emergency, while physically isolated, we can come together and collaborate through science.

These articles underwent an extensive and rigorous double-blind editorial process, including edits from our student editorial board, our faculty advisory board, and Duke Vertices editors who served as our outside-of-institution review board. We hope that you find this issue full of research papers that pique your interest and broaden the breadth of your scientific knowledge. In this issue, you will find authors both from the undergraduate and graduate levels presenting research on various topics ranging from a discussion of the healing properties of performing arts to determinants of cancer. Please join us in commending the work and contributions of these authors.

Sincerely,

Danya A. Adams
Editor-in-Chief

Nesreen Shahrour
Executive Editor

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Biological and Physical Interactions at Local Ocean Scales: Coupled Systems

Victoria Boatwright, Baylor Fox Kemper, PhD.

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Biological and Physical Interactions at Local Ocean Scales: Coupled Systems

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Abstract

Physical and biogeochemical processes that influence primary production set Earth's carbon and heat budgets. While these processes have long been the focus of research, high resolution models to investigate local phenomena have only recently been developed, and two-way coupling between oceanic physics and biology is only recently getting attention due to computational power. With these new developments, it is possible to study the mechanisms through which these processes interact at both global and regional scales to shape Earth's climate, which is the goal of this paper. This paper introduces oceanic physical phenomena at submesoscales to global scales – like mixed layer depth and turbulent structures – and the relationship of smaller scale events with biological factors. It discusses the implications of these relationships for primary production. After an introductory explanation of turbulence, primarily in the form of eddies and fronts, and the effects of internal instability and surface forcing, this paper emphasizes the contributions of those phenomena (turbulence, internal instability, and surface forcing) to vertical velocities and the influence of vertical transport on biology. Next, it introduces biogeochemical feedbacks, concerning both large scale population dynamics and increased absorption of radiation at the submesoscale, to consider their impacts on physical dynamics and regional climates. Finally, the paper compiles equations of irradiance and variables of significance, suggesting terms that could produce meaningful responses to variations in phytoplankton populations. The paper highlights the importance of understanding physical-biogeochemical relationships and suggests directions for future research, particularly areas related to global warming or abrupt climate change.

Keywords: turbulence, primary production, phytoplankton, submesoscale

1. Introduction

Oceans occupy 70% of the Earth's surface, accounting for the sequestration of 48% of carbon emissions, and its surface dwellers are responsible for roughly half of the atmospheric oxygen[1]. Marine primary producers are an integral component of the global carbon cycle, oxygen production, and marine ecosystems. Considering how marine primary production (the base of the food chain, organisms that synthesize organic compounds from carbon dioxide) accounts for twice the amount of carbon fixation performed by the open ocean (90% of the ocean surface), it is important to understand the factors promoting the growth and abundance of

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marine producers[2]. The key marine primary producers are phytoplankton, which include a range of species with various characteristics, such as surface floaters, neutrally buoyant species, species dependent on iron for nitrogen fixing, and those that are N_2-fixing[3]. While physical phenomena in the ocean are known to provide nutrients, light, and heat to plankton populations, the relationship between these physical phenomena and the abundance of marine life is still uncertain for two reasons. First, many of the interactions that impact biological abundance and spatial variability occur on the mesoscale (roughly 100km or less) or even the submesoscale (often characterized as 0.1-10km scale), depths at which resolution is currently an insurmountable computational cost to resolve, especially in two-way coupling schemes[4]. Second, the field is just beginning to understand the coupled feedback mechanisms of biogeochemical influences on physics. Coupled schemes are used in computational models to resolve the complex interactions between boundaries or systems; for example, ocean-atmosphere coupling was one of the first cases of relating two previously independent systems through heat flows, wind stress, and surface exchanges of molecules like carbon and oxygen[5]. In this paper, we demonstrate coupling between ocean physics (e.g. turbulence) and marine biogeochemistry (e.g. phytoplankton populations), which can be one-way (physics impacting biology, the more common approach to ocean models today) or two-way (physics impacts biology and biology influences physics). In doing so, this paper seeks to explain various biogeochemical-biological-physical feedbacks and their spatial scales as well as determine what parameterizations (variables or equations that can represent complex, often small-scale or unresolvable, interactions) can add to global or regional models in understanding the carbon cycle and heat exchanges. The discussion section details the impacts of global climate change and warming on submesoscale and mesoscale phenomena, identifying gaps in knowledge and potential consequences.

2. Methods

This review compiles studies that measure mixed layer depths, phytoplankton concentrations, mesoscale to submesoscale phenomena, ocean temperatures, and other physical events. Observational data in this paper are derived from several methods, including long-term hydrographic time-series, satellite imaging showing ocean color and sea surface height, in-situ measurements by Argo floats or ship-based measurements, and ocean reanalysis combining historical and computational models with observations. Ocean color indicates levels of chlorophyll-a, which is a proxy measurement for phytoplankton concentrations. Sea surface height is a proxy for eddies in the ocean, as cyclonic eddies tend to decrease surface height while anticyclonic eddies increase surface height. Models are used to determine variables of importance and predict future outcomes. This paper uses models across scales: local scales though the Large Eddy Simulation (LES), regional scales though the General Ocean Turbulence Model (GOTM) and Regional Ocean Modelling System (ROMS), and global scales though the Community Earth System Model (CESM) and MIT's General Circulation Model (MITgcm).

3. Findings

All complex dynamics discussed subsequently depend first on the fluid dynamics of the world's oceans. The Earth is a sphere covered in fluids, rotating about an axis. The winds in the atmosphere circulate about the globe and respond to pressure differentials, adding a shearing force to the ocean surface, which is then forced along with the wind's direction. Tidal currents and water density fluxes can also influence the direction of ocean surface currents. Next, we add continents and islands, and

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therefore coastlines, to the Earth. Due to hydrostatic pressure, ocean fluids will need to deflect in different directions in three dimensions as they cannot pile up on themselves along the shorelines. Since the fluids of the Earth's oceans are in a rotating frame, the fictitious Coriolis force is present in the Earth inertial frame, where the Coriolis force will deflect masses according to the cross product in

$$f = -2m(\boldsymbol{\Omega} \, x \, \boldsymbol{v}), (1)$$

where $\boldsymbol{\Omega}$ is Earth's rotation vector and $\boldsymbol{v}$ is the velocity of the mass of interest, or, broadly, to the right in the Northern Hemisphere and to the left in the Southern Hemisphere. This configuration will lead to an overall picture of surface currents, which circulate into gyres in the oceans, or smaller circulations in gulfs and bays. At smaller scales, surface currents will tend to circulate clockwise in the Northern Hemisphere and counterclockwise in the Southern Hemisphere. Incorporating density, masses of water with characteristic temperature and salinity (or isopycnals) will tend to sink or float (cold and high density waters will sink, whereas warm and less dense waters will float). This causes thermohaline circulation, which consists of deep waters with high temperature and salinity fluxes over their depth, allowing the flow of bottom waters to be circulated back into the surface currents over timescales of roughly a thousand years[6]. These differences in density and temperature can cause stratification, where two masses of water with different characteristics form an interface at which they have limited exchange. As deeper, colder waters often carry more nutrients due to sinking, this stratification is especially important when considering marine organisms as their resources will depend on the upwelling of these colder, nutrient-rich waters.

With a background of the global currents, smaller scale phenomena add to the complexity of large scale circulations and ocean gyres. Mesoscale and submesoscale interactions create turbulence (the state of fluid motion that is chaotic and unsteady) in the form of eddies (smaller vortices), mixed layer restratification, fronts, and other instabilities[7-8]. Figure 1 shows a global model of simulated submesoscale interactions with a 2km resolution, revealing this turbulence across the globe.

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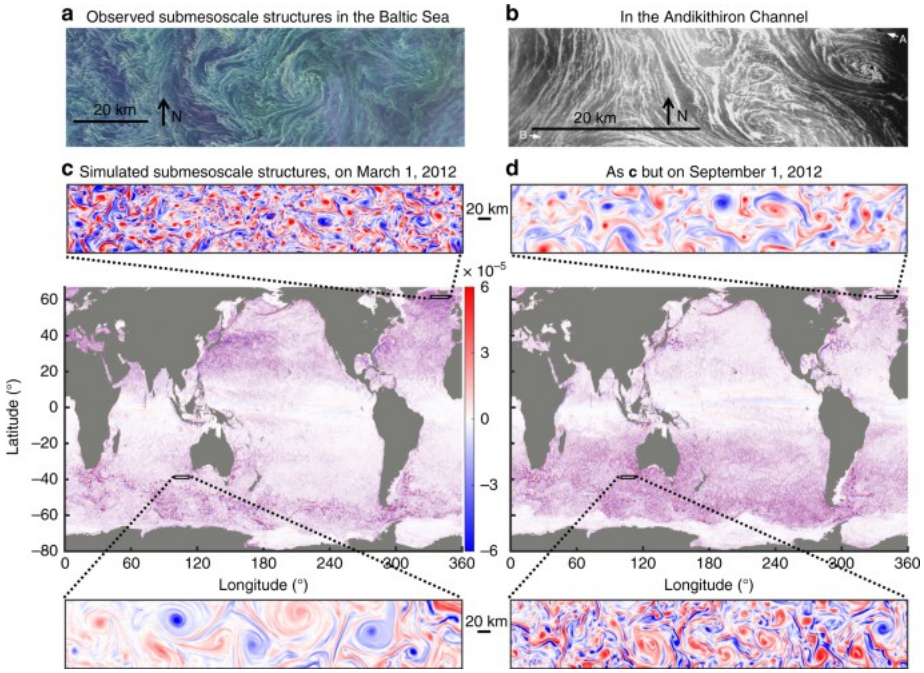

Figure 1. Visualization of global submesoscale phenomena and configuration, including eddies, front and filaments. a) Satellite image of cyanobacteria bloom using ocean color detecting chlorophyll-a concentrations as a proxy for bloom location. Shapes here indicate mesoscale and submesoscale eddies and fronts strongly affect biology. (Taken August 11, 2015, from NASA (https://landsat.visibleearth.nasa.gov/view.php?id=86449). b) Observation taken on October 7, 1984 with ship tracks labeled A and B. c, d) Simulated ocean turbulence at roughly 2km resolution, expanded at local regions. Red indicates upwelling, or cyclonic eddies, and blue indicates downwelling, or anticyclonic eddies. c) shows simulation on March 1, 2012 (Northern Hemisphere winter/Southern Hemisphere summer) whereas d) shows simulation on September 1, 2012 (Northern Hemisphere summer/Southern Hemisphere winter). Taken from Su et. al, 2018[9].

The mixed layer plays an important role in these interactions, as it absorbs and responds to the interactions at the surface. The mixed layer begins at the surface layer of the ocean, at the air-sea boundary, and continues throughout the region where temperature and salinity remain relatively uniform due to intense mixing of the upper ocean layer; once the temperature and salinity change drastically, the mixed layer ends, at which point the mixed layer is stratified from denser regions below. The mixed layer depth depends on both top mixing and bottom mixing[10]. Top mixing is driven by wind shear, waves, and buoyancy fluxes, while bottom mixing is driven by large turbulent eddies that mix denser fluid from below and shear instabilities that thicken the buoyancy interface and allow for mixing from turbulent eddies. Often, models and common perception indicate that warmer ocean surfaces yield shallower mixed layers and more stratified water beneath[11]. However, observational studies demonstrate that this relationship is more complex; stabilizing or destabilizing buoyancy forces may be the driver in regional mixed layer depth, as heating (cooling) can cause stabilizing (destabilizing) buoyancy forces leading to stratification (convective mixing and a deepening of the mixed layer).

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Overall, observational studies of global patterns in mixed layer depths show a broad correlation between surface temperature and mixed layer depth, as shown in Figure 2, although a comprehensive understanding of mechanisms shaping mixed layer depth is still under active research. With a foundational knowledge of how physical phenomena form currents and circulation patterns, the study next investigates relationships at smaller scales and their potential impact on biology.

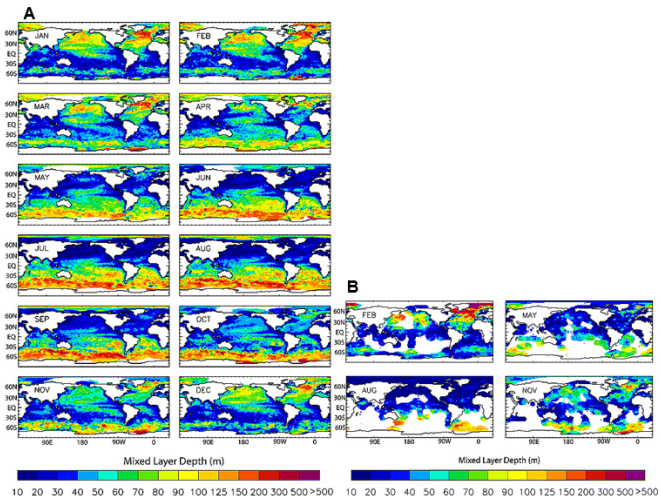

Figure 2. Global mixed layer depth climatology defined A) by temperature and B) by density. Criteria was based on computational inspection of profiles and time series data. Grid boxes are 2° and smoothing was used to fill missing data. A) Mixed layer depth for individual profiles is determined using deviation from near-surface temperature at 10m depth. A deviation of 0.2° C was used to mark the end of the mixed layer. B) Mixed layer depth is determined using deviation from near-surface density at 10m depth. Density criterion is a difference of 0.03 kg/m^3 from near-surface density, indicating the measurement is outside of well-mixed region. Figures adapted from de Boyer Montégut et. al, 2004[12]

To localize biologically productive regions in the ocean, the basic needs of phytoplankton populations must first be identified. We know phytoplankton (marine plants that perform photosynthesis) growth depends on light and nutrients. As most phytoplankton have limited control over their motion, populations predominantly flow with currents, and so physical phenomena dictate whether they have access to their basic needs. A critical disjunction between nutrient and light availability prevails; while sunlight is abundant at the surface and photosynthetically available radiation decreases exponentially with depth, nutrients are increasingly abundant in deep waters due to their sinking tendencies and storage in more dense waters. Similarly, while sunlight is abundant in subtropical regions, these regions tend to be depleted of nutrients as they tend to have shallow mixed layers that do not reach to the nutrients stored below. On the other hand, subpolar regions have deep mixed layers that can incorporate nutrients from colder, deeper waters (often reincorporated to the surface from convective mixing from the bottom), but a lack of sufficient light to support yearlong growth. This spatial consequence is shown in Figure 3[13]. For this reason, upwelling regions (where cold, nutrient-dense water is pumped to the surface from deeper

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waters by the topography of continental shelves) tend to be highly productive with increased levels of phytoplankton abundance compared to surrounding areas.

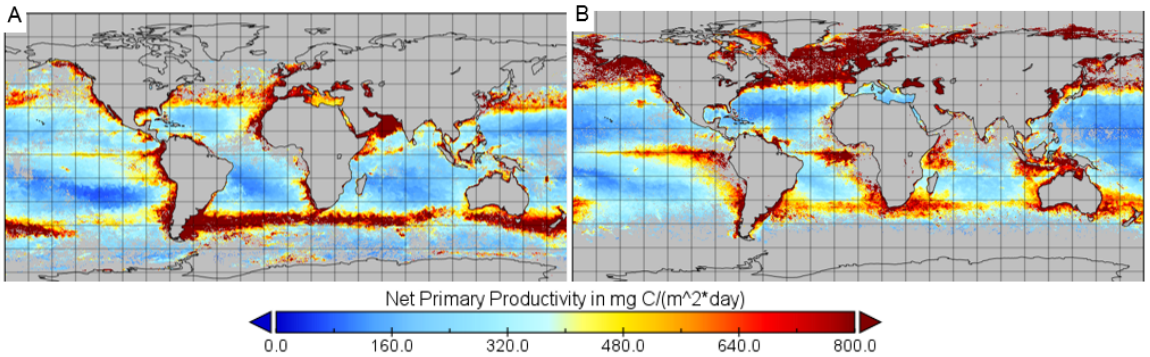

Figure 3. Observational measurements of global primary production. Satellite data from NOAA's Aqua MODIS on Chlorophyll a, incident visible surface irradiance, and sea surface temperature. Scale from 0 to 800 mg C/(m^2day), excluding extreme highs from scale for visualization. Observations from A) January 16, 2019 (Southern Hemisphere summer) and B) July 16, 2019 (Northern Hemisphere summer). Graphed by author.

Here, it will be noted that this broad confluence of light and nutrients is not the only effect on localization of primary production. Zooplankton grazing, diversity within phytoplankton species allowing for optimizations in different environments, and other factors will influence the net growth of phytoplankton populations. With the general importance of vertical transport in mind, the study turns to investigate small scale phenomena that can promote vertical velocities and fluxes of isopycnals. Focusing in on turbulence at the mesoscale and submesoscale, there are a number of mechanisms that impact vertical velocities in a more substantial way than that of global, large scale circulations. Internal instabilities and surface forcing enhance or suppress submesoscale dynamics[8]. Eddies can form at scales of 0.1-100km, created from anomalies in temperature and salinity, and carry rotational kinetic energy that can transport heat, salt, carbon, and nutrients in the horizontal and vertical planes[14]. Eddies also tend to stratify the mixed layer, which has been shown to initiate blooms in subpolar regions by keeping phytoplankton populations in upper regions with increased light exposure[15]. Fronts, produced from horizontal gradients of buoyancy (or variations in static pressure causing drag and lift forces), can form boundaries between isopycnals. These cause a cross-front ageostrophic secondary circulation to reach thermal wind balance, producing vertical velocities, as shown in Figure 4. The vertical velocities of fronts may support the transport of nutrients up into the euphotic zone where phytoplankton can access them, but they can also drive phytoplankton down away from the sunlight into lower levels without photosynthetically available radiation[13].

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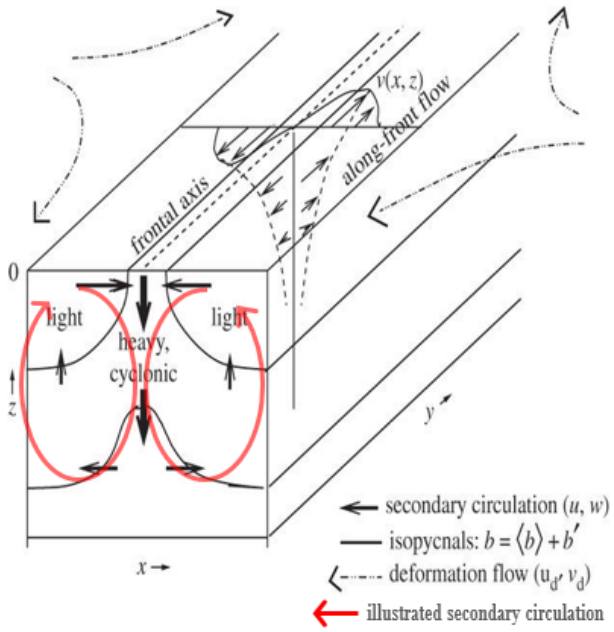

Figure 4. Depiction of submesoscale surface front configuration and the vertical velocities in frontogenesis. The along-front flow is mostly geostrophic, while the secondary circulation (in bold black and red arrows) is ageostrophic and acts to balance the system into thermal wind balance. Figure adapted from Figure 5 in McWilliams 2016[16].

However, the impact of either of these outcomes depends on frontal depth; while a deep front can access the nutricline (nutrient-rich layer often beneath the mixed layer) and upwell nutrients, a shallow front may not reach past the mixed layer and therefore have minimal effect to primary production near the surface. Further potential impact for submesoscale processes on phytoplankton populations and spatial distribution is suggested by the similarity in timescale. Phytoplankton variability develops over the course of days, aligning well with the submesoscale timescales that last roughly days. This is illustrated by the mathematical relationship between timescale and length scale, given the timescale approximation of:

$$T_s = \frac{L_s}{U}, (2)$$

where U is advective velocity, typically 0.1m/s, and L_s for submesoscale events is typically 1km[8]. In subtropical regions with shallow mixed layers, these local phenomena could be the only contribution of vertical velocities, yielding variability in regions further from the coast and allowing primary production. Nevertheless, the effect of submesoscale influences are up for debate. Some studies argue that the stirring and redistribution of the water column on the submesoscale level may not have a significant effect on global phytoplankton budgets and dynamics due to fewer submesoscale interactions in subtropical regions compared to subpolar regions, which already have sufficient nutrients in the mixed layer[13]. Evaluating the regions with shallow mixed layers where mesoscale and submesoscale interactions have potential to change phytoplankton populations should be incorporated into regional models through physical-biological coupling. This will optimize predictions of carbon cycling and of higher trophic marine populations that depend on primary production for regional fisheries.

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After investigating physical mechanisms that affect biology, it is critical to understand how biological factors can have an influence on oceanic physics and at what scale. Light can have impacts on ocean fluids by increasing sea surface temperature (SST) and affecting energy fluxes. In clear water, the shortwave radiation absorbed into the upper ocean is uniform, depending on

$$I(z) = I_0 \exp\left\{\frac{z}{\eta}\right\}, (3)$$

where I_0 is the albedo-corrected surface radiation, z is the depth, and η is the attenuation length, or a manipulation of the absorption coefficient of seawater[17]. However, waters are not all crystal clear, and varying levels of clarity must be accounted for. Jerlov defined five types of oceanic water clarity in 1968, ranging from clear to murkier, dirtier waters, which have been named Jerlov types[18]. Incorporating these different Jerlov types affects the irradiance into the ocean, which changes the equation to become:

$$I(z) = I_0[a \exp\left\{\frac{z}{\eta_1}\right\} + (1-a)\exp\left\{\frac{z}{\eta_2}\right\} B(z)], (4)$$

in which the first term concerns the red part of the light spectrum, the second term concerns the blue-green part, a is a dimensionless weighting parameter, and η_1 and η_2 are attenuation lengths. All of these parameters will change depending on the Jerlov type[19]. The $B(z)$ term represents bioturbidity, which depends on phytoplankton concentrations and detritus (dead particulate organic matter) concentration, as

$$B(z) = \exp\left(-k_c \int_z^0 P^i(z) + D(z)dz\right), (5)$$

where k_c is the attenuation constant for shelf shading, P is phytoplankton concentrations, i is the index of different plankton species, and D is the detritus concentration[20]. Equation 5 illustrates the additional absorption provided by biological presence, the importance of which is shown by the impact of the irradiance curve on physical factors:

$$\partial_t T - v'\partial_{zz}T + \partial_z\langle w'\,T'\rangle = \frac{\partial_z I}{c_P \rho_0}, (6)$$

where T denotes temperature, v' represents molecular diffusivity of heat, w' is the vertical component of velocity, c_P is the specific heat of seawater, and ρ_0 is the reference density of seawater[19]. Equation 6 highlights the aforementioned relationship between light (irradiance) and temperature in the ocean. An increase in absorbed radiation into the ocean by biological presence impacts the potential heat storage in the ocean, which in turn affects temperature differentials and triggers physical responses of turbulence or other events. We can make simple calculations to show the magnitude of the effect of biology using Jerlov types as proxies for high biological presence: We can calculate the effect of a typical level of solar radiation (500 W/m^2) in different Jerlov water types, the murkier water (Jerlov Type III) representing regions with high phytoplankton abundance and the clear water (Jerlov Type I) void of organisms. In a simple scheme without mixing, we use Equations 4 and 6 to evaluate the change in temperature over the depth in different Jerlov types. In this basic scheme where we assume the water stays in place, the magnitude of the warming across our mixed layer in Type III is greater by roughly 0.5 °C over time compared to Type I (Figure 5). However, the extent of this impact remains unclear as ocean waters do in fact mix; there is clearly missing information, as Havg in Figure 5 has a greater change in temperature, which shows the change in temperature in a well-mixed water column using the equation:

$$\frac{dT}{dt} = \frac{I_0}{c_P \rho_0 H}, (7)$$

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where H is the mixed layer depth. The increased temperature change in the mixed layer scheme shows that our simple calculation is only the beginning of the story.

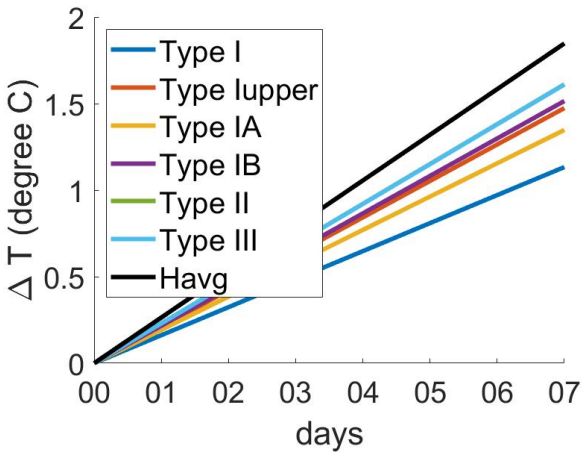

Figure 5. Change in temperature between the surface and bottom of the mixed layer for different Jerlov Types. Equation 4 was used across 100m depth from the surface to evaluate irradiance over ocean depth. Equation 6 was used to convert irradiance to temperature change over the timeframe of a week. Mixed layer depth was set to 40m depth arbitrarily and Equation 7 was used to calculate the temperature change. The difference between Type III (proxy for high biological presence) and Type I (clear water) after 7 days is 0.48 ˚C.

To fully understand the magnitude of biology's impact of heat storage in the ocean, these equations must be incorporated into small-scale models or parameterized into larger or global models. Using two-way coupled models between physics and biology, the potential physical outcomes of these additional temperature differentials could be detected. Studies have shown that an increased abundance of surface marine organisms like phytoplankton or other floaters causes both the surface albedo (amount of light that is reflected back from Earth) and absorption to be increased while momentum input from shear stress from surface winds causes it to be decreased[20]. Furthermore, research has indicated that for surfacing floating phytoplankton species, marine populations' effects of increasing absorption and reducing wind drag would outweigh the effects of an altered albedo. In regions with shallow mixed layer depth, it could be especially important to parameterize this biogeochemical-physical relationship at frontal events, as a front often develops higher biological concentrations on the side of higher density. This could theoretically lead to differential heating, changing the dynamics of the front and affecting feedback loops[21]. This phenomenon could have implications for marine ecosystems, as fisheries have long known that higher trophic marine organisms like fish populations, whales, and seabirds congregate near oceanic fronts, yet this occurrence still lacks comprehensive understanding in its relationship to physical oceanography[21]. Biogeochemical-physical coupling could help inform regional scales of heat capacity, as it has already been shown that including submesoscale interactions significantly and consistently increase upward heat transport and warms the sea surface up to 0.3˚ C at a global scale[9].

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Incorporating irradiance parameterizations into climate models could affect regional carbon budgets by changing positive feedback loops of plankton populations and other unknown physical responses[20].

4. Discussion

With an understanding of various physical and biogeochemical processes that could be parameterized, this study discussed the potential outcomes and open questions for investigation. As mentioned, mesoscale and submesoscale processes have potential to impact primary production which has serious implications for higher trophic ecosystem health. This in turn influences human economies through fisheries, tourism, and other ecosystem services. Potential heat storage in the ocean, and the coupled effects of phytoplankton growth in relation to temperature changes, is also a relevant area of study that requires further research. In terms of biological studies, future research must aim to study different species and diversity within phytoplankton populations, as abrupt climate changes could have tremendous effects on biodiversity, food chain dynamics, and spatial distributions and variations across a global scale.

Considering the looming threat of global warming, it is critical to investigate how these processes will respond to increased sea surface temperatures (SST) and higher levels of anthropogenic carbon. Some studies indicate that higher SST will lead to a more stratified ocean, decreasing nutrient fluxes from the nutricline or shallowing the mixed layer into more subpolar regions[22]. However, more frequent and stronger storms would increase turbulence in the ocean, providing vertical fluxes and nutrients[23]. While global and regional models are equipped to make predictions on specific changes in turbulence and local circulations, incorporating biological coupling into these models and understanding the various outcomes in relation to primary production require further research until they can be wellunderstood.

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Determinants of Hepatocellular Carcinoma in the United States: Differences in Risk Factor and Genetic Susceptibility by Race/Ethnicity

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the few cancers with an increasing incidence and mortality worldwide. This study aims to determine the contribution of known risk factors for HCC by race and ethnicity.

Methods: Data on race, ethnicity, age, and gender were obtained from National Health and Nutrition Examination Survey (NHANES). Population attributable fractions (PAFs) of risk factors were estimated using non-invasive scoring measures of Hepatitis B and C virus infection, excessive alcohol use, smoking, diabetes and emerging metabolic risk factors [non-alcoholic steatohepatitis advanced cirrhosis (NASH) and non-alcoholic fatty liver disease-advanced fibrosis (NAFLD-fib)] over a 10-year period, 1999-2002 and 2009-2012. Genetic analysis was performed using DisGenet platform by attaining the top enriched genes strongly related to HCC. Furthermore, cytoscape network was used to form a gene-disease network association.

Results: NASH-cirrhosis increased in the overall population and among all race and ethnic groups. Both liver fat accumulation and ALT levels vary among different populations; however, Hispanics have the highest prevalence of NAFLD and elevated ALT levels. Non-Hispanic (NH) blacks and Hispanics had a 3 to 4 times higher PAF for HCC than whites attributed due to chronic liver diseases, including NASH-cirrhosis and NAFLD-fib. Our genetic analysis demonstrated that PNPLA3 polymorphism is strongly associated with NAFLD-fib, which appears to represent susceptibility to liver disease among the Hispanic community.

Conclusion: Hispanics and NH blacks are at a disproportionately higher risk for HCC in part due to the higher prevalence of liver disease comorbidities, including NASH-cirrhosis and NAFLD-fib. Compared

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to NH whites, Hispanics and NH blacks have a higher baseline risk for liver cancer due to non-metabolic factors, which may include a genetic susceptibility. Metabolic risk factors have increased and are now contributing to nearly half of HCC cases in the US.

Keywords: non-alcoholic fatty liver disease advanced fibrosis; hepatocellular carcinoma; non-alcoholic steatohepatitis; metabolic risk factors; non-metabolic risk factors; population attributable fraction

1. Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer related deaths worldwide.[1] In the US, HCC incidence and mortality rates are increasing at a rate of 3% per year and are distributed disproportionally among certain racial/ethnic groups. [2]

HCC most often occurs among individuals who have chronic liver diseases. Nonalcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are growing and becoming the leading risk factors for HCC. NAFLD-fib and its subtype NASH-cirrhosis affect approximately 30% and 5%, respectively, of the US population.[3] The major risk factors are hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, cigarette smoking, excessive alcohol consumption, hereditary genetic diseases, and metabolic disorders (diabetes, obesity, impaired glucose tolerance, metabolic syndrome, and non-alcoholic fatty liver disease).[4] Some risk factors of metabolic diseases have been shown to be more prominent in certain ethnic groups. Data from the United States National Center for Health Statistics (2000-2006) identified chronic liver diseases as the sixth most common cause of death in the Hispanic population.[5] Obesity and diabetes are highly prevalent among both Hispanic and non-Hispanic (NH) Blacks due to lifestyle choices, diet, or genetic polymorphism, which causes all race-related genetic differences between different groups. The proportion of incident cases of heavy drinkers in the United States between 1984 and 1992 was highest among NH blacks (51%), followed by Hispanics (43%) and whites (32%). There are several lines of evidence suggesting that NH blacks who consume alcohol have greater liver enzyme elevation than whites, which further leads to liver disease .[6,7]

The prevalence of NAFLD-fib and risk of progression is higher among Hispanics than other racial and ethnic groups.[8] The higher incidence of HCC among Hispanics is driven by higher levels of sugar, carbohydrates and intake of saturated fat as compared to whites. Obesity and insulin resistance, two important risk factors for the metabolic syndrome, have been found to have a positive correlation with NASH-cirrhosis in Hispanic persons only.[5] Hispanics and NH blacks have also been shown to have higher HCC rates than whites. Cirrhosis rates are higher for NH blacks than for whites, and the highest cirrhosis mortality rates are observed among Hispanics.[7] Mortality from chronic liver disease in Hispanic people in the United States is nearly 50% higher than in NH white persons (13.7 per 100,000 in Hispanic persons vs 9.2 in NH whites and 7.5 in African American persons).[5]

In addition to known HCC risk factors, it is likely that access to preventive health education and early treatment may be a barrier to some racial and ethnic groups. The incidence of HCC varies by race and ethnicity primarily as a result of differences in the prevalence of major risk factors and also disparities in access to high-quality healthcare.[9] Socioeconomic disadvantage, lack of health insurance, and language barriers limit access to cancer screening and treatment among NH blacks and Hispanics.[10] It has also been found that

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Hispanics and NH blacks were less likely to be diagnosed with early-stage HCC compared with whites.[10]

There is a need to understand how HCC risk factors contribute to HCC prevalence rates within racial and ethnic groups, to reduce health-care disparities. The aim of this study was to determine contribution of specific known risk factors for HCC by race and ethnicity, using a nationally representative US population. A further network study of gene specificity and HCC was conducted.

1. Methods

1.1 Study Population

The National Health and Nutrition Examination Survey (NHANES) is a biennial cross-sectional survey representative of the US civilian, non-institutionalized population. Details of the NHANES methods and sampling strategy have been described by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC).[11] Briefly, subjects were recruited though a multistage probability sampling design, which was used to select participants representative of the civilian, non-institutionalized US population, with a sample weight assigned to each person.[12] Next, each subject was interviewed and underwent a physical examination, including a blood draw. General demographic characteristics, including age, sex, race/ethnicity (non-Hispanic white, non-Hispanic Black, Mexican American, other Hispanic, other race including Asian descent and multiracial (other/mixed)), and smoking behavior were collected during the Mobile Examination Center interview stage. During the examination, body measurements, including height, weight, and waist circumference (cm) were also collected. Serum samples were obtained and analyzed for albumin (g/dL), alanine aminotransferase (ALT, U/L), aspartate (aminotransferase (AST, U/L), alkaline phosphatase (U/L), fasting glucose (mg/dL), fasting insulin (uU/mL), gamma-glutamyl transpeptidase (GGT, U/L), platelet count (1000 cells/μL), total bilirubin (mg/dL), hemoglobin A_{1C} (%), total cholesterol (mg/dL), high density lipoprotein (HDL), low density lipoprotein (LDL) cholesterol (mg/dL), and triglycerides (mg/dL). All participants provided informed consent. NHANES is approved by the Institutional Review Board of the CDC. Subjects who were less than 18 years old or pregnant were excluded from this analysis (**Figure 1**).

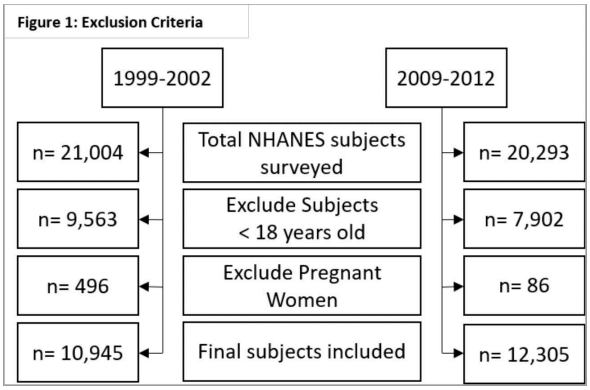

Figure 1. Exclusion Criteria

The prevalence of HCC risk factors was determined using interview, physical exam, and/or laboratory NHANES data. Hepatitis C virus was defined as having a positive hepatitis C virus antibody (anti-HCV) in laboratory testing. Hepatitis B viral infection was defined as having a positive surface antigen (HBsAg) on laboratory testing. Persons were identified as smokers when they reported current smoking on the NHANES questionnaire. Men who reported consuming more than 14 drinks per week and women who reported more than 7 drinks per week were defined as excessive drinkers.[13]

Of the metabolic risk factors, obesity was defined by BMI greater than or equal to 30 from body measurements taken on physical exam. Metabolic syndrome was defined using the

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International Diabetes Federation definition.[14] Persons with any three of the following five criteria were defined as having metabolic syndrome: 1) Elevated waist circumference (men >102cm, women >88cm) which was measured during physical exam; 2) Elevated triglycerides (>= 150 mg/dL or currently taking prescription to lower lipids); 3) Reduced high-density lipoprotein (<40mg/dL for males or <50mg/dl for females); 4) Hypertension (blood pressure measurements greater than 140mg/dL for systolic blood pressure or greater than 90 for diastolic); or 5) Elevated fasting glucose (≥100mg/dL). Diabetes was defined as having answered yes to the questionnaire question of "Have you ever been diagnosed by a physician as having diabetes?" or "Are you currently taking a blood glucose lowering medication?", and/or having a fasting glucose level greater than 126 mg/dL, or having a hemoglobin A_{1C} level greater than 6.5%.

Cirrhosis was defined as having an AST-to-platelet ratio index (APRI) >2 and any one of the following abnormal liver function tests: 1) elevated ALT levels (>40 U/L for men or >30 U/L for women); 2) Elevated alkaline phosphatase (>113 U/L); or 3) elevated total bilirubin (>1.3 mg/dL). NAFLD-advanced fibrosis (NAFLD-fib) was defined using three different noninvasive formulas: hepatic steatosis index (HSI), the FIB-4 index (FIB4), and the NAFLD fibrosis score (NFS).[15,16] Persons who had fatty liver based on the HSI and had fibrosis based on the FIB4 and/or the NFS were defined as having NAFLD-advanced fibrosis.

$$\boldsymbol{HSI} = 8 \times \left(\frac{ALT}{AST}\right) + BMI \ [+2 \ if \ Diabetes, +2 \ if \ Female]$$

$$\boldsymbol{FIB4} = \frac{Age_{\ Years} \times AST \ (\frac{U}{L})}{Platelet \ Count \ \left(\frac{10^9}{L}\right) \times \sqrt{ALT \ (\frac{U}{L})}}$$

$$\boldsymbol{NAFLD\ Fibrosis\ Score} = \frac{-1.675 + 0.037 \ (Age_{Year}) + 0.094 \ (BMI) + 1.13(Fasting \ Glucose)}{(Diabetes \ (yes = 1, no = 0)) + 0.99 \left(\frac{AST}{ALT}\right) - 0.013 \left(Platelet \ Count(\frac{10^9}{L}\right) - 0.66(Albumin \left(\frac{g}{L}\right))}$$

NHANES does not include genetic disorders which are risk factors for HCC or impaired glucose tolerance. Thus, these risk factors could not be included in our analysis.

1.2 Statistical Analysis.

This analysis used the required weighting procedures to account for the survey design of NHANES. Descriptive analyses were done to compare the NHANES population in 1999-2002 to the NHANES population in 2009-2012. Categorical variables were compared using χ^2 tests. Continuous variables were compared using the Students t-test after confirming all data were normally distributed. Age-adjusted prevalence rates for HCV infection, HBV infection, smoking, excessive alcohol use, obesity, diabetes, NASH-cirrhosis, and NAFLD-fib for the two four-year time periods were calculated using the projected population of the United States for the year 2000.[17]

To determine the predicted contribution of each risk factor towards the development of HCC, the population attributable risk was calculated. A medical literature review was done to find the relative risk (RR) of each risk factor (HCV, HBV, etc.) towards the development of HCC. The literature review was done using PubMed and with the term for each risk factor, risk, and hepatocellular carcinoma.

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Results were sorted by year of publication with US populations, and recent meta or pooled analyses preferred. The population attributable fraction (PAF) for each risk factor was then calculated using the formula developed by Levin for each sex and race group.[18]

$$Population\ Attributable\ Fraction = \frac{Risk\ Prevalence\ (RR_{Exposure} - 1)}{Risk\ Prevalence\ (RR_{Exposure} - 1) + 1}$$

In determining the PAF, the risk factors were analyzed independently, without accounting for interaction between their effects.

The combined effect of all risk factors and risk factors by type (metabolic vs. non-metabolic) were calculated using the formula below:

$$Total\ Attributable\ Fraction = 1 - \prod_{i=1}^{n}(1 - PAF_i)$$

All statistical analyses were conducted using SAS 9.4 (Cary, NC) with p <0.05 considered significant. Figures were developed using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA.

The top enriched pathogenic genes associated with liver diseases were analyzed using the DisGenet platform (Table 1).[19] The DisGeNet database uses information of human gene-disease association (GDAs) and variant-disease association (VDAs) from expert curated repositories. The GDA score was calculated using the formula developed by DisGeNet. Scoring (gda) was used to rank the gene-disease according to their level of evidence.[19]

The DisGeNET Score (S) for GDAs is computed according to:

$$S = C + M + I + L$$

$$C = \begin{cases} 0.6 & if\ N_{sources_i} > 2 \\ 0.5 & if\ N_{sources_i} = 2 \\ 0.3 & if\ N_{sources_i} = 1 \\ 0 & otherwise \end{cases}$$

where:

$N_{sourcesi}$ is the number of CURATED sources supporting a GDA

i ∈ CGI, CLINGEN, GENOMICS ENGLAND, CTD, PSYGENET, ORPHANET, UNIPROT

$$M = \begin{cases} 0.2 & if\ N_{sources_j} > 0 \\ 0 & otherwise \end{cases}$$

where:

j ∈ Rat, Mouse from RGD, MGD, and CTD

$$I = \begin{cases} 0.1 & if\ N_{sources_k} > 0 \\ 0 & otherwise \end{cases}$$

where:

k ∈ HPO, CLINVAR, GWASCAT, GWASDB

$$L = \begin{cases} 0.1 & if\ N_{pubs} > 9 \\ N_{pubs} * 0.01 & if\ N_{pubs} < 9 \end{cases}$$

where:

N_{pubs} is the number of publications supporting a GDA in the sources LHGDN and BEFREE

DisGeNET uses two other metrics to facilitate the ranking of the genes associated with hepatocellular carcinoma. The Disease Specificity Index (DSI) was used, which is inversely proportional to the number of diseases associated to gene. A gene associated with multiple diseases

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gets a score close to zero, and a gene associated with only one disease has DSI of 1.[18] It is computed according to :

$$DSI = \frac{log_2(\frac{N_d}{N_T})}{log_2(\frac{1}{N_T})}$$

where:

N_d Is the number of diseases associated to the gene/variant

N_T is the total number of diseases in DisGeNET

The Disease Pleiotropy Index (DPI) was the second metric used to rank the genes. It ranges from 0 to 1 and is proportional to the number of different (MeSH) disease classes a gene is associated with. The DPI is computed according to :

$$DPI = (\frac{N_{dc}}{N_{TC}}) * 100$$

where:

N_{dc} is the number of the different MeSH disease classes of the diseases associated to the gene/variant

N_{TC} is the total number of MeSH diseases classes in DisGeNET.

Furthermore, cytoscape network was used to form a gene-disease association to visualize interaction among different genes (Figure 2). Cytoscape is an open-source platform for visualizing molecular interactions.[20]

1. Results

There were 10,945 individuals in the 1999-2002 sample and 12,305 in the 2009-2012 sample (**Figure 1**). The groups did not differ among the distribution of sex, age, or race/ethnicity. The mean age at screening was 45.4 (1999-2002) and 46.4 (2009-2012) and 50.7% of the participants were female (**Table 2**).

Figure 2: *HCC hub gene*: Gene network representing the interaction of most significant genes involved in HCC

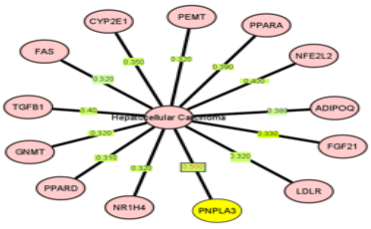

Figure 2: Each gene is associated with a score generated by DisgGeNET platform. Green score (0.500)represents how strongly associated each gene is with HCC. Based on the scoring and our genetic analysis demonstrates PNPLA3 gene represents the strongest gene associated with HCC. Each gene node in the figure is representing association of the gene in the process of tumorigenesis and have an important influence on the occurrence of HCC.

Table 1. Genes associated with hepatocellular carcinoma from the DisGeNET database

Gene	DSI	DPI	GDA score
PNPLA3	0.556	0.692	0.500
NFE2L2	0.357	0.885	0.400
PPARA	0.432	0.885	0.390
ADIPOQ	0.376	0.885	0.380
CYP2E1	0.459	0.692	0.350
TGFB1	0.287	0.962	0.340
FGF21	0.485	0.769	0.330
LDLR	0.449	0.885	0.320
FAS	0.372	0.923	0.320
PEMT	0.653	0.538	0.320
NR1H4	0.513	0.808	0.320
GNMT	0.626	0.462	0.320
PPARD	0.513	0.846	0.310

DSI = disease specificity index

DPI = disease pleiotropy index

Score $_{GDA}$ = ranking of the gene-disease association

In the overall NHANES population, obesity, excessive alcohol consumption, and smoking were the most prevalent HCC risk factors in both 1999-2002 and 2009-2012. The prevalence of HBV, HCV, or excessive alcohol consumption did not change over the ten-year period. All metabolic risk factors (obesity, diabetes, NAFLD-fib, NASH-cirrhosis) increased over the ten years of study in the overall population (**Table 2**). Concurrently, BMI, waist circumference, fasting glucose levels, and triglyceride levels all increased between the two time periods (all $p < 0.001$, **Supplemental Table 1**).

Table 2. Participant Characteristics

Characteristics		Survey Period		
		1999-2002	2009-2012	p-value
Demographics	Gender			0.15
N %	Male	5395 (49.3)	6072 (49.4)	
	Female	5550 (50.7)	6233(50.7)	
	Age at Screening (years) *	45.4(0.33)	46.4(0.51)	0.22
	Race/Ethnicity			0.19
	Non-Hispanic White	5132(46.9)	5158(47.9)	
	Non-Hispanic Black	2163(19.8)	2734(22.2)	
	Hispanic	3273(29.9)	3065(24.9)	
	Mixed/Other	377(3.44)	1348(10.9)	
	Smoking Status			<0.001
	Current Smoker	2125(24.5)	2441(20.3)	
	Former Smoker	2625(26.8)	2768(23.7)	-
Disease Prevalence	Hepatitis C Virus	2.0(54)	1.6(48)	0.37
% Prevalence (Population Attributable Fraction)	Hepatitis B Virus	0.2 (5)	0.4(7)	0.67
	Excessive Alcohol	23.8 (12)	21.6(12)	0.83
	Current Smokers	24.5 (12)	20.3(10)	<0/001
	Diabetes	9.8(9)	11.8(11)	<0.001
	Obesity	29.9(25)	35.2(28)	<0.001
	NASH-Cirrhosis	0.07(3)	0.2(6)	0.02
	NAFLD-fib	1.5(1.4)	4.0(3.7)	<0.001

NASH = non-alcoholic steatohepatitis; NAFLD-fib = non=alcoholic fatty liver disease advanced fibrosis

Supplemental Table 1: Metabolic Measures

Metabolic Measures	1999-2002		2009-2012		
	mean	s.e.m.	mean	s.e.m	p-value
BMI (kg/m^2)	27.9	0.13	28.6	0.49	<0.001
Waist Circumference (cm)	95.3	0.16	98.1	0.16	<0.001
Mean Systolic Pressure (mmHg)	123.2	0.42	121.1	0.41	<0.001
Fasting Glucose (mg/dL)	104.3	0.55	107.1	0.45	<0.001
Fasting Insulin (uU.mL)	13.0	0.34	13.8	0.26	0.17
Cholesterol, total (mg/Dl)	197.9	0.78	193.1	0.73	<0.001
HDL-cholesterol (mg/Dl)	51.2	0.36	52.9	0.59	<0.001
LDL-cholesterol (mg/Dl)	122.4	0.96	115.0	0.71	<0.001
Triglycerides	144.6	2.62	150.4	2,24	>0.001
Fasting Glucose > 100 (%)	16.0	0.56	19.5	0.63	<0.001
Albumin(g/dL)	4.37	0.01	4.29	0.01	<0.001
ALT (U/L)	25.9	0.42	25.4	0.23	0.44
AST (U/L)	24.4	0.22	25.9	0.16	<0.001
GGT (U/L)	84.9	0.49	69.0	0.24	<0.001
Platelet count (SI)	268.5	1.39	239.0	1.13	<0.001
Total Bilirubin (mg/dL)	0.58	0.01	0.74	0.01	<0.001
GGT (U/L)	30.3	0.46	26.6	0.50	<0.001
Iron (ug/dL)	87.2	0.39	84.2	0.34	<0.001

s.e.m. = standard error of the mean; ALT= Alanine aminotransferase; AST=Aspartate aminotransferase; GGT= Gamma-Glutymyl Transpetidase;

Both NASH-cirrhosis and NAFLD-fib increased in prevalence between 1999-2002 and 2009-2012. The prevalence rates of NAFLD-fib increased from 1999-2002 (1.53%) to 2009-2012 (4.0%) (p<0.001), while NASH-cirrhosis

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increased from 0.07% to 0.20%, p=0.03). This represents a 164% increase for NAFLD-fib and 186% increase for NASH-cirrhosis overall. The largest increase of NAFLD-fib occurred among the Hispanic population (a 296% increase, p<0.001) (**Table 3**).

Table 3A and B: Hepatocellular Carcinoma Risk Factor Prevalence and PAF by Sex and Race/Ethnicity

Table3a: Hepatocellular Carcinoma Risk Factor Prevalence and PAF Stratified by Sex and Race/Ethnicity Subgroup (1999-2002)

		HCV		HBV		Excessive alcohol		Current smoker		Diabetes		Obesity		NASH cirrhosis		NAFLD-fib	
		Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev (%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF (%)
Overall	Total	**2.0**	**54**	**0.2**	**5**	**23.8**	**12**	**24.5**	**12**	**9.8**	**9**	**29.9**	**25**	**0.07**	**3**	**1.5**	**1**
	M	**2.6**	**52**	**0.3**	**7**	**22.3**	**11**	**26.9**	**13**	**11.2**	**10**	**26.7**	**23**	**0.05**	**2**	**1.5**	**1**
	F	**1.4**	**53**	**0.2**	**2**	**26.9**	**13**	**22.1**	**11**	**8.5**	**8**	**32.8**	**27**	**0.09**	**4**	**1.6**	**1**
NH White N=5132	**Total**	1.9	53	0.1	1	24.5	12	25.0	12	8.4	8	28.7	25	0.08	4	1.3	1
	M	2.4	60	0.0	0	24.1	12	26.1	13	10.7	10	27.2	24	0.04	2	1.1	1
	F	1.3	34	0.1	0	25.3	13	24.0	12	6.1	6	30.1	25	0.13	6	1.4	1
NH Black N=2163	**Total**	4.1	70	0.8	19	22.9	11	26.5	13	14.2	13	38.9	31	0	0	1.9	1
	M	6.0	78	0.8	10	18.8	9	33.9	16	10.6	10	26.8	23	0	0	1.2	1
	F	2.6	62	0.7	3	30.9	14	20.5	10	16.8	15	48.7	35	0	0	2.5	1
Hispanic N=3273	**Total**	2.0	44	0.0	0	22.3	10	22.9	11	14.1	12	30.1	25	0.10	4	1.3	1
	M	2.0	53	0.1	2	22.1	10	27.2	13	13.5	12	23.1	21	0.23	9	1.2	1
	F	1.8	52	0.0	0	24.1	9	18.9	9	13.9	12	36.5	29	0	0	1.3	1
Other/Mixed N=377	**Total**	1.1	40	2.3	8	28.4	13	22.3	11	17.9	15	20.3	19	0	0	0.8	1
	M	1.4	35	3.6	5	27.6	12	31.4	15	18.3	16	22.3	20	0	0	0.7	1
	F	1.0	36	0.9	21	28.2	16	14.3	7	17.5	15	19.2	18	0	0	0.9	1

Table 3B. Hepatocellular Carcinoma Risk Factors Prevalence and PAF Stratified by Sex and Race/Ethnicity Subgroup (2009-2012)

		HCV		HBV		Excessive Alcohol		Current Smokers		Diabetes		Obesity		NASH cirrhosis		NAFLD-fib		
		Prev (%)	PAF (%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev (%)	PAF(%)	Prev(%)	PAF(%)	Prev (%)	PAF(%)	
Overall	**Total**	**1.6**	**48**	**0.4**	**7**	**21.6**	**12**	**20.3**	**10**	**11.8**	**11**	**35.2**	**28**	**0.20**	**8**	**4.0**	**4**	
	M	**2.2**	**47**	**0.4**	**10**	**27.4**	**11**	**22.7**	**11**	**13.7**	**12**	**34.3**	**28**	**0.37**	**14**	**4.1**	**4**	
	F	**1.1**	**46**	**0.3**	**4**	**23.7**	**13**	**18.0**	**9**	**10.1**	**9**	**36.0**	**29**	**0.06**	**3**	**4.0**	**4**	
NH White N=5158	**Total**	1.6	49	0.1	2	21.3	12	21.1	10	9.3	9	33.2	27	0.19	8	3.5	3	
	M	2.1	56	0.2	1	27.1	11	21.9	11	11.5	10	34.0	28	0.43	16	3.6	3	
	F	1.2	31	0.0	0	26.6	13	20.3	10	7.4	7	32.4	27	0	0	3.4	3	
NH Black N=2734	**Total**	2.5	59	0.9	21	23.1	12	24.4	10	18.1	15	48.8	36	0.21	8	4.8	4	
	M	3.3	66	1.2	14	31.8	11	30.0	11	18.3	16	38.2	30	0.00	0	4.5	4	
	F	2.0	55	0.7	2	23.7	14	20.1	9	18.0	15	57.2	39	0.41	15	5.1	5	
Hispanic N=3065	**Total**	1.4	35	0.1	0	23.1	11	17.0	10	18.9	16	40.8	32	0.23	9	4.0	4	
	M	2.1	55	0.1	3	25.3	11	23.1	11	20.4	17	38.4	30	0.29	11	3.4	3	
	F	0.6	27	0.2	2	22.1	12	10.7	10	17.4	15	43.2	33	0.17	7	4.5	4	
Other/Mixed N=1348	**Total**	0.8	34	2.4	9	20.3	10	17.1	12	17.2	15	19.8	18	0.10	4	0.9	1	
	M	1.8	41	2.5	4	19.4	9	22.3	14	19.8	17	21.1	19	0.23	9	0.9	1	
	F	0.0	0	2.3	41	19.2	10	12.5	10	15.2	13	18.5	17	0	0	0.9	1	

Prev=prevalence (%); PAF= Population Attributable Fraction (%); M=males; F=Females; SEM=Standard error or the mean; HCV= Hepatitis C Viral infection; HBV= Hepatitis B viral Infection; NASH=Non-alcoholic steatohepatitis; NAFLD-fib=Non-alcoholic Fatty liver disease advanced fibrosis

The risk factors to decrease over the ten-year period were the number of current smokers (prevalence 24.5% to 20.3%, p=<0.001) and access alcohol. There were no temporal changes in the prevalence of excessive drinking behaviors overall, which remained between 23.8% and 21.6% (p = 0.83, **Table 2**).

1.1 Population Attributable Fractions

Overall, 77.9% of HCC cases from 1999-2002 and 76.2% of HCC cases from 2009-2012 could be attributed to the risk factors analyzed in this study. In the overall population, metabolic risk factors (diabetes, obesity, NAFLD-fib, NASH-cirrhosis) composed 35% of HCC cases in 1999-

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2002 and increased to accounted for 42% of HCC cases in 2009-2012. Synchronously, non-metabolic risk factors (HBV, HCV, excessive alcohol use, current smokers) decreased from a PAF of 66% in 1999-2002 to 59% in 2009-2012 (**Table 4**). Other/mixed category was removed from the study because it was not statistically significant enough to make conclusions about the PAF due to low sample size. Among both metabolic and non-metabolic risk factors, HCV was the single highest attributable cause of HCC in both time periods; 54.1% of HCC cases were estimated to be attributable to HCV in 1999-2002 and 48.4% in 2009-2012.

Table 4. PAF by Risk Category Stratified by Sex and Race/Ethnicity

Table 4A: PAF by Risk Category Stratified by Sex and Race/Ethnicity 1999-2002

		Metabolic	Non-Metabolic	Unknown	Total
Overall	**Total**	**34.9**	**66.0**	**22.1**	**77.9**
	M	**33.4**	**65.4**	**23.1**	**76.9**
	F	**36.4**	**64.6**	**22.5**	**77.5**
NH White	Total	13.1	63.7	31.5	68.5
	M	32.8	69.3	20.6	79.4
	F	34.5	49.0	33.4	66.6
NH Black	Total	40.4	81.1	11.3	88.7
	M	31.5	85.0	10.3	89.7
	F	46.1	71.3	15.5	84.5
Hispanics	Total	38.2	54.7	28.0	72.0
	M	37.3	64.1	22.5	77.5
	F	38.7	60.1	24.5	75.5

Table 4B. PAF by Risk Category Stratified by Sex and Race/Ethnicity

Table 4B. PAF by Risk Category Stratified by Sex and Race/Ethnicity 2009-2012

		Metabolic	Non-Metabolic	Unknown	Total
Overall	**Total**	**42.0**	**59.0**	**23.8**	**76.2**
	M	**46.3**	**60.9**	**21.0**	**79.0**
	F	**37.8**	**61.7**	**23.8**	**76.2**
NH White	Total	39.5	61.0	23.6	76.4
	M	45.1	65.7	18.8	81.2
	F	34.0	46.7	35.2	64.8
NH Black	Total	47.9	67.6	16.9	83.1
	M	43.5	78.9	11.9	88.1
	F	51.0	69.6	14.9	85.1
Hispanics	Total	47.6	49.7	26.4	73.6
	M	49.5	64.0	18.2	81.8
	F	45.1	43.8	30.8	69.2

M=males; F=Females; Metabolic Diseases = Diabetes, Obesity, Nonalcoholic steatohepatitis, Non-alcoholic fatty liver disease-advanced fibrosis; Non-Metabolic Diseases = Hepatitis B, Hepatitis C, Excessive alcohol use, Current smokers

Differences in PAF magnitudes were observed among race/ethnic subgroups. The highest HCV PAF was observed among non-Hispanic Blacks (59% in 2009-2012), while the lowest was among other/mixed (34% in 2009-2012). These values did not significantly decrease over the ten-year period. Non-Hispanic Blacks had the highest obesity PAF (31% in 2009-2012 compared to 25% in the overall population), which decreased from 36% in 1999-2002.

NASH-cirrhosis or NAFLD-fib could be attributed to, at most, 8% of HCC cases; these liver diseases had the largest fold change of any risk factor over the study period. Specifically, NAFLD-fib had the greatest fold increase of any risk factor studied, increasing 2.6-fold. NASH-cirrhosis had the second greatest PAF, increasing to a similar degree of 2.4-fold. NASH-cirrhosis

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increased from 3.8% of cases to 5.8% and NAFLD-fib increased from 1.4% to 3.7% (p=0.022 and p<0.001, respectively, **Figure 3**).

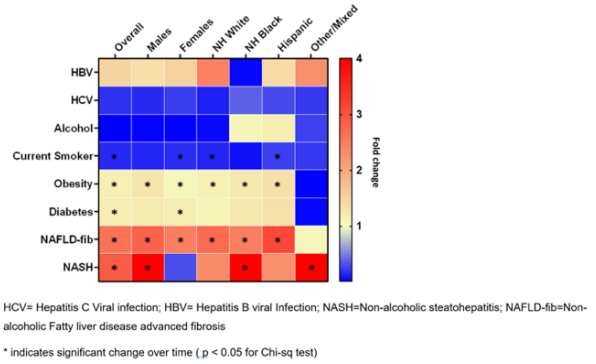

Figure 3: Fold Change in Hepatocellular Carcinoma Risk Factor Population Attributablr Fractions 1999-2002 and 2009-2012

Stratification by race/ethnicity showed that obesity and NAFLD-fib increased across all groups. The largest increase was seen among Hispanics with a 208% increase in NAFLD-fib. NASH-cirrhosis was more common among men than women in 2009-2012 (relative risk = 6.2). The largest increase in NASH-cirrhosis was among non-Hispanic Blacks, increasing from PAF of 0% in 1999-2001 to 8% in 2009-2012. NASH-cirrhosis increased significantly, accounting for 6.3% of HCC cases in 2009-2012 (**Table 3, Figure 3**).

1.1.1 Genetic Analysis.

There are differences in HCC outcomes between different ethnic groups. The gene *PNPLA3* was shown to play a major role in the development of liver disease such as NAFLD-fib and NASH-cirrhosis. *PNPLA3 represents* a GDA score of 5.00(figure 2). The specificity for HCC was 0.556, and the association of the PNPLA3 with HCC specifically is 0.692. This is based on an evidence score of 0.500, which was calculated from DisGenet . *PNPLA3* gene represents a cytosine to guanine substitution, resulting in an isoleucine to methionine switch at codon 148 and individuals with the G allele have a higher hepatic triglyceride level and elevated serum of ALT.[21] Our study found a total of 13 pathogenic genes from DisGeNET platfrom with a DSI , DPI and a GDA score based on DisGeNet ranking system. Our analysis represents high frequency of *PNPLA3* gene among Hispanic groups. Furthermore, gene-diet interaction plays a vital role in the pathogenesis of liver cancer in Hispanics.

Figure 2 represents the 13 gene strongly associated with HCC. Based on the GDA score from DisGeNET, *PNPLA3* (0.500) holds a strong association with increased risk of HCC. The GDA score of *PNPLA3* (0.500) is the highest as compared to other genes in list. The lowest GDA score based on DisGeNET ranking is *PPARD*. Genes *LDLR, FAS, PEMT, NR1H4, GNMT* represents the same GDA score of 0.320 which means they all are equally associated in the development of HCC.

1. Discussion

This study attributed nearly 80% of HCC cases in 1999-2001 and 2009-2012 to eight known risk factors in a large nationally representative sample of the U.S. population. Metabolic risk factors are now contributing to nearly half of HCC cases in the US. Metabolic diseases (diabetes, obesity, NASH-cirrhosis, and NAFLD-advanced fibrosis) increased from contributing an estimated 35% of HCC burden in 1999-2002 to 42% in 2009-2012. Concordantly, non-metabolic risk factors decreased from 66% to 59% of total HCC burden over the same period. Stratification by race/ethnicity showed a similar shift across all groups. The HCC risk factor prevalence rates of obesity and NAFLD-fib increased for all groups and were particularly high among Hispanics and non-Hispanic Blacks. A genetic variant in *PNPLA3* was identified as strongly associated with HCC.

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These findings highlight vulnerabilities within certain racial and ethnic groups within one country's population of HCC, which is increasing in the US as well as worldwide. [25-28] As the obesity epidemic had increased from 1999 until 2012, metabolic risk factors (NAFLD-fib and NASH-cirrhosis) became contributers to HCC development in the US more so than non-metabolic risk factors (HBV, HCV), excessive alcohol use, and smoking. [22,23]

It is likely that there is an interplay between lack of access to health care, racial disparities, and genetics leading the whole pathogenesis and playing a major role in the development of liver disease that eventually forms into HCC. Specific genetic contributions may help explain differences observed in PAFs for HCC between race/ethnic groups.[29] While insulin resistance likely plays a role in its pathogenesis, oxidative injury and inflammatory reactions could be influenced by genetics. A study performed in the US among Hispanic, NH black and NH white individuals identified the variant, rs73809, (148M) in patatin-like phospholipase domain-containing protein 3 (*PNPLA3)* as a predictor for hepatic fat content.[30] The study confirmed that patients with NAFLD-fib who carry an allele of the gene (rs73809) *PNPLA3* have an increased risk of developing advanced diseases, including NASH-cirrhosis. Risk allele (rs73809) was the main common genetic determinant of hepatic fat content and of progressive NAFLD-fib, and this allele was mostly observed among Hispanic groups. It is not clear as to why this allele is increased among Hispanics. The variant has been reported to manifest in early life among Hispanic adolescents, [31] as well was having a prevalence of 80% in a single center study in Mexico.[32] This is consistent with the current study's findings showing higher burdens of NAFLD-fib among Hispanics. Overall, evolving knowledge in genetics along with epidemiological studies focused on race/ethnic backgrounds may help identify patients at higher risks for HCC.[33] This study demonstrates that PNPLA3 influences liver fat accumulation early in life in Hispanic children and adults. [34] This analysis also represents, individuals carrying the GG genotype of the *PNPLA3* gene are susceptible to increased hepatic fat when dietary sugar intake is high. The role of *PNPLA* gene may have an association in the development of NAFLD-fib and NASH-cirrhosis in the Hispanics. Other studies have also confirmed that this gene predispose obese children and adolescents to exhibit hepatic damage.[35]

Among non-metabolic risk factors, HCV has long been recognized as a major predictor of HCC risk.[36] This study is also consistent with previous studies indicating a largest risk of HCV in the non-Hispanic Black population. [36-37] A cross-sectional study utilizing Medicare databases have shown that the proportion of HCC cases attributable to HCV and HBV have doubled over approximately the same study period.[37,38] The differing estimations in previous studies compared to the current is likely due to the broader age of subjects surveyed presently in NHANES compared to SEER-Medicare databases. The known birth cohort effect of those born between 1945-1965 reaching the age of peak HCC risk had been previously reported, [37,39] and the greater proportion of HCV contribution was towards HCC among the older US generation.

The current study does have certain limitations. While this study has assumed independent causation of HCC for each risk factor, the course of disease from obesity, diabetes, NAFLD-fib, and NASH-cirrhosis is not a mutually exclusive path toward malignancy. [25,26,40-43] Patients can often present with multiple risk factors, raising the question of how to accurately weigh the contribution of each risk and handle overlapping interactions with other, possibly concurrent, risk factors. While this study served to focus on contributions of each specific risk factor,

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future studies are needed to disentangle these interactions using real-world data .[44] Importantly, the average age of HCC onset is 65 while the study's cohort had an average age of 46. While this study did adjust for age, the burden of each metabolic risk factor may have been an underestimation.[16] This study also assumes that people do not change their lifestyle habits as they grow older, thereby potentially decreasing their HCC risk.[29] Thus, these results should be interpreted as epidemiological evidence for prevention strategies and public health education on risk factors.

In conclusion, these results display the changing contributions as well as the proportions of known HCC risk factors among specific racial and ethnic groups in a representative sample of the US population. The results of this study show that the increasing HCC rates are due to modifiable causes; this can be used to inform prevention and education programs with awareness as to racial and ethnic genetic and lifestyle differences.

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The Preventative and Healing Properties of Performing Arts in Female Genital Mutilation

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The Preventative and Healing Properties of Performing Arts in Female Genital Mutilation

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Abstract

Despite being outlawed in many of the countries where it is the most prevalent, female genital mutilation (FGM) still persists. It is critical that innovative interventions be adopted in order to better address the cultural roots of this gender violence epidemic. The aim of this study is to explore the use of performing arts to fill this gap in effective preventative and treatment interventions. Due to the lack of data in this field, this study comprises of an extensive literature review. Existing programs were evaluated through thorough web searches, interviews of program leads, and analyses of the results. After reviewing existing evidence, it has been concluded that performing arts interventions provide positive outcomes in the field of FGM due to their ability to engage with cultural assumptions, incite empathy, and cross educational boundaries, all through community-connected approaches. Local outcomes were connected to government intervention in the recommendations to conclude that all governments should ban FGM, allocate public funds to the field of arts and health, and increase the validity of performance-based interventions through increased and improved research.

Keywords: Female Genital Mutilation (FGM), Performing Arts, Performance, Theatre for Development

1. Introduction

In the fight to end FGM, outlawing the practice has proved to not be enough. For example, in Egypt, FGM has been a crime since 2008, but the number of women between the ages of 15 and 49 who have undergone FGM is still as high as 91% in 2020[1,2]. In order to work towards the abandonment of FGM, interventions must target attitudes and behaviors at the individual and community levels. By honing in on the root causes of FGM, these targeted interventions can initiate open and honest discussions about the negative impacts of FGM in order to contribute to meaningful change[3].

FGM, while a tradition in many cultures, is a violation of human rights. By directly engaging the complex driving forces behind this phenomenon, performative art has been promising in the process of changing the behavior and beliefs of those abetting the practice of FGM, including mothers, religious leaders, and circumcisers. While there is a very limited amount of research on the quantitative effectiveness of using performing arts to reduce the practice of FGM, the existing accounts are hopeful for the future of this nexus.

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This study serves to gather existing accounts and research to propose performance as an underutilized but nonetheless helpful intervention for the prevention and treatment of FGM, because of its abilities to cross language barriers, engage empathy, empower self-expression, and challenge culturally ingrained issues.

The field of performative arts (including theater, dance, music, and radio) and its role in health interventions is becoming more relevant in research. Just over the past two decades, academia, NGOs, and many governments have increasingly conducted research on the effects of the arts overall on health and well-being[4]. The *Health Evidence Network Synthesis Report 67*, which focused on the evidence of the arts in improving health and well-being, synthesized evidence from over 900 publications at the intersection of arts and health and gives recognition to the positive role of arts on health. The results are clustered into two themes: prevention and promotion, and management and treatment. Under prevention and promotion, the report found that the arts can affect the social determinants of health, support child development, encourage health-promoting behaviors, help to prevent ill health, and support caregiving[4]. In the theme of management and treatment, the findings displayed that the arts could help people experiencing mental illness, support care for people with acute conditions, help to support people with neurodevelopmental and neurological disorders, assist with the management of non-communicable diseases, and support end-of-life care[4]. This report serves as proof of the tangible and impactful effect the arts can have on individual well-being and global health.

FGM is one health issue in which the arts can benefit survivors and those at risk by facilitating healing and targeting incidence. While Finn and Fancourt's *Health Evidence Network Synthesis Report* does not cover FGM in its evidence, arts interventions were successful in improving other culturally embedded global health issues, such as the stigma against LGBTQ communities. As FGM also requires culturally sensitive solutions, the arts have the potential to be transformative in this health issue's outcomes.

The topic of FGM is especially pertinent in 2020 as a result of COVID-19 lockdowns. There are severe long- and short-term implications of these policies. In the long term, estimates provided by Avenir Health, Johns Hopkins University, and Victoria University predict that lockdown-related disruption over six months will disrupt programs to end FGM, potentially resulting in two million additional cases of FGM over the next 10 years[5]. The Kapenguria Theater Group, a theater group fighting FGM, reported in July 2020 that the number of girls being circumcised has drastically risen since schools were closed due to COVID-19[6]. Given these increased rates of FGM and the subsequent need to find unique interventions, it is critical to produce effective and innovative projects combatting FGM.

Sections 3 and 4 will introduce the topics of FGM and performance for development. Section 5 will assess existing performance programs working in FGM prevention and health promotion through covering existing research and giving an overview of existing programs through an in-depth internet search and interviews with professionals working at the programs. Section 6 will look at the ways in which performance is used to improve mental health outcomes of survivors of FGM.

Due to a lack of reporting of data and awareness of positive evidence of the arts in health interventions, there has been little translation from projects to policy[4]. Section 7 will report on current policy revolving around FGM and arts interventions and give recommendations on moving towards the solution.

2. Methodology

In order to achieve the aim of this research, the chosen methodology integrated literature review, expert background knowledge, quantitative data in health databases, and primary source surveys and interviews with organizations who work in the intersection of FGM and performing arts.

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Primarily, the aim was to establish a cause-and-effect relationship between performance and FGM, stipulating that the implementation of performance interventions prevents FGM and improves outcomes for survivors. In order to do so, both quantitative and qualitative data were required. The literature review allowed for synthesis of already existing research, though limited, to include a blend of quantitative and qualitative data. It was especially important that journals from countries where FGM is the most prevalent were included. Other sources outside of journals could include newspaper articles, in particular theater reviews, and books on the subject of FGM or performing arts in development and health.

Background research that was conducted also included speaking with experts in the broader area of arts and health. The interviews incorporated both short surveys to collect basic information and longer interviews encompassing a comprehensive review of the organization's work. Criteria for interview was kept to representatives of organizations with performance-based FGM interventions. Due to the limited number of research and practice in this specific area, no other factors for interview criteria were restricted. Interviewees were based in several different countries, including Egypt and Italy. As seen in Table 1, each interviewee answered a list of questions over a Zoom interview. The interviews were later transcribed in order to search for keywords and themes across programs and descriptions.

Table 1. FGM Organization Interview Guide

Main Question	Probes and Further Question
1.What kind of programming does your organization do at the intersection of performance and FGM?	-Please describe the programs in more detail. -Where do you work? -Approximately how many people have you served/how many people does each project reach?
2. Do you utilize a specific methodology in your organization?	-What does community participation look like during the programs? -Which populations do you try to reach? -Where did this methodology come from?
3.What are your measures of success?	-Does your organization have public reports of project evaluations? - What are the results of the programs in these measurements?
4.Have you seen any translation of work in performing arts and FGM into legislation? Is so, where?	
5.Where do you see the future of your programs going? Where do you see the future of this intersection going?	
6.Do you have any helpful article, book, or video recommendations for further research?	

2.1 Health Promotion Models and Theories

The analyzed and proposed interventions in this paper fall under the broad category of global health promotion, as they seek to enable people to "increase control over, and to improve their health"[7]. As such, it is important to consider health promotion theory and models in the application of performance as a tool to prevent FGM. The social-

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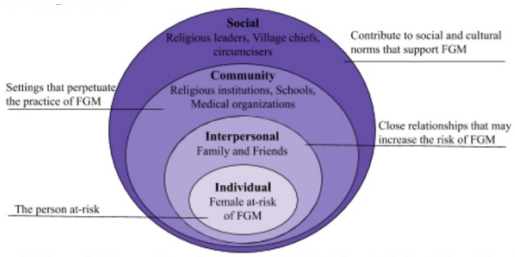
ecological model traditionally reflects the range of factors that put people at risk of violence. In Figure 1, this model was applied to reflect the different groups influencing the practice of FGM, centered on the individual as the female at risk of FGM, in order to understand the range of audiences for consideration in approach. Each group, with its own motivations and backgrounds, may require different approaches in order to shift beliefs and attitudes toward FGM.



Note. Adapted from the *Center for Disease Control and Prevention's Social-ecological model used for violence prevention* (The Social-Ecological Model, n.d.).

Figure 1. FGM Groups Social-Ecological Model

2.1.1 Individual and Interpersonal.

The theory of planned behavior (TPB) serves as a useful framework to evaluate the potential impact of interventions on the individual and the effects of interpersonal relationships. The focus of this model is behavioral intent, which predicts actual behavior. The theory states that behavioral intentions are influenced by the attitude towards a behavior, subjective norms, the perceived societal approval rating of the action, perceived behavioral control, and the individual perception of one's own agency[8]. The TPB has successfully been used to predict other behaviors and intentions including smoking, drinking, and health service utilization.

2.1.2 Community and Social.

Social network theory benefits the understanding of an individual's larger networks. This theory posits that social networks can positively or negatively influence an individual's health behaviors or outcomes. The network's effects are attributed to the types of connections an individual has, based on technical measures such as density, size, centrality, homogeneity, and frequency of ties. Types of interventions include enhancing existing networks through the development of new social linkages, creation of community health workers, and advancing community capacity building.

2.2 Measures of Success

FGM is very difficult to monitor, because it is unethical to check for its occurrence (invasion of privacy). Instead, data is reliant on self-reporting, which in turn, raises issues in the data collection. Data is underreported due to many factors, including a lack of criminal procedure in both developed and developing countries, the controversial practice of having children who have undergone FGM speak against their parents in court, and a lack of infrastructure for monitoring capabilities[9]. Furthermore, self-reporting has been recognized as very unreliable[10]. As such, organizations more frequently measure the success of interventions through qualitative means, including testimonials, increased rates of female education, and overall spending on healthcare. As performance-based interventions are variously implemented and rarely evaluated by the same means, it is necessary to interpret results as indicators, in lieu of definitive results.

This study will focus on these indirect measures in order to evaluate the success of interventions.

3. Background on Female Genital Mutilation

The World Health Organization (WHO) classifies FGM as "all procedures that involve partial or total removal of the external female genitalia, or other injury to the female genital organs for non-medical reasons"[11]. The practice is primarily carried out on young girls between infancy and age 15[11]. WHO is firm in stating that FGM has no health benefits and only harms girls and women. The United Nations Population Fund-United Nations Children's Fund (UNFPA-UNICEF) Joint Programme on FGM estimates

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that more than 200 million women and girls worldwide have been deliberately mutilated, specifically in 30 countries[12].

Out of the 30 countries listed, the rates of FGM differ per location. In countries such as Somalia, Guinea, Djibouti, Egypt, and Mali over 90% of women and girls aged 15–49 have undergone some form of FGM[12]. Table 2 (in the appendix) shows the aforementioned and other relevant countries with high prevalence rates. Meanwhile, other countries qualify as practicing FGM primarily due to the presence of diaspora communities[13]. Approximately 180,000 girls and women within large African diaspora communities in Europe are at risk each year[14].

However, as reported by *The Guardian* in early 2020, the number of FGM survivors could be much higher due to the failure of countries to record cases[15]. While the UNFPA-UNICEF report primarily records data from 30 countries, research from Equality Now, the End FGM European Network, and the US End FGM/C Network reports "hundreds of thousands" of cases across 92 countries in Asia, the Middle East, Europe, North America, and Latin America[15]. The lack of data reduces the urgency of public officials to act, resulting in harmful inaction from governments. In the United States alone, over 500,000 women and girls are survivors of FGM or at risk of being victim[15]. Beyond the European Union and UK, cases have also been found in Iran, Israel, and Russia. Even these numbers could be underestimated, as the data largely focuses on diaspora communities and ignores other prevalent ones, such as Christian communities in the United States[15]. The exact number of girls and women who have undergone FGM is still mostly unknown.

3.1 Types of FGM

The health risks and other adverse effects of FGM vary based on the type of FGM conducted[11].

1. Type I describes the partial or total removal of the clitoral glans and/or the clitoral hood.
2. Type II describes the partial or total removal of the clitoral glans and the labia minora, sometimes with the removal of the labia majora.
3. Type III is also known as infibulation, which is the narrowing of the vaginal opening by sealing it. The seal is created through repositioning and stitching.
4. Type IV includes other non-medical, harmful procedures to female genitalia, including nicking, piercing, incising, and more.

Estimates from 2004 predict that around 90% of FGM cases include Type I, II, or IV, and about 10% include Type III[16]. The present study is not concerned with the eradication of a particular type of FGM, but rather the entire practice, as all types are a violation of human rights and cause harm to females.

3.2 Types of FGM

The risks of FGM vary from physical to psychological to economic, with lasting negative effects. While the type of FGM, described above, determines all associated risks, overall health risks include severe pain, hemorrhage, genital tissue swelling, fever, infections, urinary problems, wound healing problems, injury to surrounding genital tissue, shock, and death[17]. Beyond these risks, long-term complications may include urinary problems, vaginal problems, scarred tissues, menstrual problems, and increased risk of childbirth complications, including newborn deaths, and psychological trauma.

Girls are expected to undergo FGM in order to avoid stigma and isolation from family[18]. Because of this, refusal to be cut can also lead to harmful socioeconomic effects. Overall, however, preventing FGM provides major benefits for women, communities and economies. The health outcomes of performing FGM results in high healthcare costs for the individual and the state[19]. Dr. Ian Askew, Director of WHO's Department of Sexual and Reproductive Health and Research, claims that FGM is extremely harmful to a

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country's economic resources. WHO reports that "the total costs of treating the health impacts of FGM would amount to $1.4 billion USD globally per year, if all resulting medical needs were addressed." FGM presents a significant economic burden for both the individual and the state.

3.3 Reasons for Performing FGM

3.3.1. Individual and Interpersonal.

There are many overlapping factors that contribute to the ongoing practice of FGM. People often tend to suspect that FGM is attributed to religion. Nevertheless, there is no evidence that supports this stereotype. In Egypt, it is a "centuries-old tradition," or "tribal ritual" embedded in the culture. Though there are no writings in religious texts that prescribe the practice of FGM, practitioners often believe that the practice has religious support[11]. Furthermore, in some locations, FGM is still more prevalent in certain religious communities. For example, in Burkina Faso, FGM is higher among Burkinabe Muslims than in other religious communities[20]. While stereotypes can conflate Islam and FGM, it is practiced by all major religions[21]. Furthermore, the practice of FGM predates the establishment of all major religions. Thus, while some religious communities may have higher rates of FGM, religion is not the origin or driving force for FGM.

The views of religious leaders vary: some promote it, others consider it irrelevant, and some actively contribute to its abandonment. Religious leaders and beliefs are featured in many drama skits and other advocacy efforts in order to target religious misconceptions and change attitudes. NCA Ethiopia has worked with faith leaders for over a decade, releasing joint statements condemning FGM, driving community conversations, and leading trainings. In a partnership with the Inter Religious Council of Ethiopia (IRCE), the umbrella organization for seven faith-based organizations in Ethiopia, Norwegian Church Aid released this joint statement in 2011:

"We religious fathers and leaders will seriously teach that female genital mutilation, early marriage, abduction and related harmful practices committed against women have severe consequences on the lives of our daughters, sisters, and mothers have no support in any religious teaching. We have reached an agreement for religious admonition to be administered on all people committing the practices in violation of the Call"[22].

This statement summarizes the efforts of anti-FGM organizations to collaborate with religious leaders. Performance-based interventions featuring religious leaders can be especially effective in targeting a common community misconception/assumption about FGM.

3.3.2. Fear and Taboo.

Ostensibly, reasons for its practice vary across region. In Ijurin, Nigeria, primary beliefs behind its practice include it being taboo not to be circumcised, an association of the clitoris and a woman being too sexually active is cultivated, the need to preserve a family's honor, a concept that the procedure widens the vagina to make birth easier, and lastly the idea that an uncircumcised woman is a sex slave[23]. Other factors involve the marriageability of women, a vague religious association — although, as mentioned previously, no religious text explicitly endorses the practice — local structures of power, and traditions of neighboring groups[11]. The practice is typically conducted by older women in the community who are traditional circumcisers[11]. More recently, the practice has evolved to include health care providers and medical professionals carrying out FGM, because of a belief that it could be safer than having it done by local circumcisers. Because this issue is largely cultural, interventions must be sensitive and empathetic in their approach.

The underlying causes for the perpetuation of FGM are reflected in Figure 2 through the socio-ecological model. This model emphasizes that no

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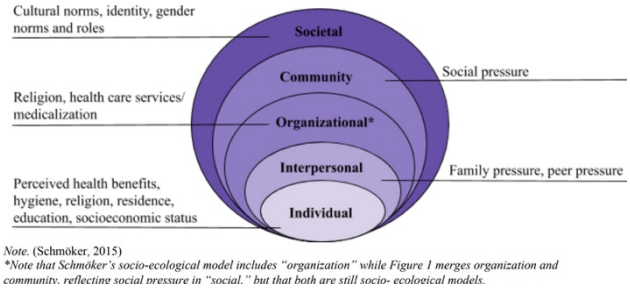
single factor can explain why FGM still exists or why some females face a higher risk of undergoing FGM[24]. Considered in this way, potential interventions can be specialized to target the varying social dynamics of each group. Strategies addressing a larger cultural norm on the societal level should look different from working with medical professionals to inform them of the harmful implications that come from medicalized FGM. This figure can be a guide to the different approaches an organization should take in order to be attentive to each level of organization.



Note. (Schmöker, 2015)
**Note that Schmöker's socio-ecological model includes "organization" while Figure 1 merges organization and community, reflecting social pressure in "social," but that both are still socio- ecological models.*

Figure 2. ***Socio-ecological model of the underlying factors of FGM***

On the individual level, ideas involving a person's perceived health, religion, residence, and socioeconomic status are all important for understanding the reasons for undergoing FGM. All of these factors control information and knowledge a person can access at an individual level. The interpersonal level reflects the communication of an individual's beliefs, which contributes to family or peer pressure. The organizational level reinforces the existing beliefs through medicalization and religion, validating the practice through the institutions' credibility. The community and society levels reflect the desire of an individual and family to "maintain ethnic identity and social unity" by carrying out what is recognized as a tradition in their community[24].

Other factors, such as gender, cross socio-ecological divides in their impact. The gender of the individual and genders of those in interpersonal interactions influence attitudes towards FGM. According to data compiled in Table 3 (in the appendix), women and men have separate attitudes towards FGM, with differing opinions on whether FGM should end. Additionally, the sexuality and gender of partners greatly influences the practice of FGM. In two studies it was found that men preferred women who were circumcised based on the belief that the men would have enhanced sexual enjoyment[24]. With the consideration of marriage, in a study in Somalia, 96% of men preferred to marry circumcised women, while just 2.8% said they would possibly consider marrying uncircumcised women.

The methodology in Section 2 explained the intervention models which match each group. In general, the reasons for carrying out FGM are largely based in tradition that originates from misconception. In this context, performance serves as an educational platform in addition to a method of addressing deeply ingrained cultural beliefs. The explanation of its function and evidence for its effectiveness are covered in Section 5.

3.4 Progress

The progress toward achieving fewer instances of FGM and shifting attitudes can be measured in a few ways: the number of girls and women who want FGM to stop, the number of men who want FGM to stop, the number of women ages 15–49 who have been cut vs other age groups, and the overall number of women who have been cut (per country or otherwise). It is difficult to gather country data on each national decline because of poor data collection. Neither the World Bank nor UNICEF have consistent data for countries over the past 2–40 years[25,26]. Still, UNICEF was able to conclude that among girls and women in high-prevalence countries, within the last two decades, the proportion of girls and women who want the practice to end has increased by 100%, as displayed in Figure 3[25].

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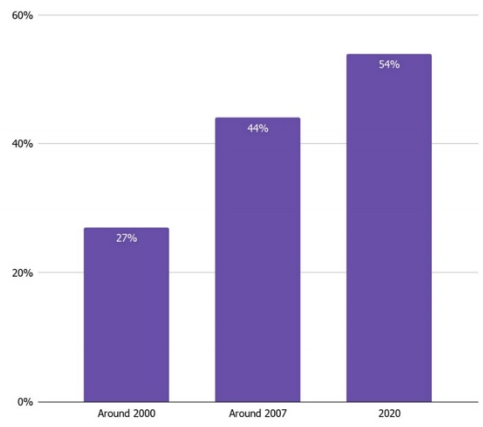

*Data gathered in 2020
Source: Female Genital Mutilation (UNICEF, 2020)

Figure 3. Percentage of girls and women aged 15 to 49 years familiar with FGM and believe that the practice should stop, in high-prevalence countries*.

At the same time, it is important to note that although the trend is still positive, the progress has slowed. Between 2000 and 2007, there was a 16% increase in reported cases followed by a 10% increase from 2007 to 2020. Furthermore, the data in Figure 3 include a wide range of ages, 15 to 49, though laws and interventions have changed significantly in the decades between those age groups. In most countries listed in Table 2, except for The Gambia and Somalia, the prevalence of FGM among 15- to 19-year-olds is recorded to be lower than the prevalence among the entire range of 15- to 49-year-olds[27]. These changes are most likely due to the activism, research, and legislation of the last few decades[28].

The inclusion of FGM in the UN Sustainable Development Goals (SDGs) reflects the prevalence of FGM and the ongoing global battle to end it. Target 5.3 of the SDGs attempts to "eliminate all harmful practices, such as child, early and forced marriage, and female genital mutilation"[29]. Established in 2015, the SDGs have until 2030 to do so. The practice has become less common in many countries, but in order to reach the global target of elimination by 2030, progress would need to be 10 times faster[30]. Moreover, progress is not yet universal. It is particularly difficult to address FGM in locations where prevalence is unknown.

4. Performance for Development and Health

"Performance for development" is not an established term within the arts, but one that this study will use to encompass the performative art forms beyond theater, including radio, film, puppetry, and public demonstrations. This term draws on "Theater for Development (TfD)" which is defined as "an alternative communication strategy which is people-centered and is deeply rooted in community development by empowering marginalized groups to consciously take up the onus of effecting some change within their immediate environment"[31]. Performance for development aims to provide people with an improved quality of life, in line with the SDGs mentioned above. This chapter will describe the theoretical frameworks that guide impact in each art form.

4.1 Prevention

TfD can encompass the art forms of drama, comedy, spoken-word, music, singing and/or dance, miming, and participatory or improvisation forms. This field is rooted in two concepts: a critical pedagogy and participatory theater[32]. The former was developed in the late 1960s and articulated by Paulo Freire, whose literacy campaigns for adult education in South America led to the development of a pedagogy of liberation. Freire proposes learning centered on the reality of the learner with dialogue between teacher and student. These values led to the formation of the Freirean dialogic model, which engages learners, facilitators, and the community in a critical reflection of its situation in order to create social change[32]. In his evaluation of TfD, Tim Prentki connected Freire's student-centered pedagogy to community-centered theater, or "theater which takes as its starting point an issue or set of issues that are revealed as important by research processes

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that set a premium on listening to the experiences of all sections of that community"[32]. Freire's work generates critical community consciousness and empowers its members to take action.

The second root, participatory theater, comes from the work of Augusto Boal, who built upon the work of Freire to bring theater back to styles more similar to those of medieval European carnival forms, before the ruling class commanded theater[32]. In his book *Theatre of the Oppressed*, Augusto Boal introduces forms of theater where "the spectator starts acting again," including forum theater, image theater, and invisible theater[33]. Boal describes the central purpose of participatory theater by explaining its function within illiteracy. He relays that theater can serve the oppressed as a language[33]. His design for this communication, named forum theater, involves the spectator assuming the role of the protagonist to change the dramatic action, try out solutions, and discuss plans for change. In this way, the performance is a "rehearsal for the revolution," as Boal famously declared. He adds that he believes that "theater is a weapon, and it is the people who should wield it"[33]. Boal is clear in his interpretation of theater as a tool by which people can create change.

Boal's model of participatory theater, as shaped by his design for the theater of the oppressed, has impacted the function of existing programs today. Hara TV 3, an interactive theater-based FGM intervention, describes interactive theater as breaking the "fourth wall" in order to "illustrate real-life political and moral debates"[34]. They explain that interactive theater gives the audience an opportunity "to become the main characters on the stage." This type of engagement is crucial because it allows audience members to empathize with the actors, linking their own lives and stories to the one being told. In this way, TfD prevents FGM through its ability to educate and empower survivors and their communities to speak and act out against FGM, proactively reducing the practice before it occurs.

4.2 Treatment

In the realm of treatment, the concept of drama therapy is central to the nexus of theater and treatment. The first theoretical account of drama therapy is based in Greek theater, specifically the tragedy[35]. Aristotle portrays tragedy as a catharsis which releases deep feelings[61]. Building on Aristotle's work, in 1857, Jacob Bernays proposed a theory that "catharsis" is a medical metaphor, connecting the purge of the soul through tragedy[33]. In a modern context, author Emma Brodzinski describes drama therapy as a psychological therapy in her book *Theatre in Health and Care*[36]. The drama therapist combines the art form with psychotherapy practice to enhance well-being by building trusting relationships, developing communication and social interaction skills, exploring feelings to overcome negative mental health effects, and developing creative skills for self-advocacy[37]. It is a creative and clinical procedure. That is, in the case of treating FGM survivors, drama therapy is primarily utilized as a method of mental health care, focused on increasing self-esteem and working through trauma.

Film is recognized as a method of providing a common language for communication in multilingual societies. Much like with theater in non-literate communities, film acts to bridge gaps, though its mass distribution is less accessible in rural villages. In his 1971 UNESCO report on the role of film in development, Peter Hopkinson notes the ability of film to "create a climate for practical innovation, stimulate the thirst for knowledge, and provide instruction, in particular fields, such as agriculture and health." Film, in its capacity to contribute to better health outcomes, can support educational efforts, target stigma through empathetic appeal, and foster awareness for a particular issue[66]. In the *Health Evidence Network Synthesis Report 67*, film is reported as being effective in reducing ethnic tensions and improving cultural competence, reducing pain and distress (through virtual reality relaxation), and

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improving parental attitudes towards LGBTQ-identifying children[4].

4.3 Awareness

Performing arts also have the ability to increase advocacy, reaching new audiences and increasing private donor funding to the cause of FGM. There are a plethora of shows and films depicting the story of FGM. In her play *Emotional Creature*, Eve Ensler features the monologue of a girl who runs away from her family to avoid undergoing FGM. The monologue depicts the girl praying for her god to spare her. These types of work may be the first point of exposure of many communities to FGM. In 2016, there was a popular play named *Cuttin' It* that toured in the United Kingdom. This play specifically exposes the prevalence of FGM in developed countries and introduces the audience to that specific experience in a "sensitive exploration"[38]. While many of these performances are exclusively shown in high-income countries, their influence on public opinion and increased attention is invaluable. Performance has the unique ability and great potential to cover a range of work in the fight against FGM, by using a community-based approach, providing education experiences, inciting activism, and including sensitization work.

5. Assessing Existing Prevention Programs

The theories of planned behavior and social networks effectively demonstrate that in order to intervene in a holistic manner that addresses all levels of the socio-ecological model as demonstrated in Figures 1 and 2, interventions must target planned behavior, change subjective norms, and establish community health workers or activists. These theories also connect to the ideals set forth by the developers of TfD, emphasizing the need to incorporate the whole community in a grassroots approach that meets their cultural needs, while pushing towards the abandonment of FGM. Beyond this overarching framework of health promotion, performance interventions should also meet the needs of FGM specifically.

Plan International, an independent development and humanitarian organization focused on advancing children's rights and equality for girls, presents seven ways to end FGM. NGOs that counter FGM use these methods to better understand the existing programs working in performing arts and FGM and what they accomplish. Note that the methods often do not exist in isolation of each other, but often cross over.

Out of the programs observed, many fall into two models. The interventions are either performance-oriented or workshop-oriented with performance aspects. In the performance-oriented design, the projects usually focus on a play, radio show, or other performance then incorporate a forum for discussion. The second design often features a central workshop oriented around performance with other components, sometimes concluding in a performance. Both models work as effective methods because of their connection to traditional health promotion frameworks, through their ability to fulfill the seven following methods of ending FGM.

5.1 Challenging discriminatory reasons for FGM

The first method is to challenge the discriminatory reasons underpinning the practice of FGM[3]. These include the need to control female sexuality and gender roles. By targeting these underlying assumptions, traditions can be challenged in a way that understands cultural perspective. Through interactive theater, drama workshops, and puppet shows, organizations fighting FGM target underlying beliefs about girls that lead to FGM. The different art forms depict alternate realities where women are empowered, allowing communities to imagine a future with new gender norms and treatment of women.

Tostan International, a West African-based development organization working directly with rural communities, does not focus specifically on FGM, but their programs report changes in FGM

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practices[39]. Their workshops primarily focus on education in democracy and human rights, which facilitate conversations about women's rights. Participants learn materials that encourages them to challenge preexisting notions and decide how they feel about FGM based on shared values and concepts of human rights. The participants engage with FGM by choice, after being introduced to the broader field of human rights and shape their curriculum through their own actions. Although Tostan did not intend to end FGM, between August 1997 and December 2009 its educational programs, which involved theater as a method of learning about human rights and encouraged public theater as a form of protest, challenged traditional notions on a widescale, fostering numerous collective declarations abandoning FGM.

Organizations successfully reproduce this methodology in other settings: Plan International works with Sahar Education, an international nonprofit providing education to Afghan girls, to produce educational puppet shows to target FGM in Egypt; ARC Theater works in East London to train teachers about FGM, targeting any biases that might exist and training them to account for the underlying reasons of FGM; and Active Voices uses dramas to address the needs of youth specifically[40, 41, 42].

5.2 Change Traditions

The decision to abandon the practice of FGM must come from the communities themselves, reflect a collective choice, be reinforced publicly, and be grounded on a firm human rights foundation[18]. In doing so, communities can direct their own social transformation, thus changing traditions so that individuals and families do not feel as if they are breaking away from their community by denying FGM. Through community workshops and programs, organizations involve all members of the community, including village leaders, religious leaders, circumcisers, and families. Tostan and Hara TV present two great examples of how these programs manifest and yield effective results.

Tostan's Community Empowerment Program exemplifies work that directly involves communities in the transformation of traditions. The class honed in on human rights, democracy, and governance and incorporated multiple teaching methods. Diane Gillespie from Tostan International believes that theater exercises in particular kept people coming to class, because they engage everyone involved[39]. Exercises include everything from simple "invisible ball" exercises, where participants pass an invisible ball to connect with each other, to writing and performing their own skits.

As a result of these classes, participants came to the understanding that FGM could change. Sessions in human rights and democracy, showed participants that their voices have a place in society and can effect change. Learning about an international human rights framework empowered participants to challenge existing social norms such as FGM[43]. In a report Gillespie wrote with her sister, the founder of Tostan, Molly Melching, on the transformation of Tostan's approach, they report that the culmination of a workshop in 1997 involved a community effort that collectively abandoned the practice of FGC[43]. The data collected reports abandonment in 4,121 communities in Senegal, 364 in Guinea, 48 in The Gambia, 34 in Somalia, and 23 in Burkina Faso[43].

Hara TV is a fast-paced, interactive, comedic project in Egypt that uses theater to educate people about FGM. Like the example in Ijurin, Nigeria, this project also incorporates community participation. While the show is performed by two actors from the project, the director asks the audience questions throughout the performance to ignite discussion. The article "Using comedy to combat a cruel tradition," describes the past performances of the Hara TV troupe. In one performance depicted, the group interacts with an audience of 40 circumcised girls varying from ages 13 to 20[1]. The director of the project states that the troupe's goal is to "use our performance to create

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an opportunity to talk about the difficult topic of female genital mutilation, beyond the confines of religion or medicine, in very practical terms." The performance comedically depicts a mother warning her daughter about the effects of not going through FGM, a girl being told what not to do and wear, and more. These scenes are followed by conversations where the audience is able to open up about their experiences, from not being able to play in the streets like boys to having many duties in the home. Actress Sherin Hegazy, who performs in the show, believes 70% of the message is communicated through the conversation after the show, explaining that in the conversation they are able to disagree, clarify misunderstandings, and answer questions from the audience[44].

After the event, one 18-years old female expresses, "Now that I've seen the play, I understand the problems circumcisions cause for girls." Another woman in the audience with a 13-year-old-daughter explains that while she has been thinking of having her daughter circumcised, after seeing the performance, she will not. Even though those in the audience had all already undergone FGM, the play encouraged them to stop the practice in the future. In this way, the play takes a grassroots approach, reaching communities through individuals within them.

After the director Nada Sabet sold her piece to the United Nations, they decided to fund an additional 160 performances in Egypt in 2014. Approximately 200 performances have taken place in Egypt since its commencement[34]. In a 2016 report "Hara TV: The Journey," written by Noon Creative Enterprises and UNFPA, the organizations report that in villages in upper and lower Egypt, the interactive theater activities achieved efficient communication of anti-FGM and anti-early marriage messages as well as establish the importance of creative methods of learning[44].

In a 2016 report on their programs, Noon Creative Enterprise explains its methodology behind Hara TV 3:

"As such their engagement is not based on laughing about FGM; it rather laughs at the many wrong facts, myths and misconceptions that communities hold onto to maintain the practice. Making those facts into a public and collective laughing matter, communities become accomplices in the change movement."

Noon Creative enterprise highlights the idea that FGM is most effectively abandoned by a community when they work together rather than individually[18]. Both Hara TV and Tostan work within communities to produce approaches that are non-judgmental and encourage collective action.

5.3 Educate girls on their bodily autonomy

Several recent studies have demonstrated that female education writ large is associated with a decline in FGM[45, 46, 47, 48]. Sanaba, a 24-year-old mother from Mali, who was one of the last girls in her family to undergo FGM, asserts, "No child who is well informed and able to stand up for himself or herself wants the practice of genital cutting to continue." As a mother's level of education rises, the likelihood that her daughter undergoes FGM declines[47]. Inversely, when girls undergo FGM they are more likely to drop out of school[56]. Plan International specifically emphasizes the need for curriculum teaching girls to understand their rights and autonomy over their own body[3]. Through creative expression, confidence building, and artistic empowerment exercises, performance can facilitate this learning for girls and women.

A core value of Tostan is dispelling the notion that people are unable to learn. Gillespie explained that some women who attend Tostan's workshops have never spoken outside of the home before and assume that they are "stupid" and cannot learn. Gillespie reports that when theater is incorporated into their practice, "people get so engaged in the plays they forget that they're speaking"[39]. This assertion also relates back to Boal's recognition of theater as a language to serve oppressed communities. In this way, artistic expression is

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uniquely positioned to engage previously untapped learning capabilities.

Beyond theater, Hara organizes music, dance, and visual art workshops for young children[44]. They present a perfect example of the workshop design at the beginning of this chapter. The workshop lasts three days, for a total of nine hours, and culminates in a final hour of a performance. Through singing instruction, participants learn songwriting, composing, and singing to produce songs focused on advocacy. The instructor of this program, Ahmad El Sawy, says "I believe the desired awareness was met along with the participants' acquiring a new skill…" Through dance, Hara indirectly addresses FGM without discussing it. Using dance to establish a relationship with their bodies, participants identify its dimensions and capabilities, gaining ownership and comfort with their physical self. One exercise asks participants to recall painful memories associated with their body parts, connecting girls understanding of FGM to their physical body, and empowering bodily autonomy.

The YouTube video "Ending Female Genital Cutting in Guinea" features a workshop presented by Plan International and AFAF, an NGO educating girls about the dangers of excision[49]. In the workshop, the girls learn about excision, reproduction, and more using song and music. The workshop utilizes a participatory and community approach based on dialogue. At the end of the program the students march, dance, and sing to campaign against FGM. As a result of this program, the village came out against excision. In this example, education translated to a public demonstration which increased awareness of the negative risks and FGM in a way that communicated to the whole village.

5.4 Speak out about the risks and realities of FGM

Through non-judgmental and non-coercive public discussion, reflection, and storytelling, communities affected by FGM can come to understand the risks of FGM[18]. The Girl Generation, the world's largest collective of organizations working together to end FGM in the current generation, recommends positive storytelling as an effective intervention, reporting that "sharing what is happening is essential to building awareness. Research shows that stories are more effective than facts, explanations, or arguments in influencing behavioral change[50]. Their effectiveness is attributed to their ability to transport the reader, engage empathy, garner attention, and leave a memory. As mentioned in Section 4, theater and film act as natural forms of storytelling, facilitating awareness as unique platforms for advocates against FGM.

The international non-profit organization Right to Play utilizes radio dramas to empower youth to speak out against FGM[51]. Girls in the program write and perform the radio dramas to share stories that work towards gender equality. In the feature radio drama, the story focuses on a teacher who stands up for a girl, Matinde, bullied for not being circumcised. In the end, the other children accept her decision. The young Matinde is depicted as a champion for girls' rights in her school. There is no impact measurement for this specific program, but Right to Play claims that through their programs, "more girls are finding their voice, claiming their right to education, and learning to defy dangerous traditions such as female circumcision and child marriage"[52]. They also train teachers how to build trusting relationships so "children gain the confidence to talk about threats to their safety... like female genital mutilation."

The Global Media Campaign, created by former journalists at *The Guardian*, works to end FGM through innovative media methods across seven countries/territories: Kenya, Mali, Nigeria, Sierra Leone, Somalia, the Gambia, and Puntland[53]. Their work is primarily focused on a grassroots approach that empowers activists through summits and workshops to provide them with training and education on the subject and in the use of film and radio resources[53]. In their Virtual Media Academy, created in May 2020 in response to COVID-19, they have created an extensive online library which includes films and

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webinars. This program was successful in attracting 500 new activists and 175 grants for media campaigns. The Global Media Campaign (GMC) reports reaching 870 million people through the work of their activists working both locally and internationally[53]. Examples of grant programs in Mali include a national slam poetry contest, YouTube education campaigns, and radio shows and debates. Their method of using film and radio to create FGM activists is an especially effective way of exposing the negative effects.

The Kapenguria Theater Group is an anti-FGM advocacy program in Kenya that combats the practice of FGM and its risks through theater and song on social media[6]. They are using this moment during lockdown to spread their message widely online, as they expect many teenagers are online more now. The group first records their skits and songs on CDs, then they deliver them to girls in villages to view and discuss. They identify lack of exposure as the primary reason for the continued practice of FGM. Like other programs, their interventions are centered around educating communities on the harmful effects of FGM. Mr. Walufa, the leader of the group, accounts that "many people still don't know that we are using digital methods in the anti-FGM drive"[6]. Furthermore, by involving community members in the arts programming, these programs create advocates against FGM, both youth and otherwise.

5.5 Spread understanding that religion does not demand FGM

Misconceptions regarding religion are often targeted in different performing arts campaigns that address existing beliefs. It is such an involved and prevalent component of existing beliefs, that it merits its own category. Furthermore, by including religious members of the community in the attendance or participation of the arts program, they are effective in addressing religious concerns.

Sadia Hussein, a graduate from the GMC, led a 10-day program in Kenya, featuring religious leaders condemning FGM on radio[54]. The program presented three key results:

1. More than one of four listeners changed their minds and no longer thought FGM was necessary;
2. 87% of respondents said that FGM had been discussed more than usual in the past month;
3. 100% of respondents could name at least two harmful effects of FGM after the media campaign, when only 67% could before the intervention.

In addition to impressive measured results, the media campaign also prides itself in having cost-effective interventions, described in further detail in Section 7 on financing. The media campaign plans to continue its work with over five new programs in the next six months.

Cuttin' It, the play by Charlene James that advocates for FGM survivors, combats assumptions about religion by featuring two girls of the same religion, one who undergoes FGM and one that does not[55]. This inclusion of religion in both girls' lives makes the statement that religion is not the cause of FGM. Performing arts interventions are not often centered on targeting religious misconceptions but have the ability to sensitively challenge established beliefs through its programming.

5.6 Address the secrecy that allows cutting to continue

Performing arts programs do not directly expose the secrecy of FGM, referencing that it occurs behind closed doors and is seldom the subject of public dialogue, but their public nature encourages open and candid discussion about the topic. For some, a language barrier prevents them from learning about the consequences of FGM and from advocating for themselves and their communities. After participating in the Plan International and AFAF workshop, referenced above, one girl praises the publicity of the intervention, saying, "seeing my mothers and grandmothers campaigning against excision makes

https://doi.org/10.48091/BNKO5024

me happy, because something that was once hidden has now come out"[49]. Public demonstrations are effective in drawing attention to an issue and engaging with non-literate cultures.

Diane Gillespie from Tostan International says "'Seeing is believing' is a huge thing in a non-literate culture. And female genital cutting is unseen." Tostan is specifically praised for their pedagogy, which builds on cultural traditions of the communities' oral tradition in West Africa. The oral tradition includes storytelling, strong memories, and a variety of languages. In the interview conducted with Gillespie, she explained why Tostan's work necessitates the use of theater[39]. When working in illiterate communities, it is important to rely on oral forms of education and communication, which have the added effect of being interactive and engaging. As mentioned in the previous section, theater often made it easier for participants to engage with the topics. While it is difficult to directly address the issue of genital cutting happening behind closed doors, by encouraging public activism and empowering people to speak on the topic, the reluctancy towards conversing about FGM can be addressed.

5.7 Keep pushing for FGM to be banned

Lastly, the seventh method is to "keep pushing for FGM to be banned." In its capacity for advocacy, described in methods 4 and 6, performance-based interventions push for policy change in FGM. In the interview conducted for this study with Chiamaka Uzomba, program director at Active Voices — an organization that tackles critical issues of health and development that has used theater as a form of youth activism against FGM — Uzomba spoke about her experience serving on a national technical working group on FGM in Nigeria. She relayed that within her position in Active Voices, she has the ability to affect the policy choices surrounding FGM[42]. Though Active Voices has only run one program utilizing theater, Chiamaka hopes to explore it further, once she has more funding, to advocate for a total ban on FGM.

The ability of the arts to impact the abandonment of FGM beyond just the local level, as demonstrated in many aforementioned cases, is incredibly evident through the use of a film in Iraqi Kurdistan. In 2013, two filmmakers in Kurdistan spent almost a decade persuading citizens to talk about the effects of FGM, including impacts on marital sex and family dynamics[57]. The activism of the film helped the Iraqi Kurdistan Parliament outlaw FGM in 2011. In the three years after it was outlawed, there was about a 60% reduction in the number of girls being cut in the autonomous region[57]. Section 7 focuses more on the translation of performing arts work in FGM to policy affecting broader change.

5.8 Results of Performance-based Interventions

Table 4 depicts the varying outcomes of the programs for those that gave any form of measurement. As previously mentioned, many of the programs did not include measures of success. Out of six observed programs/organizations, only two organizations recorded statistics of FGM abandonment following their programs. However, all eight reported positive results in reaction to their projects, either a reduction in reported cases of FGM, community commitments to end FGM, or individual audience proclamations of abandonment.

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Table 4. Results of FGM and Performance Programs, with their respective outcome measurements

Program	Country/ies where they work	Result	Year
Active Voices	Nigeria	Testimonial of community members asking for Active Voices to replicate the program, but no recorded data	N.D.
ARC Theater	United Kingdom	"Workshop evaluations found that the approach was highly popular and effective, with many scoring 100% on indicators of knowledge"	2013-2016
Associazione Italiana Donne Per Lo Sviluppo (AIDOS)	Italy, Belgium and other European countries, Burkina Faso, Egypt, Guinea, Mali, Mauritania, Senegal	One of their advocacy videos has been used by 5 African anti-FGM organizations in the last 2 years	2018-2020
Global Media Campaign (GMC)	Mali	Measures programs in an estimated total reach on people (varies from 600,000 to 2 million people)	2020
Hara TV III	Egypt	Reported proclamations to not carry out FGM by audience members	2014
Injurin Intervention (Adeseke, 2019)	Nigeria	Reported 0 cases after the intervention	2018
Plan International/AFAF	Guinea	The village came out against excision	2009
Tostan International	Senegal, Guinea, The Gambia, Somalia, and Burkina Faso*	Data collected reports abandonment of FGM in 4,121 communities	2010

*listed by highest to lowest number of communities that abandoned FGM
Note. (Uzomba, 2020; Fanelli, 2020; Brown and Porter, 2016; Global Media Campaign, 2020; Lehmann, 2014; Adeseke, 2019; Plan International, 2009; Gillespie and Melching, 2010)

5.9 Results of Performance-based Interventions

5.9.1. Monitoring and Evaluation

Monitoring and evaluation of performance-based interventions varied greatly among the observed programs. The methods included informal interviews/testimonials, focus groups, questionnaires, or nothing. All interviewees (representatives of Tostan International, AIDOS, and Active Voices) recognized monitoring and measurement as major challenges of implementing a performing arts program as a health intervention. Measurements are not consistent among art forms either. For radio shows, the measurement might take the form of estimated listeners, but surveys are difficult to conduct because the listenership is not directly recorded. If surveys were to be used, the radio show would have to be played for a controlled group, with the surveys conducted after listening. For a theater performance, there is a more controlled audience, allowing for post-show surveys and a post-

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program follow-up later on. The same is true for workshops in which the participants are contained.

Tostan International primarily relies on surveys and testimonials in order to measure attitudes towards FGM. The choice of these measurements is based on the idea that if a community comes to believe that a practice is harmful, then their attitudes will change, resulting in behavioral change. Unfortunately, the results of surveys are not publicly accessible. Gillespie reported example questions that might be on the surveys to measure prevalence: asking, for example, whether respondents think that most of the people in their community support cutting girls, and if they believe people approve of people who cut their daughters. The development of those questions relies on the idea that an individual is more likely to perform an action if they believe it is socially acceptable.

AIDOS, an Italian organization that combats FGM, by empowering women to create videos fighting against FGM, monitors their success by the number of organizations that use their videos and the views on their videos. The videos are posted on YouTube and periodically monitored for views. Their most popular video has 1,600 views. Without more context from other programs, however, this number is difficult to assess comparatively. Additionally, as behavioral change is a long process, it can be challenging to conduct effective data collection over time[44]. The lack of data reported out from the existing programs presents a challenge for program analysis. These challenges can be mitigated by partnering with local organizations to conduct long-term surveying of the community after the intervention has ended.

5.9.2. Funding

Another highly cited issue was funding — for example, one interviewee from Hara TV reported that the program closed in 2020 due to a lack of funding[58]. In light of the COVID-19 pandemic and subsequent government spending to combat its adverse impacts, there is less funding going towards anti-FGM programs. Uzomba from Active Voices and Fanelli from AIDOS both reported that their organizations are dependent on grants in order to conduct more programs[59]. The topic of funding, including the implications of this grant-based funding structure, will be explored further in Chapter 7.

5.9.3. Research

Throughout the research process, only one peer-reviewed study was found that measured the prevalence of FGM in a society after using performance as an intervention. Given the scarcity of research on the topic, the topic was investigated through other sources, such as interviews, articles, and video reviews. When there is very little to no previous reported knowledge on the effectiveness of an intervention, it is difficult to gain legitimacy for a program proposal. The lack of reporting and research in general also limits the potential for performance interventions to become more prominent in contributing to the abandonment of FGM.

5.10 Case Study: Addressing the Menace of Rape and Female Genital Mutilation through Theater for Development, Nigeria

In 2018, Adefolaju Eben Adeseke conducted one of the first case studies to research the effects of TfD as an intervention in FGM. This study stands out compared to the short-term methods of evaluation used by program interventions because of its long-term and wide-ranging evaluation. Adeseke's study, "Addressing the Menace of Rape and Female Genital Mutilation through Theater for Development" deployed TfD in two Nigerian communities in order to educate people on the issues of FGM and violence against women. In Ijurin, Nigeria, those conducting the study first identified and acquainted themselves with the issue, then developed a solution through the creation of a drama skit[23]. The cast of the play incorporated traditional songs, community actors and singers, and the elderly.

https://doi.org/10.48091/BNKO5024

The research culminated in a performance for the community that highlighted the story of two girls, one who goes through FGM and one who does not and stays in university. The play directly contrasts the two as a warning and motivation to the audience, while simultaneously educating the viewers on the negative effects of FGM. After the show, the audience, consisting of chiefs, men, women, and children, participated in a post-performance discussion that sparked reflection on the practice of FGM. At the end, Adeseke writes that, "Four of the female circumcisers said that now that they have seen the outcome of FGM in the performance, they would definitely stop the practice." This verbal report is the first measure of success. When the study conducted a "follow-up" a month after the performance, the researchers found that there was no record of circumcision in Ijurin since the facilitators had left. Another check-in three months later displayed the same results. Although the study does not include a numerical record of FGM practiced before the theater program was implemented, Adeseke writes that Ijurin was selected as a site because of "the serious negative impact FGM can have on the female children…" and reports that the study's findings "revealed that many children had died in the past in the village due to hemorrhage." From these explicit mentions of FGM in Ijurin, it is clear that FGM was prevalent beforehand and posed a high risk for young girls. Therefore, the post-intervention shift to zero incidence of female circumcision is remarkable.

In this study, Adeseke proposes the ability of performing arts to intervene in FGM, exemplifying many of Plan International's methods of ending FGM. The study challenges the discriminatory reasons of FGM, changes traditions, and speaks out about the risks and realities of FGM through the performance of the play, which combats misinformation and preconceived notions of FGM. Additionally, by drawing community members to the production, including chiefs, the intervention brings the topic of FGM to the forefront of community interest. The abandonment of FGM in the community also mirrors the push to ban FGM worldwide. While the study does not provide sufficient pre-intervention data, it serves as a strong example of how to conduct monitoring and evaluation after a program, thereby helping solve one of the most significant challenges in the field.

6. Treatment of FGM Patients using Performance Therapy

"*If health is about adaptation, understanding, and acceptance, then the arts may be more potent than anything medicine has to offer*"[60]

Thus far, the ability of performance to act as preventative intervention targeting FGM in a health promotion context has been highlighted. Another area of unexplored potential for performance and health is the capability of performance to serve as therapy. The research study, "Use of Drama Therapy in Unlocking the Voices of Female Genital Mutilation Among the Kenyan Maasai" by Zippora Agatha Okoth, is one of the only existing studies on this specific intersection of drama therapy and FGM. Nonetheless, the *Health Evidence Network Synthesis Report* on the role of the arts in health reports the effectiveness of art therapy in the case of other health issues, the effects of which are transferrable to the area of FGM. After reviewing both of these documents, it is evident that performance therapy is effective due to its ability to create a safe environment for survivors to process their trauma and to ease mental health symptoms.

6.1 Safe Spaces, Storytelling, and Trauma

In her PhD thesis, Okoth argues that drama therapy, through techniques such as story-telling, role playing, song, and dance, can be used as an effective tool to empower the voices of FGM survivors[35]. After data collection, she reports that theater is particularly effective because "it creates a safe and playful environment where the survivors are able to act out their anxieties, fears,

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and mental conflicts due to FGM"[35]. In this way, theater acts as a buffer for survivors to tell their stories. This strategy works to both help survivors process their emotions and to dispel the stigma of the trauma that arises from being subjected to FGM. The results of the study conclude that after the program, the survivors regained self-confidence, self-esteem, and trust.

In general, expressive art therapy is reported to be effective in helping children and adults experiencing the effects of traumatic experiences, including abuse (physical, emotional, sexual), addiction to drugs, and accidents[63]. FGM falls under the categories of physical, emotional, and sexual abuse. Okoth's study took place at a girls' primary school shelter in Kenya for survivors and escapees of FGM. Girls aged 9–15 participated in drama therapy exercises, including physical warm-ups, imagination exercises — in which participants imagine themselves in different settings or doing different activities — games, and storytelling[35]. Overall, the study found several techniques to be helpful in bringing about therapeutic healing to FGM survivors. Through dramatic reenactments, including improvisation and role playing, the participants were able to look at the situation from new perspectives and feel united in their emotions as a group. After testing dance as a method of breaking down boundaries created after FGM, researchers discovered that dance and music as drama therapy techniques proved to be valuable as they helped the participants feel comfortable with their bodies, have physical contact with one another, and dance in front of each other[35]. The removal of these inhibitions allowed the participants to feel more open with their personal experiences. Furthermore, storytelling and games encouraged self-exploration and empowerment.

In order to measure the results of the study, the researchers used the Rosenberg Self Esteem Scale to measure change in attitudes towards self, relationships, and the future. The scale uses a four-point scale from "strongly agree" to "strongly disagree" to assign a value to statements reflecting study found that surveyed girls' attitudes towards their lives, relationships, and their future all improved after the project[35].

6.2 Mental Health Impacts

The *Health Evidence Network Synthesis Report 67* on the role of the arts in health includes successful examples of the ability of drama therapy programs to address psychological impact. The report cites art therapy (art form unspecified) as improving self-confidence, self-esteem, and self-concept with children who had experienced sexual abuse, developmental delay, or emotional disturbance[4]. For example, dance therapy encourages healthy living by incorporating confidence-building and physical exercise — weekly dance therapy over several months improved body consciousness, body image, and confidence in obese youth. When considering mild to severe mental illness, music and dance therapies were able to reduce anxiety, depression, and other symptoms in children and adolescents. An example of the physical impact of drama therapy is observed in stroke patients. Listening to music and dancing was found to help the development of new neural pathways, improve upper and lower-limb motor function, muscle weakness, balance, grip strength, cadence, and more. Furthermore, music therapy reduced blood pressure in diabetes patients[4].

There are many other instances of the remarkable effects of drama therapy on physical and emotional wellbeing. Unfortunately, the understanding of performance therapy's impact on FGM survivors is limited. More research should be conducted on the potential physical impact drama and other art therapies can have on this population. Despite the lack of research, though, the wide-ranging impact that performance therapy has been proven to have on similar traumatic experiences holds encouraging implications for interventions regarding FGM.

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7. Further Considerations and Recommendations

7.1 Government Involvement

Despite the preceding emphasis on grassroots interventions that create community and local programs with the ideals of performance for development, it is also essential that subsequent interventions involve all levels of government in order to generate sustainable and institutional change. While grassroots movements foster awareness of and direct engagement with the threats posed by FGM, these interventions should be followed by codification of their values into law. In this way, the relationship between the government and the broad movement against FGM have a significant, cyclical relationship. As the movement gains traction, it puts increasing pressure on the government to enact meaningful change — when the government incorporates these reforms, it further empowers and validates the movement. An example of this relationship includes the HIV/AIDS movement in the United States, in which case activists succeeded in influencing the government to address the epidemic. The combination of grassroots interventions and institutional change can be very effective in targeting FGM.

In general, politicians tend to be hesitant to establish policies relating to the intersection of arts and health overall, not because they do not care about the health issues, but because of the lack of legitimacy and recognition. Lara Dose, the director of the National Network for Arts in Health, observed in 2005 that, "Politicians appear to be sufficiently brave to set targets high enough to raise eyebrows and expectations, but too scared to try anything innovative to ensure these are achieved"[36]. Her criticism comes after the Department of Health in England launched a review of arts and health, and politicians were unresponsive. In 2006, the network ran out of funding and it was suspended. The next paragraphs will examine the ways in which the government has supported FGM and performing arts efforts and give recommendations going forward.

7.1.1. Emergency Preparedness

In order to address the increasing rates of FGM described in the introduction, it is essential that preparedness and response plans incorporate FGM in their considerations regarding gender-based violence. With the current spate of instances of FGM owing to the COVID-19 pandemic, future emergency responses should anticipate the extra burden and plan ahead to prevent it. They can do so through health promotion activities and community awareness initiatives that incorporate the techniques enumerated in Section 5.

7.1.2. Law

Out of the countries with the highest reported rates of FGM, seen in Table 2, 11 of 16 have made FGM illegal in national policy. According to End FGM Network's March 2020 report, "Female Genital Mutilation/Cutting: A Call for a Global Action," of the population of 92 countries where FGM is practiced, about 55% (approximately 51 countries) specifically outlaw FGM through national law, either through a specific anti-FGM law or through domestic laws[17].

In this report, the legal status of FGM in countries is split into three categories: countries that have enacted a special national anti-female genital mutilation/cutting (FGM/C) law; countries in which FGM/C is specifically mentioned/covered within other laws; and countries that do not specifically address FGM/C within their laws. The layers of policy are complex and cross over the realms of constitutional, national, and local law. Table 5 reports the law coverage from the countries listed in Table 2. Note that all countries included have national representative surveys.

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Table 5. *FGM laws in the countries reporting the highest incidences of FGM*

Country	Specifically address FGM within their laws*	Constitution Prohibits FGM	National legislation (NL) criminalizes act of FGM	NL criminalizes participation of medical professionals in acts of FGM	Government has a strategy in place to end FGM	Source
Somalia	No**	✓	X	X	X	Somalia, 2018
Guinea	Yes, other laws	X	✓	✓	✓	Guinea, 2018
Djibouti	Yes, other laws	X	✓	X	✓	Djibouti, 2018
Egypt	Yes, other laws	X	✓	X, prohibits act without penalty	✓	Egypt, 2018
Mali	No	X	X	X	✓	Mali, 2018
Eritrea	Yes, national law	X	✓	✓	✓	Eritrea, 2018
Sudan	Yes, other laws***	X	X	X	✓	Sudan, 2018
Burkina Faso	Yes, other laws	X	✓	✓	✓	Burkina Faso, 2018
Gambia	Yes, other laws	X	✓	X	✓	Gambia, 2018
Ethiopia	Yes, other laws	X	✓	X	✓	Ethiopia, 2018
Mauritania	Yes, other laws	X	✓, but only on those under 18	✓, not directly	✓	Mauritania, 2018
Guinea-Bissau	Yes, national law	X	✓	X, not directly	✓	Guinea-Bissau, 2018
Senegal	Yes, other laws	✓	X	✓	✓	Senegal, 2018
Nigeria	Yes, other laws***	X	✓	X	✓	Nigeria, 2018
Kenya	Yes, national law	X	✓	✓	✓	Kenya, 2018
Uganda	Yes, national law	X	✓	✓	✓	Uganda, 2018

*Note. *(Female Genital Cutting, 2020)*
***The Somali Constitution expressly states that the "circumcision of girls is prohibited." However, there is no national legislation that expressly implements this Constitutional provision, and there are no known instances where FGM/C offenses have been prosecuted under general criminal provisions (Female Genital Cutting, 2020).*
****While Sudan and Nigeria have specific criminal provisions against FGM/C, these provisions do not apply in all states within the country* (28 TOO Many, 2018).

Other countries where FGM is still legal include Norway, Greece, Poland, Hungary, and 15 states in the United States. As mentioned previously, because the numbers in these countries are not regularly reported, it is difficult to assess how many women and girls are affected[17]. This murky legislative environment reinforces the need for governments around the world to establish laws specifically banning the practice of FGM. Nada Sabet, co-founder of Noon Enterprise — which runs the Hara TV Project — explains the importance of policy through her quote:

"My biggest challenge in the struggle against female genital cutting is the passing of legislation that will outlaw it. Then, and only then, will we be able to put an end to FGM. But it will take a lot of lobbying and advocating, at all levels: in government, in parliament, and in villages and communities"[1].

https://doi.org/10.48091/BNKO5024

Governments must explicitly ban FGM in order to strengthen and legitimize the implementation of FGM interventions overall, which naturally includes performance-based interventions.

Beyond the establishment of laws, it is essential to consider the effectiveness and enforcement of those laws. Despite every country in Tables 2 and 5 having a strategy in place to end FGM, the prevalence of FGM is still high in the countries listed. The unambiguous gap between the enactment of laws and the practical enforcement of those laws demonstrates the necessity of interventions that target the root causes of FGM's persistence. Uprooting a harmful societal tradition requires sensitivity and care, which is effectively accomplished through performance.

In the interviews with AIDOS and Active Voices, both organizations stated that work was being done in their respective countries, Italy and Nigeria, to allocate funds to FGM abandonment, but not specifically to performance interventions. Italy passed a law in 2007 that enacted guidelines to health and social work professionals working with migrants from countries where FGM is practiced[63]. In the same year, the Italian government drafted strategic plans aimed at programming initiatives and measures. These campaigns comprised of a documentary, theatrical play, radio shows, TV ads, and more, conducted by seven different Italian anti-FGM organizations, including AIDOS[59]. The involvement of the Italian government in anti-FGM policy and budgetary allocation illustrates the multifaceted role that the legal environment can play in the implementation of anti-FGM performance programs.

7.1.3. Funding

As mentioned previously, one of the most significant challenges in performance-based program implementation is receiving proper funding for anti-FGM programs in general. In 2019, Ethiopia spent approximately 78.21 million USD on healthcare costs associated with FGM[64]. This cost is projected to grow to 123.4 million USD in 2048 if Ethiopia does not pursue abandonment more vigorously. If they were to pursue full abandonment, they would lower this projection to 48.03 million USD in 2048. Partial abandonment would lower the costs to about 91.37 million. Considering the heavy financial burden FGM causes for governments, it is within their best financial interest to pursue cost-effective interventions.

There is very little data reporting the cost effectiveness of performing arts programs due to an overall lack of research in the area. Even so, the projected costs presented by the GMC's radio and television campaigns are provided in Table 6 to demonstrate the cost efficiency of similar programs.

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Table 6. The cost per person for radio and TV anti-FGM interventions in Mali

Intervention	Estimated Reach (in number of people)	Total Estimated Cost (USD)	Cost per person (USD per one hundred thousand)
1 Hour Radio Show with education specialist	600,000	$650	$108.33
1 Hour Radio Debate with medical doctor	600,000	$750	$125
1 Hour TV Debate on Mali TV	1,200,000	$750	$62.50
20 anti-FGM ads over 2 weeks on popular tv show, Emission Baroni	2,000,000	$2,700	$135
National Slam Poetry Contest	2,000,000	$2,300	$115
Average cost per person per one hundred thousand			$109.66

Note. (Why Media Could be The Fastest, 2020)

Since radio shows, TV ads, and televised debates account for four of five of the interventions in Table 6, and are primarily indirect interventions, it follows that their successful implementation could contribute to a partial abandonment of FGM. It is projected that if Ethiopia does not put more funding and effort into abandoning FGM, then the country would have about 39.24 million cases by 2048[64]. If the Ethiopian government pursues partial abandonment, however, they can lower this projection by 9.86 million cases to have 29.38 million cases in 2048. Applying the average cost per one hundred thousand people of a GMC campaign ($109.66) to the projected number of reduced cases if partial abandonment is adopted (9.86 million), it would cost about $10,812 to accomplish this result. In theory, Ethiopia would only need to spend $10,812 USD to save 32.03 million USD in health care costs attributed to FGM. Though these calculations are fairly simplistic, even if the interventions were only half as effective as projected then it still would only cost $10,812 USD to save about 16 million dollars in healthcare costs. The Ethiopian government has a limited scope to put FGM policies into practice due to constraints in budget allocation and human resources dedicated to targeting FGM, but with cost-effective strategies such as those of the GMC, the government can work with NGOs to lower their prevalence of FGM[65].

Valentina Fanelli, a program officer working on FGM, gender stereotypes, and gender- based violence at AIDOS, projects that in the future, in light of the COVID-19 pandemic, the already severely limited funding will be diverted to support economies and emergency efforts[59]. Even so, she also projects that as a result of social distancing measures, more funding will go into media (TV, film, radio) work targeting FGM. Fanelli further explained that AIDOS' programs are grant-dependent, as is the case for many other anti-FGM organizations. Since grant-based funding is relatively inconsistent, this situation puts a great deal of these organizations in vulnerable positions.

These organizations would benefit greatly from an increased government budget allocation, which would provide a more consistent stream of funds. Governments are also financially incentivized to invest in performance interventions in the immediate term, to save on health care costs related to FGM later. In order to support existing performance interventions and contribute to their expansion in the future, governments should allocate more funds to the intersection of arts and health.

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7.2 Recognition through Research

One of the primary barriers to effective and innovative work at the nexus of performing arts and the treatment and prevention of FGM is the lack of research. There was only one accessible study on the effects of performing arts on the prevention of FGM and only one on the effects of drama therapy. Furthermore, the former paper included little to no data to substantiate the positive change that it reported. In order for the performing arts to become relevant as an intervention in FGM, there must be more field studies conducted with rigorous and standardized methods of measurement and data reporting. Only then will there be an increase in its legitimacy in academia, policy, and beyond.

In general, there is a lack of research and therefore authority surrounding the role of arts in health. To combat this phenomenon, the same recommendation as above applies with a suggestion that the research agenda be elevated to the multilateral realm. Performance must be considered as a tangible and legitimate health intervention by the WHO, UN, and national health institutions. Improved recognition will translate to increased funding for performance-based programs, hopefully leading to an increase in organizations incorporating the arts in their work.

As of December 2020, only about eight organizations report any link to conducting performing arts intervention to target FGM. In general, these organizations lack standardized measurement and evaluation mechanisms for their programs. Thus, individual organizations should prioritize research in conjunction with their programs in order to increase the overall breadth of research in this area. Furthermore, it is recommended that the WHO establish a formal working group to study and evaluate existing arts and health programs, including those that focus on addressing FGM. While *Health Evidence Network Synthesis Report 67* was a great start, it was published only by the regional Europe office. This work must be brought to all regions of the world.

7.3 Program Pedagogy

More NGOs, governments, and local health organizations should learn from the success and creativity of the pedagogy of TfD and incorporate more elements thereof in their interventions. These organizations can collaborate with practiced applied theater experts to structure their programs and train volunteers. Furthermore, techniques utilized in performing arts therapy and performance for development should be taught to NGOs through large-scale initiatives and workshops. While the health and performing arts fields are — with only a few notable exceptions — isolated from one another, they must initiate cooperative dialogue in order to tackle culturally embedded issues such as FGM.

8. Conclusion

An in-depth literature review and assessment of performing arts interventions demonstrates the ability of performance-based interventions to potentially improve conditions for those at risk of FGM as well as survivors. Drawing upon the socio-ecological model, performance can positively impact outcomes by creating awareness, challenging existing norms and underlying assumptions, and empowering girls and community members to speak out against FGM. Performing arts interventions stand out in their natural community engagement, wide potential reach, emotional approach, and cost effectiveness.

In order to pursue performance interventions, there must be a commitment from NGOs to further utilize performance, more robust research and analysis, and increased funding for organizations that facilitate programs in this field. In order to commit to eradicating FGM, both local and national governments must set it as a priority. The criminalization of FGM and enactment of anti-FGM laws legitimize the cause, leading to the opportunity for increased research. With more research, the field can grow in strength, and therefore practice. All of the

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interventions require funding, of course, which is easier to allocate and distribute when there is significant proof of the effectiveness of performing arts interventions. From this paper, the hope is that others will be inspired to research not only the intersection of FGM and performing arts but also the ability of performing arts to effect real change.

The ideal performing arts intervention works within social, local, and institutional networks to create an environment that does not allow for the practice of FGM. In order to further the field, it incorporates effective and thorough monitoring and evaluation before, during, and after the central program. The program itself does not attack culture and tradition, but rather, through its art, invites the audience into a dialogue about FGM. With the precious ability to incite empathy, empower, and educate, performance is a humane solution to this significant global health challenge.

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Policy Brief: Comparison and Recommendations for State COVID-19 Responses of New Mexico and Utah

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Policy Brief: Comparison and recommendations for state COVID-19 responses of New Mexico and Utah

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Abstract

New Mexico has seen a steady increase in SARS-CoV-2 (COVID-19) cases, but compared to Utah, New Mexico has kept case numbers low due to important mitigation policies passed by Governor Michelle Grisham. The goals and priorities of each state's governor contributed to the policies and severity of restrictions in each state.[1, 2] Governor Grisham's mask, mass gathering, and interstate quarantine policies are restrictions that she deems necessary in order to mitigate the spread and save lives.[2] Governor Gary Herbert has valued economic recovery as opposed to virus mitigation, which is reflected in his lenient restrictions.[2] In the immediate future, Governor Cox, Governor Herbert's successor as of January, should enact the same mask, mass gathering, and interstate quarantine policies as New Mexico; both states should then reassess the list of states that people need to quarantine from, given that 21 states hit records for 7-day average of COVID-19 cases, as of October 11, in order to get transmission under control.[3] Additionally, if COVID-19 transmission is slowed, mass gathering restrictions can be reassessed to allow for larger gatherings but should stay tightly restricted until that point.

Keywords: COVID-19, Policies, New Mexico, Utah

1. Background

The two states being compared are New Mexico and Utah. These two states were chosen for comparison because they are two adjacent states in the U.S. with differing gubernatorial policy responses to the COVID-19 pandemic. The gubernatorial responses are important policy actions to observe and study, regarding the pandemic in the U.S. This is because there has been limited and fractured federal response to the pandemic, leaving much of the leadership to pandemic response in the hands of state governors.[4] Governor Michelle Grisham of New Mexico has enforced stricter policies, such as a statewide mask mandate, in order to slow the spread of the SARS-CoV-2 virus in New Mexico.[5] Governor Gary Herbert had not enacted the same statewide level policies to slow the spread of the virus, enacting policies that affect only certain locations and counties.[2, 6] Due to the differences

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between the two policy paths, the states have seen quite different results in terms of COVID-19 mitigation.[7] The differences in New Mexico and Utah's gubernatorial policies regarding COVID-19 demonstrate the various choices states are taking; the choice is either maximizing mitigation of the virus through statewide, strict measures or giving more freedoms to citizens, while slowing the virus's spread to an extent and ensuring the economy is able to stay open. These two adjacent states are an example of these two policy strategies, and the comparison of the outcomes of these policies serve to show which plan is more beneficial to states and the subsequent effects the laws have on lowering daily cases.

2. Comparison of Epidemiology, Policies, and Outbreaks

Compared to Governor Herbert's policies, Governor Grisham's COVID-19 policies have been far more stringent. New Mexico's stricter policies have translated into comparatively lower current and overall COVID-19 infections throughout the pandemic.[7] Governor Grisham passed a statewide mask mandate on May 15, 2020, requiring a face covering in public at all times except when eating, drinking, exercising, or advised otherwise by a physician.[5] The strict statewide face covering mandate was instituted to slow the spread of COVID-19 following evidence that the disease can be transmitted through spit and water droplets. Face coverings create a cloth barrier between people, and in the case of coughing, sneezing, or spreading water particles while speaking, the covering would reduce transmission of the virus.[8] A study measuring the effectiveness of U.S. mask mandates showed a significant decline in the growth rate of COVID-19 cases after a public face covering mandate was issued, further demonstrating the importance of Governor Grisham's decision.[9] Conversely, Governor Herbert instituted a policy for mandatory use of face coverings within state buildings.[6] This was later paired with a mask requirement in K-12 schools.[10] Utah's new Governor, Spencer Cox, who has replaced Governor Herbert, said in a recent debate that Utah had yet to enact a statewide mask mandate because it was unlikely to be followed and that "it doesn't make that much of a difference".[11] Cox's statement directly contradicts the findings in the previous study; these included that the growth rate of the virus continued to decline after a mask mandate was enacted and that U.S. states with statewide face covering mandates had a greater decline in the growth rate of COVID-19 compared to states that did not sign the same mandates.[9]

Another difference in the choices made by the two governors is the capacity restrictions passed regarding mass gatherings. Suspension of mass gatherings is a mitigation strategy that helps early in pandemics when medical countermeasures may not be adequately researched and available for use.[12] Governor Grisham has passed a policy on mass gatherings that limits an enclosed space gathering to 5 people; this law lasted from March 23 to August 28, when it was increased to a limit of 10 people on August 29, 2020.[13] Since large gatherings can facilitate transmission due to the lack of social distancing, this order was meant to limit such gatherings and slow the spread of COVID-19.[12] In May, Governor Herbert passed a law that limited mass gatherings to 20 individuals, a policy that was soon replaced by a reopening plan in June 2020, prioritizing the reopening of businesses.[2] Medical experts and epidemiologists were cut out of the process of drafting reopening guidelines, which led to policies that allowed indoor gatherings of 3,000 people and outdoor gatherings of 6,000 people.[2, 14] Governor Herbert claimed that the relaxed COVID-19 mitigation policies on mass gatherings avoided enacting overly strict policies on less affected counties in

https://doi.org/10.48091/LYQC3557

Utah. His decision led to a ranking system in which counties were rated by transmission risk (low, medium, and high), with the lowest tier counties being allowed gatherings of up to 3,000.[2] The choice to allow for such large mass gatherings most likely contributed to a rise in cases in Utah, which can be seen in the uptick of cases during the same time frame. Between June 1 and July 18, daily cases state-wide rose from 197 to 858.[7] This is because mitigation of a pandemic level virus, like COVID-19, is impossible without stopping mass gatherings through policy choices.[12] In comparison, with New Mexico's more restrictive policies in place, daily cases state-wide only rose from 111 to 280 between June 1 and July 18.[7]

A third difference in gubernatorial policy is the differences in quarantine requirements for air travelers to each state. Air travel is a large source of importation of COVID-19 to new areas, especially through asymptomatic travelers.[15] Governor Grisham passed a quarantine policy, on March 27, 2020, which requires air travelers to New Mexico to self-quarantine for a minimum of 14 days.[16] The policy was adapted to limit COVID-19 transmission from individuals arriving from states with high transmission rates and daily case counts and prevent unnecessary deaths due to such spread.[1] This mitigation strategy is fueled by CDC guidance indicating that air travel can lead to COVID-19 infection due to extended time spent in security lines and terminals, as well as the lack of social distancing on crowded flights.[17] Conversely, Governor Herbert passed a policy on April 8 dictating that any individuals, 18 years of age, arriving by air or road must fill out a declaration of entry form but does not require quarantine of individuals.[18] The goal of this policy was to contact trace individuals coming to Utah who could possibly spread COVID-19. The aim was to allow citizens to enter Utah without forcing them to quarantine upon arrival, but this is a less effective policy measure than quarantining due to the fact that Utah does not have enough contact tracers to handle the current active case numbers in the state.[19] There are currently 8.32 total contract tracers per 100,000 residents in the state of Utah.[19]

Governor Grisham's three outlined policies enacted to slow COVID-19 were strict due to the fact that she believed policies requiring quarantine, masks, and mass gathering restrictions limited transmission pathways of the virus; she believed this was essential to save lives and reduce hospitalizations that were overwhelming state hospitals.[1]

Governor Herbert chose not to make uniformly strict and statewide policy decisions, since some counties were less affected by the virus.[2] However, the virus has not been and cannot be contained by county borders, so uniform policies are needed to ensure transmission does not continue to flare up in some parts of the state, with a possibility of spreading to the rest. Governor Herbert made policy decisions based on state hospitals' capacities to handle cases, instead of effectiveness of mitigation strategies.[2] Moreover, Governor Herbert also drafted policies based on the capabilities of state contact tracers, emphasizing that the policies were focused on the state's capacity to respond to outbreaks, rather than mitigating transmission.[2] Governor Herbert's policy decisions, such as allowing up to 3,000 individual mass gatherings inside, were aimed at aiding businesses that could not survive under lowered capacity limits.[2] Since these laws were passed, Utah's new unemployment claims fell by 78% in early July, in comparison to the peak in claims that occurred in April.[2] In July 2020, Utah's unemployment rate was at 4.5%, after reaching 10.4% in April.[21] In comparison, New Mexico's unemployment rate reached 12.7% in July 2020.[21] Utah's policies have lowered the unemployment rate and prioritized economic recovery. However, this recovery has occurred at the expense of mitigating the virus.

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The effectiveness of Governor Grisham's policies can be seen in the state's case numbers; the outcome of New Mexico's policies has led to lower case numbers, compared to Utah.[7] New Mexico had 485 daily cases of COVID-19, as of October 9, with a seven day average of 320 cases; Utah had 1,332 daily cases, as of October 9, with a seven day average of 1,162 cases.[7]

3. Proposal for Future Coronavirus Policy

In the next month, Utah should echo the stricter policies of New Mexico and start by passing a statewide policy that makes facial coverings necessary in public, in order to be in accordance with CDC guidance.[8] A mask mandate would be beneficial for lowering numbers in the state because evidence has found that a mandate can slow the growth rate of COVID-19, with the decline in cases increasing the longer the mandate is in place (Lyu and Wehby 2020). Additionally, Utah should require travelers coming from out of state to quarantine for 14 days because 21 states have reached their records for 7-day average of new COVID-19 cases, as of October 11.[3] Travelers, especially those arriving via air travel, from outside the borders of Utah can possibly spread COVID-19 through asymptomatic transmission, introducing the virus to new populations and parts of the state.[15] Since cases are rising in states across the U.S., travelers from outside the borders of Utah pose a risk to Utah's citizens; the need for a mandatory quarantine policy is further intensified because of Utah's lacking contact tracing workforce.[19] Moving forward, if states are able to get case counts under control, Utah and New Mexico should reassess the transmission risk of some states and make an updating list of states from which travelers are allowed to enter without quarantine, due to the low case count of the state from which travelers come. As mentioned, mass gathering restrictions are necessary to slow the spread of a pandemic, and therefore Utah should institute a statewide mass gathering restriction of 10 people, similar to New Mexico.[12] Mass gatherings have been shown to exacerbate the spread of COVID-19, as the virus transmits easily in crowds where social distancing of six feet apart is not followed or attainable.[12] As time progresses, New Mexico and Utah can reassess case counts in their states, and if the virus has been controlled and spread mitigated through policy decisions, they could possibly increase the number of individuals per mass gathering.

3. Conclusions

As shown, Governor Grisham's mask, mass gathering, and interstate quarantine policies have contributed to the amount of COVID-19 cases in New Mexico being lower than that of Utah. Governor Herbert's policies reflect the fact that the governor values economic recovery over virus mitigation.[22] Utah's governor should implement the same three policies outlined for New Mexico, in order to better mitigate the spread of COVID-19. If instituted, both states can reassess the strictness of their interstate quarantine, updating the list of states that people need to quarantine from; this should only occur if states can decrease case numbers. With the hope of more research and COVID-19 control, mass gatherings may be reassessed and possibly relaxed depending on Utah and New Mexico's ability to slow the spread of COVID-19.

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Interplay of Nicotine and Social Stress Mediate Dopaminergic Neuron Firing in the Ventral Tegmental Area —Nucleus Accumbens Pathway, Contributing to Stress and Depressive Mood Disorders

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The Interplay of Nicotine and Social Stress Mediate Dopaminergic Neuron Firing in the Ventral Tegmental Area - Nucleus Accumbens Pathway, Contributing to Stress and Depressive Mood Disorders.

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Abstract

Nicotine use and social stress have a complex interplay, which has been shown to be mediated by cholinergic neurons in the ventral tegmental area (VTA). Social stress is often comorbid with nicotine consumption, and the presence of either stress or nicotine use significantly increases the risk of developing the other. In fact, it has been shown in mice that nicotine injection is sufficient to increase susceptibility to social defeat, a reliable model for stress and anxiety-like behavior. Stressful events can molecularly remodel cholinergic synapses, inducing the production of more cholinergic transporters and increasing the number of nicotinic receptor binding sites. One way stress and nicotine remodel cholinergic synapses are through long-term potentiation (LTP) in cholinergic pathways in the VTA, enhancing the experience of stress and the effects of addiction. Despite both primarily acting on the $\alpha 7$ subtype nicotine receptor, nicotine and stress induce LTP in vastly different ways: nicotine acts quickly via ligand-gated ion channels while stress activates a slower hormonal-induced G-protein coupled receptor pathway. These findings suggest that dopaminergic VTA neurons may be a useful therapeutic target for depression, anxiety, and other stress-related disorders. Deep brain stimulation has preliminarily shown to be a potential therapeutic treatment for untreatable depression, especially when it targets the medial forebrain bundle within the VTA-NAc pathway. Sleep patterns are also partially regulated by dopaminergic VTA neurons, and sleep deficits may contribute to social stress and other depressive symptoms. The role of nicotine dependence in stress-related mental illnesses is especially important to consider given the recent increase in adolescent nicotine use with the advent of vaping. Adolescents already have an increased risk for developing mental illnesses, and it is important that young people are made aware of the potential psychological harms of nicotine use.

Keywords: $\alpha 7$ subtype nicotine receptor, cholinergic synapses, long-term potentiation, nicotine, stress, nucleus accumbens, and ventral tegmental area

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1. Introduction

According to the National Institute on Drug Abuse, nicotine is classified as a highly addictive substance, ranking among other drugs of abuse such as cocaine, amphetamines, and heroin.[1] The US Department of Health and Human Services estimates some 20% of all Americans are addicted to nicotine, whether by smoking cigarettes, chewing tobacco, or vaping.[2] While many—up to seventy percent each year—will try to quit, only around three percent will succeed.[3] The smoking rate is doubled in individuals who have mood or stress disorders, like clinical depression, and although these individuals will attempt to quit at the same rates, their chances of success are even lower than their neurotypical counterparts.[4] Each condition heightens the risk of developing the other: depressed individuals are more likely to begin smoking and experience more potent withdrawal symptoms when they try to stop, and smokers have a higher chance of becoming depressed at some point in their lifetimes than non-smokers.[5] This pattern seems to follow for other drugs of abuse as well, as it has been reported in both human and animal studies that stress is sufficient enough to significantly increase the likelihood of drug self-administration and/or relapse following a period of abstinence from the drug.[6] The difficulty that many face in trying to quit using tobacco products and the comorbidity between stress disorders and nicotine dependence can be explained in part by the biological effects nicotine and stress have on certain areas of the brain, particularly in the reward pathway.

The brain's reward pathway - the dopaminergic mesolimbic system - typically functions to reinforce survival-promoting behaviors, such as eating, or positive social interactions, producing positive feelings, and serving to motivate such behavior.[7] Drugs of addiction, however, act on the same pathways to produce their characteristic "high" and reinforce drug seeking-behaviors and dependence.[7] Nicotine, for example, is an agonist of the endogenous neuronal nicotinic acetylcholine receptor (nAChR), an ionotropic receptor type found widely dispersed throughout the CNS. nAChRs have been found to be critical in the modulation of dopaminergic activity throughout the mesolimbic system.[8]

Several brain regions have been linked to the encoding of reward-related behaviors, including the nucleus accumbens (NAc), ventral tegmental area (VTA), amygdala, and hypothalamus, with a particular emphasis on the dopaminergic projections from the VTA to NAc. The VTA and NAc are strongly implicated in both nicotine addiction and many stress and mood disorders.[5, 9] The variety of inputs that both the VTA and NAc receive - glutamatergic, cholinergic, peptidergic, and serotonergic - function to modulate dopaminergic neuron firing between the VTA and NAc (Figure 1).[10] At the core of encoding reward-related behaviors in these two regions are the distinct modes of the firing of the VTA dopaminergic neurons innervating the NAc: tonic and phasic. Tonic firing is low-frequency and regular, whereas the phasic mode is characterized by high-frequency bursts of firing. Cholinergic signaling - especially as mediated by nAChRs - is critical in facilitating the switch between VTA neurons' tonic and phasic modes and, therefore, in encoding reward-related information and behaviors.[10] Further, both stress and drug addiction have been reliably shown to be key in determining the basal firing rate for dopaminergic neurons.[10] Combined, this evidence points toward an important connection between stress, addiction, and cholinergic signaling in the dopaminergic mesolimbic system.

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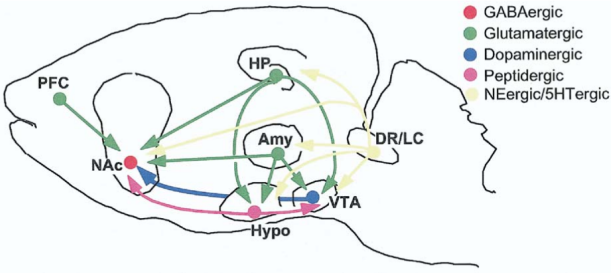

Figure 1. A variety of complex neural inputs modulate dopaminergic signaling from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). The illustration above shows a simplified summary of several well-established circuits that influence mood and have been implicated in the dysregulation of mood. Glutamatergic areas include the prefrontal cortex (PFC), hippocampus (HP), and amygdala (Amy), where the dorsal raphe/locus coeruleus (DR/LC) mainly transmit serotonin and norepinephrine (5HT, NE). The hypothalamus (Hypo) also influences the VTA and NAc with peptidergic inputs. (Figure taken from Nestler and Carlezon, 2006).

Like all complex neural systems, the ability of the mesolimbic system to encode nuanced information relies on the amount and type of afferent input it receives. nAChR subtypes are differentially expressed on different types of neurons throughout the VTA and NAc; depending on the subunit composition of the receptor, its affinity for endogenous or pharmacological agonists varies. These receptors' unique expression patterns lend each region the ability to integrate a variety of signals and encode complex behaviors and are differentially implicated in the pathology of both nicotine dependence and stress. This paper explores these cholinergic influences in the mesolimbic system and the convergence and divergence of the pathologies of nicotine and stress, implicating mechanisms of long-term potentiation (LTP) and exploring potential therapeutic targets.

2. Nicotinic Receptors and Social Stress' Role on the VTA

Interestingly, mood disorders and social stress have also been shown to affect dopaminergic signaling in the same pathways.[9] Several studies have found that the effects of nicotine use and stress can exacerbate each other, independent of the withdrawal effects common in nicotine addiction.[12] Morel et al., 2018 found that this bidirectional relationship between nicotine and stress is likely mediated by dopaminergic (DA) VTA neurons.[5] Because the etiologies of stress-related and depressive symptoms are often quite varied and complex, studying these in animal models can pose a difficult challenge.

Both nicotine exposure and social stress have been shown to increase the frequency and bursting activity of VTA DA neuron firing.[12] Interestingly, this study found that the effects of stress seem to depend upon the same nAChRs that nicotine acts on. Specifically, both nicotine and stress interact with the $\alpha 7$ homomeric and $\beta 2$-containing heteromeric nAChRs, the two primary nAChRs in the brain. $\alpha 7/\beta 2$ double knockout mice who experienced the social defeat (SD) paradigm did not show the same increased VTA DA neuron firing as wild type mice who experienced SD.

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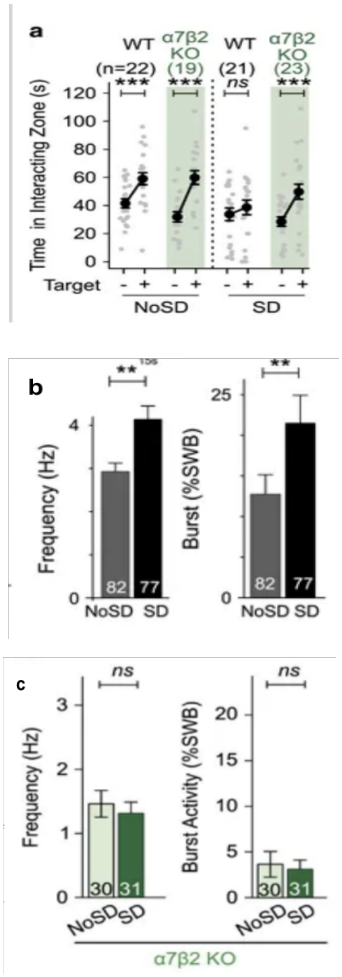

Figure 2. Knockout of nAChRs eliminates the effects of social stress on VTA DA neurons and social behavior. The 7/ 2 knockout is a knockout for both of the primary nAChR subtypes in the brain. For graph a, less time spent in the interacting zone is taken to mean higher effects of social stress on the mouse. The frequency and burst activity measurements seen in graphs b and c are measurements from VTA DA neurons. Graph b shows VTA DA neuron frequency and bursting activity in mice without the nAChR knockout, while graph c shows VTA DA neuronal activity for mice with the knockout. (Figures taken from Morel et al., 2018.5).

Additionally, knockout mice did not exhibit social aversion, contrasting the typical results of SD on wild type mice (Figure 2). Corroborating this information suggests that nAChRs in the brain are key to both the cellular and the behavioral effects of stress. Unsurprisingly, nicotine injection also failed to induce increased VTA DA neuron firing in knockout mice, confirming that stress and nicotine interact with the same nAChRs.

Furthermore, mice given acute nicotine along with subthreshold SD (SubSD) exhibited much higher VTA DA neuron excitability compared to control mice who experienced SubSD with no nicotine administration. This demonstrated a clear remodeling of nicotinic synapses on the VTA. Mice who underwent SD also had a greater number of cholinergic transporters and nAChR-binding sites, indicating a comprehensive effect of stress on nicotinic pathways in the VTA.

Morel et al., 2018 employed PNU, an $\alpha 7$ nAChR positive allosteric modulator, to explore subtype-specific effects. PNU-treated mice who experienced SubSD exhibited DA VTA firing increases equivalent to that found in mice experiencing the full SD paradigm. Additionally, these mice exhibited social aversion quite similar to those undergoing the full SD paradigm. Mice who underwent SubSD and were pre-treated with NS9238, a $\beta 2$ nAChR positive allosteric modulator, exhibited DA VTA firing and behavioral changes similar to WT mice who underwent SubSD. Thus, it seems that activation of the heteromeric $\beta 2$ nAChR does not significantly modulate the effects of stress. Both PNU and nicotine administered via a cannula led to increased social aversion when paired with SubSD, confirming that this stress-nicotinic relationship was occurring specifically in the VTA.

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These results implicate α7 nAChRs as the primary receptor upon which stress has its synaptic effects in the cholinergic VTA system. α7 nAChRs can therefore be considered as possible therapeutic targets for stress and mood disorders going forward.

Interestingly, stress and nicotine both seem to have a significant effect on long-term potentiation (LTP) of synapses on DA VTA neurons. Mice who underwent SD were found to have an increased AMPAR/NMDAR ratio for their DA VTA neurons, signifying LTP occurring at these synapses. AMPAR/NMDAR ratio serves as a marker of LTP, as the insertion of AMPARs, and thus an increase in AMPAR/NMDAR ratio, is the primary biomarker of LTP. LTP at these synapses helps to complete the picture of the cholinergic-stress-VTA pathway as it likely contributes to greater firing rates and bursting activity in DA VTA neurons for mice under stress. Mice treated with PNU also experienced increased LTP on DA VTA neurons (Figure 3).

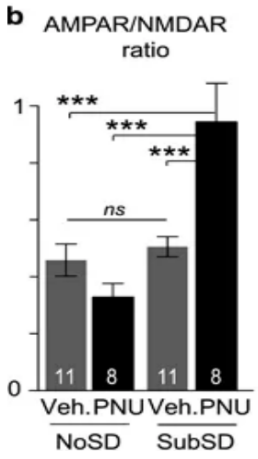

Figure 3. Administration of PNU, an α7 nAChR positive allosteric modulator, to mice increases LTP in VTA DA neurons following the SubSD paradigm. Interestingly, mice did not experience increased LTP when given PNU in the absence of SubSD exposure, implying the enhanced LTP here is indeed primarily caused by the effects of stress. (Figures taken from Morel et al., 2018.5)

Changes in LTP may be the main way in which stress and nicotine remodel VTA DA synapses. They both initiate molecular changes which result in AMPAR insertion into VTA DA neurons, leading to an increase in the firing rate of their neurons and likely mediate the behavioral changes associated with stress. Being that these neurons are highly implicated in addiction, LTP at these neurons may also increase the susceptibility of an individual to addiction, nicotine or otherwise. This implicates stress as a risk factor for addiction and also implicates nicotine use as a risk factor for increases in stress response and potentially mood dysregulation.

3. Synaptic Mechanisms: Role of LTP in the Reward Pathway

Morel et al., 2018 found that dopamine firing is likely modulated through LTP as a response to nicotine and/or stress. Here, the mechanisms of modulated dopamine firing through LTP are examined. Through this mechanism, LTP amplifies the reward associated with dopamine release in the mesolimbic pathway. Both stress and nicotine affect the signaling strength of excitatory and inhibitory synapses on dopamine neurons in the VTA through NMDAR-dependent LTP and $GABA_AR$-dependent LTP, respectively.[12, 13] The mechanisms by which stress and nicotine alter these pathways are very different, but these same pathways play a large role in addiction and reward association.

NMDAR-dependent LTP is crucial for increasing the strength of excitatory synapses. Glutamate signaling on dopaminergic neurons in the VTA drives this process through the reinforcement of excitatory synapses via insertion

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of AMPARs postsynaptically.[14] This LTP pathway involves both AMPAR subtype GluR1 and NMDARs (Figure 4). When glutamate is released from the presynaptic terminal, it binds to GluR1 receptors on the postsynaptic terminal. The binding of glutamate onto GluR1 allows the influx of sodium (Na^+) and potassium (K^+), which depolarizes the postsynaptic cell. Once the membrane is depolarized, the magnesium (Mg^{2+}) voltage-dependent block on NMDA receptors is released and glutamate binds to these receptors with the help of a glycine or serine cofactor. Once glutamate binds to NMDA, there is an intracellular influx of calcium (Ca^{2+}), which activates Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) through Ca^{2+}-dependent autophosphorylation. Following this, CaMKII phosphorylates GluR1 to increase conductance and targets stargazin-like transmembrane AMPAR regulatory protein in order for AMPA localization and clustering to occur, leading to an increase in AMPARs.

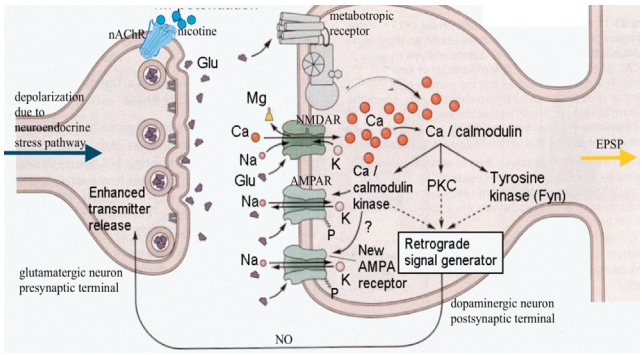

Figure 4. NMDAR-mediated LTP pathway.[15, 16] (Adapted from Dr. Mann.)

The way nicotine and stress contribute to this LTP pathway differs. In the VTA, some nAChRs are located on the presynaptic terminals of glutamatergic neurons.[17] Once nicotine binds to nAChRs on the presynaptic terminal, it allows for the influx of Na^+ and Ca^{2+}, which depolarizes the presynaptic neuron. Depolarization causes further Ca^{2+} influx, which mediates the docking and release of glutamate. Following its release, glutamate binds to AMPARs on the postsynaptic membrane of dopaminergic neurons, leading to NMDAR-mediated LTP as described previously.[18]

For stress-induced NMDAR-mediated LTP, G-protein coupled (GPCRs) glucocorticoid receptor (GR) signaling is crucial.[19] Exposure to acute stress leads to a sympathetic nervous system response in which adrenaline is released from the adrenal medulla.[20] Following this, there is an increase in noradrenaline (NA) in the CNS. With the increase of NA in the brain, corticotropin-releasing factor (CRF) neurons in the hypothalamus are stimulated, which stimulates the anterior pituitary, and adrenocorticotropin is released. This activity in the hypothalamic-pituitary-adrenal (HPA) axis elicits the release of corticosteroids from the adrenal cortex into the bloodstream. These corticosteroids can pass through the blood-brain barrier, and once in the brain, they bind to GRs. GRs in dopaminoceptive neurons of the nucleus accumbens (NAc) communicate with dopaminergic neurons in the VTA through glutamatergic interneurons.[21] When the corticoids bind to these GRs on dopaminoceptive neurons, glutamate release through this positive feedback pathway excites the dopaminergic neurons in the VTA, which when stimulated leads to LTP. While nicotine and stress both increase DA VTA firing, nicotine acts quickly through an ionotropic receptor while stress acts slower on a metabotropic receptor that requires many different neuroendocrine pathways.

Conversely, both nicotine and stress inhibit $GABA_A$R-mediated LTP, which leads to decreased inhibition of dopaminergic neurons in the VTA (Figure 5). This form of LTP is characterized by an enhancement of inhibitory postsynaptic potentials (IPSP) via increased

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GABA release.[22] When GABAergic neurons release GABA from their presynaptic terminals, GABA binds to $GABA_AR$s on the postsynaptic terminals of dopaminergic neurons in the VTA, hyperpolarizing these neurons via an influx of intracellular chloride (Cl^-). Following numerous IPSPs, there is a rebound depolarization of the membrane, and, subsequently, voltage-gated Ca^{2+} channels (VGCC) on the dendrite are activated. With the activation of VGCCs, there is an influx of Ca^{2+}. Furthermore, when glutamate activates NMDARs, $GABA_AR$s in neighboring synapses may be potentiated. The influx of Ca^{2+} is crucial for the mechanism of LTP. It is suspected that this increase of intracellular Ca^{2+} leads to the release of nitric oxide (NO), a retrograde messenger. NO activates presynaptic soluble guanylate cyclase, which produces cyclic guanosine monophosphate (cGMP). cGMP activates the cGMP-dependent protein kinase, which increases GABA release from the presynaptic neuron. When this type of LTP is inhibited, GABA release decreases, resulting in less inhibition on DA neurons in the VTA. Research shows that there is an impairment of this form of LTP following the injection of nicotine and acute stress.

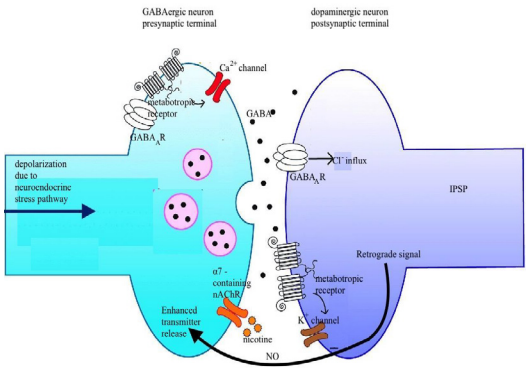

Figure 5. $GABA_AR$-mediated LTP pathway.[23] (Adapted from Govindpani et al.)

The mechanism by which nicotine and stress lead to inhibition of $GABA_AR$-mediated LTP is not completely known. α7-containing nAChRs are localized on the presynaptic terminals of GABAergic neurons in the VTA.[13] When nicotine binds to these nAChRs, the GABAergic presynaptic terminals desensitize quickly. This leads to less release of GABA, which inhibits the LTP pathway on postsynaptic terminals of DA neurons in the VTA. For acute stress, the glucocorticoid pathway described in NMDAR-mediated LTP contributes to reduced GABA firing.[24] The initial response to this acute stress is an increase in GABA firing mediated through the NO-cGMP-PKG pathway, but the long-term effect of this acute stress is an inhibition in this form of LTP. After sustained hyperpolarization, there is a shift in Cl^- reversal potential, making GABA become excitatory rather than inhibitory. Although more research needs to be performed to elucidate the specific pathway, both nicotine and stress affect this LTP pathway.

These two LTP neural mechanisms play a key role in how nicotine and stress increase DA VTA release on a synaptic level. By increasing excitatory input on dopaminergic neurons via NMDA-receptor mediated LTP and decreasing inhibitory input on dopaminergic neurons via GABA-mediated LTP, there is an increase in excitatory postsynaptic potentials (EPSP) on the dopaminergic neurons, leading to enhanced dopamine firing. With greater DA firing, the reward association increases as the mesolimbic pathway of reward is more active. Since nicotine and stress have similar results on DA VTA firing via different pathways involving nAChRs and GRs, respectively, showing that nicotine and stress exacerbate each other. With nicotine and stress's underlying LTP mechanisms, which modulate the firing rates of DA VTA neurons, long-term nicotine use and stress can both have detrimental effects on the excitation of DA neurons and transmitter release; in this way, increased

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dopamine signaling underlies the neural mechanism for addiction. Furthermore, with LTP changing neural connections, this can lead to effects such as depression during withdrawal of nicotine due to not enough stimulation of dopamine pathways in the brain.

4. Future Directions: Decreasing Dopamine Firing as a Potential Therapy

Morel et al. 2018 demonstrate that social defeat triggers increases in VTA DA neuron spontaneous activity. This is exacerbated by nicotinic binding to the $\alpha 7$ nAChR in the VTA. Both the frequency and burst activity increases significantly in mice exposed to SD and nicotine. This novel finding demonstrates that local VTA exposure to nicotine and SubSD is sufficient to trigger social aversion. Given that activation of nAChRs has an effect on DA VTA signaling, DA signaling is implicated. Altogether, this data suggests that dopamine signaling is necessary for the behavioral manifestations of social stress. Preventing this increased dopamine firing could, therefore, be a potential therapy for social aversion, which has been established to be a symptom in depression.

To test how dopamine modulation directly impacts the VTA, Chaudhury, et al. 2013 selectively altered levels of dopamine in the VTA using optogenetics to test social avoidance in order to evaluate depression-like symptoms in mice.[25] While Morel et al. found that nicotinic binding leads to alterations in DA signaling, it did not directly establish that modifying DA signaling changes social aversion-like symptoms. This is why optogenetic inhibition of dopamine in the VTA is a powerful tool to investigate the potential therapeutic opportunities for altering dopamine signaling.

Induction of phasic firing in VTA neurons in the VTA-NAc pathway in mice that were undergoing SD induced a susceptible depressive phenotype with increased social avoidance. Mice that were resistant to social avoidance, after being exposed to SD, became depressed when DA phasic firing was optogenetically induced. Stimulation of phasic dopamine firing corresponded to a rapid onset of the susceptible phenotype after stress exposure. The fact that social stress was a precursor to dopamine alteration in rendering a depressed phenotype shows the context-dependent alterations that occur. This finding emphasizes that dopamine is being directly acted on, which confirms that VTA DA neurons serve in stress-response modulation, contributing to a depressive-like phenotype.

Deep brain stimulation (DBS) therapies to the VTA could be an effective way to reduce dopamine firing and mediate anti-depressive effects. The circuit and context-specific nature of this pathway make it a prime therapeutic target. If dopamine activity is restored to tonic firing following stress, social defeat scores decrease.[25] In DBS surgery, an electrode is stereotactically implanted into specific neuroanatomical regions, and electrical stimulation is provided via a pacemaker-like stimulator.[26] DBS is used in movement disorders such as Parkinson's disease. Recently, DBS has been studied for neuropsychiatric disorders.

Open-label studies have convincing data that suggest that DBS is effective in mediating antidepressant effects in individuals who do not respond to conventional treatments for depression.[26] Data from clinical studies of the neurophysiological effects of DBS suggest that electrical stimulation leads to both short-term and long-term effects on firing rates and patterns. Although the direct mechanism of action by which DBS works is unknown, it has been proposed that DBS inhibits neurons being stimulated, potentially by modulating the electrical activity of potassium and sodium channels. Another

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proposed mechanism of DBS is that it disrupts neuronal signals and activity from being propagated in neural pathways.

Recently, a review was published that investigated DBS for treatment-resistant depression.[27] The review highlights clinical trials where DBS was used to treat depression in different brain regions. The results from this review emphasize the importance of the medial forebrain bundle (MFB)—a pathway between the VTA and lateral hypothalamus—as a potential therapeutic target for DBS in the VTA-NAc pathway (Figure 6).

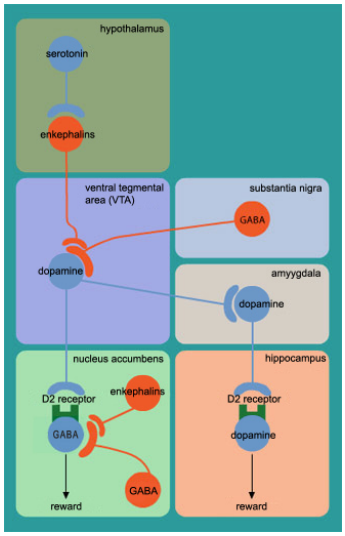

Figure 6. The medial forebrain bundle (MFB) connects the VTA and lateral hypothalamus. The VTA is connected to subcortical and cortical prefrontal regions. The mesolimbic reward pathway is contained within the MFB, composed of dopaminergic axons that project from the VTA to NAc.[28] (Figure taken from McGill University)

The nerve fibers of the reward circuit are located in this pathway, which is composed of dopaminergic neuron axon projections that go from the VTA to the NAc. Even though this pathway involves brain regions beyond the VTA - NAc connection that Morel et al. focus on, it is useful to evaluate DBS as a potential therapy.

Despite being limited to 11 patients, findings indicate that DBS of the MFB could induce antidepressant effects. Short-term bilateral stimulation of the MFB led to a rapid reduction of depressive symptoms in 6 out of 7 patients in one study. In other studies, the antidepressant effects were also consistent, with no evidence of cognitive impairment following months of stimulation. The hypothesized mechanism of action for these encouraging results is that DBS activates the mesocorticolimbic system by modulating dopaminergic glutamatergic and neurotransmission. The circuit that achieves this neurotransmission may be that DBS alters the firing of glutamatergic fibers from the mPFC to the VTA, which indirectly modulates dopaminergic firing at the VTA. Even though the mechanism has yet to be fully elucidated, the results show that DBS reduces antidepressant-like symptoms without yielding severe or common side effects.

DBS is a promising future direction given that phasic dopamine firing in the VTA – NAc pathway leads to depressive features in mice, which optogenetic alterations reduce. The study cited makes evident that DBS is surgically viable and potentially modulates the VTA – NAc projection. If DBS can alter dopamine in the VTA, as proposed, then this therapy is especially promising.

5. Sleep Disorders: Contribution of Dopamine in the VTA - NAc Pathway

It has been shown that nicotine users experience decreased sleep quality.[29] Since Morel et al. focus on how nAChRs mediate the combined effect of stress and nicotine by altering the activity of DA neurons in the VTA, this activity may also affect sleep disorders. In addition to the stress and depression effects of altered DA signaling in the

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VTA, DA regulates motivational processes via this pathway. DA neurons project from the VTA to many brain regions (Figure 6). To study how the VTA—NAc pathway specifically alters sleep, researchers used chemogenetic and optogenetic manipulations with polysomnographic recordings.[30] These recordings are a diagnostic test used in sleep medicine to comprehensively record physiological changes during sleep. Chemogenetics is similar to optogenetics but uses chemically engineered molecules and ligands instead of light and light-sensitive channels (opsins). These methods provide incredible techniques to study the relationship between neuronal activity and behavior.

VTA dopamine neurons have been found to undergo changes in firing in rapid eye movement (REM) sleep and non-REM (NREM) sleep, making them interesting candidates to further examine. Through complex analysis of DA neuron activity in VTA projections, researchers found that these neurons are altered by different arousal states: in NREM sleep their activity is reduced, and when active they maintain long-term wakefulness. Overall, dopamine neurons that project from VTA – NAc promote wakefulness and suppress sleep. Selectively optogenetically activating the neurons maintained wakefulness and suppressed nest-building behavior, which is where mice sleep. Inhibiting the activity of these neurons promotes sleep-related nesting behavior. Even though this study examined the VTA projections to the NAc, prefrontal cortex, amygdala, and dorsolateral striatum, the NAc was the only projection that stimulated arousal. The NAc increased wakefulness and decreased NREM and REM sleep. While other pathways such as the prefrontal cortex have a larger effect on REM duration, NAc was still significant in modulating arousal.

These findings are important because theyimplicate VTA dopamine in mediating stress and sleep-like behaviors. Nicotine, as established by Morel et al., increases the activity of these DA neurons, which induces stress-like behavior as a symptom of depression. It is also possible that the same mechanism leads to heightened arousal-like states, hurting the ability to sleep. A recent survey-based study found that college students who use electronic cigarettes report significantly higher difficulty sleeping compared to non-users.[29] Interestingly, electronic cigarette users also report more difficulty sleeping compared to traditional cigarette users, suggesting that electronic cigarettes may be more potent for nicotine than traditional cigarettes and thus may pose an increased risk for the side effects of nicotine. Taken together, nicotine's effect on DA firing has extensive implications, which likely contribute to sleep difficulty.

Conclusion

The combination of social stress and nicotine binding to acetylcholine receptors in the VTA modulates DA firing through various synaptic mechanisms. Modulations in DA firing in the VTA have broad implications related to several mood and sleep disorders. This social stress-nicotine bidirectional interplay supports and partially explains the strong association between behavioral disorders and nicotine addiction. The data presented in this paper thus demonstrate a comorbidity between social stress and nicotine dependence: a pattern wherein both pathologies enhance the other. Elucidating the synaptic link between the disorders has therapeutic implications as well, where modulation of dopamine activity via DBS or antagonistic binding to acetylcholine receptors may reduce the stress and depressive symptoms that these mechanisms are sufficient to induce. The complexity of these mood-responses

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are mediated by diverse neural, endocrine, and physiological pathways, which leaves a lot of mechanisms and connections unknown. Further investigating the connections between drugs and the environment, and the effects they have on synapses, will uncover more of the mechanisms by which a broad range of mood disorders arise.

The importance of understanding these neural processes is especially important today, as nicotine vaping rates have been rapidly increasing in the United States. In 2018, a steep increase in vaping was observed, with 37% of high school seniors reported vaping activity.[31] In 2019, 10.5% of middle schoolers and 27.5% of high school students reported vaping, an alarming increase from past years.[32] In many cases, these students do not know that nicotine is contained in their electronic cigarettes, or do not think that nicotine alone is harmful. Discovering these synaptic mechanisms will demonstrate to uninformed teens how nicotine is detrimental and can be a risk factor for mood and sleep disorders, especially when stressors are involved. It is imperative that information on these dangers of nicotine becomes more widespread so that society will have a comprehensive understanding of how consuming nicotine can put people at risk for adverse behavioral disorders, independent from addiction.

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Evaluating the Efficacy of Targeted Inhibitor Therapeutics for Sonic Hedgehog Medulloblastoma: Significant Milestones and Current Limitations

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Abstract

Medulloblastoma (MB) is the most common pediatric brain tumor with the Sonic Hedgehog (SHH) subtype accounting for 30% of all diagnoses. The current standard treatment regimen includes high doses of toxic chemotherapy and radiation, as well as surgical resection, motivating the need for alternative therapies which do not generate deleterious effects on patients. The purpose of this literature review was to evaluate the most recent developments in the efficacy of targeted therapeutics in treatment of SHH MB, specifically focusing on small molecule inhibitors targeting the Sonic Hedgehog pathway. The sources analyzed in this review include case studies, preclinical and clinical studies, and other review papers that investigate the mechanism and value of five SHH inhibitors: vismodegib, sonidegib, glasdegib, temozolomide, and GANT-61. Novel discoveries have highlighted that inhibitor therapeutics effectively target aberrant activity of the SHH pathway at various stages, thereby diminishing tumor progression and metastasis. Through evaluation of the inhibitors, it was determined that they are promising targeted therapeutics for SHH MB, despite their limitations. These limitations include drug resistance, molecular heterogeneity of SHH-driven tumors, and poor drug properties. More research will be needed to overcome these obstacles for clinical use, but the investment is warranted given the promise of these inhibitors. Future research should seek to establish optimal dosage and timing of intervention, further delineate the genetic basis for SHH MB, and investigate potential combination therapies with SHH inhibitors.

Keywords: Medulloblastoma, Sonic Hedgehog Pathway, Inhibitor Therapeutics, Vismodegib, Sonidegib, Glasdegib, Temozolomide, GANT-61, Pediatric, Cancer, SHH, Sonic Hedgehog, SMO, PTCH

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1. Introduction

Medulloblastoma (MB) is a malignant brain tumor first described by Harvey Cushing and Percival Bailey in their classification of central nervous system (CNS) tumors in 1925. Although it is the most common pediatric malignant brain tumor, representing about 20% of all pediatric brain tumors,[1] it is very rare in adults, representing 0.4 to 1% of adult brain tumors.[2] Most cases are diagnosed under age 16 and it is rarely seen after age 40. In the United States, an average of 500 children and 200 adults are diagnosed with MB every year, and it is more common in males than females.[3] Medulloblastoma is part of the primitive neuroectodermal tumors (PNET) group, which is classified within the embryonal subtype of CNS tumors. It originates in the cerebellum, in the posterior fossa, and may spread to other regions of the brain and spinal cord. The World Health Organization classified medulloblastoma into four non-histological subgroups according to molecular profiling of the tumors: Wingless (WNT), Sonic Hedgehog (SHH), and groups 3 and 4. This review will focus on the SHH subtype because it accounts for 30% of all MB diagnoses and is the most prominent subtype in both infants (< 3 years of age) and adults (> 17 years of age).[4]

First introduced in the 1930s, surgery was the initial treatment approach. However, the mortality rate following the operation exceeded 30%.[5] In 1953, craniospinal irradiation was introduced following surgery, and although there was improvement to a 3-year survival rate of 65%, the development of significant motor and cognitive side effects was observed. Non-specific cytotoxic chemotherapy complemented with surgery and/or radiation was then introduced in the 1970s, and it is still the standard treatment today. Unfortunately, the unforeseen long-term use of this high toxicity treatment therapy has caused extensive toxic damage to patients, particularly in younger patients.[6] Cerebellar mutism, dysarthria, and neurocognitive disorders result in over 25% of patients following treatment, as well as the growth of secondary tumors due to high intensity of radiation and chemotherapy. Moreover, the current standardized treatment fails to address the root cause of tumor growth, and along with the occurrence of serious adverse effects, validates the need for non-toxic, individualized therapy which tackles the substantial variability of pathological mechanisms among the four subtypes.

Currently, a substantial amount of research is focused on developing targeted therapeutics for SHH MB in order to meet this need. Since the overactivation of the SHH pathway may play a critical role in the formation of this MB subtype, creating therapeutics that can mitigate aberrations to the SHH pathway serves as a putative treatment strategy. Evidence indicates that SHH signaling directs developmental processes, such as cell differentiation and morphogenesis, and has been implicated in several cancers.[7] Importantly, proper SHH signaling is crucial for neural tube formation and normal cerebellar development, as the pathway mediates the proliferation of cerebellar granule cells (GCs) during embryonic development. The heightened activation of the SHH pathway leads to the overproliferation of cerebellar GCs, which may culminate in tumorigenesis.[8] This pathway shows the most promise for developing effective inhibitors, as shown by the extensive amount of research that has been dedicated to understanding its mechanism and the role of SHH signaling in generating medulloblastoma.

The purpose of this literature review is to evaluate the most notable recent developments in targeted inhibitor therapeutics as a possible treatment for SHH MB and suggest further research that would improve the efficacy of the inhibitors. The sources examined describe the mechanism of action and value of five SHH inhibitors: vismodegib, sonidegib, glasdegib, temozolomide, and GANT-61. Significant work has been recently devoted to the development of these inhibitors, and researchers need to understand the extent of this recent progress in order to plan their next steps. Therefore, this review serves as an important resource because it

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presents a summary and evaluation of findings regarding the efficacy of SHH inhibitors, as well as potential future steps to eventually use these therapeutics in clinical practice. Overall, novel findings suggest that inhibitors of the SHH pathway exhibit efficacy in suppressing the pathway and diminishing tumor growth in SHH MB; however, further investigation is needed to overcome the limitations that arise with these therapeutics.

2. The Hedgehog Pathway

SHH-MB is named as such because the overactivation of the SHH pathway is the mechanism driving the formation of this tumor. Evidence suggests that mutations in genes that contribute to the SHH pathway may generate tumorigenesis. Some of these genes include the patched homologue 1 gene (PTCH1), smoothened homologue gene (SMO), and the suppressor of fused homologue gene (SUFU). Heightened expression of the GLI zinc finger transcription factors (GLI1, GLI2, GLI3) and MYCN, an oncogene, has also been associated with the formation of SHH MB. When the ligand for the SHH pathway is not present, the Patched 1 (PTCH) protein, a 12 transmembrane receptor protein, represses the smoothened receptor (SMO), thereby inhibiting the pathway. As shown in Figure 1, when a PTCH ligand, such as sonic hedgehog, is present, it binds to PTCH1 and activates the pathway, as SMO is no longer suppressed. Next, SMO is moved to the primary cilium, and it then activates a GLI zinc finger transcription factor, which could be GLI1, GLI2, or GLI3. Once a GLI factor is stimulated, the transcription of target genes for the SHH pathway (e.g. GLI1, PTCHI1, cyclin D1, BCL-2, SNAIL) is promoted. Anomalous activity at any stage of this pathway may spur the formation of medulloblastoma. The most prominent drivers of SHH MB include aberrant expression of SHH target genes, PTCH dysfunction, and SMO promotion.[6] To counter the deleterious effects of these SHH pathway permutations, targeted small molecule inhibitors that prevent the SHH ligand from binding to PTCH or antagonists of SMO have risen as potential therapeutics against SHH MB.

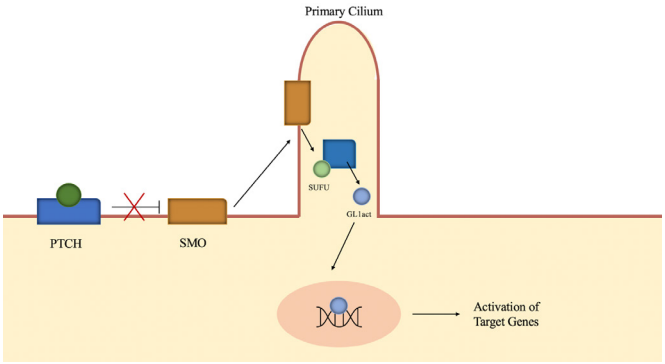

Figure 1. An overview of the hedgehog signaling pathway. In the absence of a PTCH ligand (e.g. sonic hedgehog), PTCH represses SMO. Once sonic hedgehog binds, PTCH no longer inhibits SMO, and SMO is translocated to the primary cilium, where it subsequently activates GLI transcription factors that promote target gene expression of the hedgehog pathway.

3. Mechanism of Action for Small Molecule Inhibitors of The Sonic Hedgehog Pathway

The following SHH pathway inhibitors are potential targeted therapeutics for SHH MB: vismodegib, sonidegib, glasdegib, temozolomide, and GANT-61. As seen in Figure 2, they target and block specific parts of the hedgehog pathway. They are not yet approved for treatment for MB.

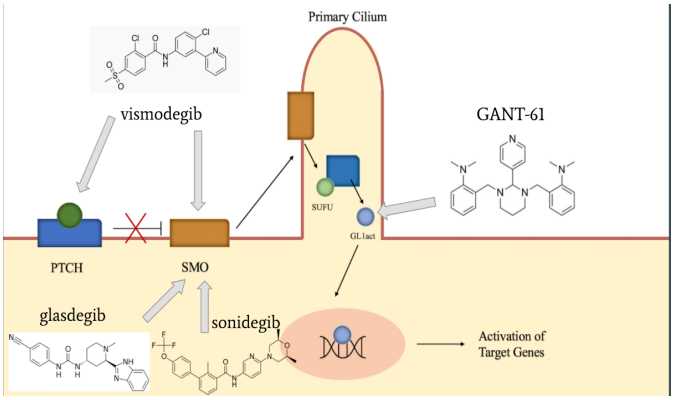

Figure 2. Continued on next page.

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Figure 2. Overview of where the inhibitors target the pathway. As shown, vismodegib targets PTCH1 and SMO. Glasdegib and sonidegib are SMO antagonists. GANT-61 is a GLI inhibitor.

However, both vismodegib and sonidegib were approved for metastatic non-resectable Basal Cell Carcinoma in 2012 and 2015, respectively. Glasdegib was approved in 2018 for acute leukemia treatment.[6]

Vismodegib is a small molecule drug taken orally. In regard to the SHH pathway described above, it interacts with SMO and PTCH by specifically blocking activities of hedgehog-ligand cell surface receptors PTCH and/or SMO. By blocking the process here, hedgehog signaling is suppressed. When vismodegib (also known as GDC-0449) blocks PTCH, SMO can continue to be repressed, and not further the cycle.[9] Vismodegib acts as an SMO antagonist, blocking the SMO receptor. This prevents SMO from activating the GLI zinc finger transcription factors, further inhibiting the pathway. Vismodegib is also a kinase inhibitor, meaning that it inhibits activity of the enzyme kinase (kinases can add phosphate groups to proteins and change their functions). In an *in vivo* study, vismodegib has been shown to affect complete tumor regression in mice with doses of 12.5 mg/kg administered twice per day.[10,11] Additionally, vismodegib has been shown to yield vast but impermanent tumor regression and relief of symptoms when given orally with a dose of 540 mg/day for 3 months. This data is important in evaluating the efficacy of targeted inhibitors to the SHH pathway as therapeutics for SHH MB.

Sonidegib is also a SMO antagonist. Sonidegib works by penetrating the blood-brain barrier and blocking the SMO receptor and therefore the SHH pathway, making it a potentially effective treatment for SHH MB. It has inhibited tumor growth in mice when administered at 5 mg/kg/day and is shown to allow for more regression at higher doses. However, these are also transient effects, and after being exposed to sonidegib for a long time, it has been shown that resistance/relapse occurs due to mutations in SMO that are formed. Moreover, while preclinical research has demonstrated momentary efficacy of sonidegib in SHH MB, the beneficial effects dwindle over time due to SMO mutations and serve as a significant limitation.[12] Additionally, glasdegib is a SMO antagonist. It functions due to its benzimidazole scaffold which has been shown to be used as an anticancer agent.[13] It has a very high potency, indicating that it may effectively inhibit tumor growth in SHH MB. As mentioned before, it has been FDA approved along with low doses of cytarabine for treating acute leukemia.[14,15]

In addition to preclinical research, clinical trials have evaluated the efficacy of sonidegib and vismodegib at mitigating MB tumor growth, as well as their safety profile. [16-21] In a phase I trial conducted from 2007 to 2008, vismodegib was administered to 68 patients who had refractory, locally advanced, or metastatic solid tumors caused by aberrant hedgehog pathway signaling.

One of these patients had SHH MB. The SHH MB patient demonstrated a partial but unconfirmed response to increasing doses of vismodegib, as well as an acceptable safety profile. Resistance to vismodegib occured in this patient as a result of SMO mutations, revealing vismodegib's potential as a therapy for SHH MB while highlighting mutations as a drawback. This clinical trial is not recent, however. Much more research has been conducted on vismodegib's efficacy in SHH MB patients since 2008, producing conclusions that are more revealing.[17] A more recent clinical trial regarding vismodegib was conducted in 2013. Vismodegib was provided to 6 patients at a dose of 85 mg/m2, and 7 patients received a 170mg/m2 dose. There was no bone

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toxicity documented from the drug, although dose-limiting toxicities did manifest. This study served to evaluate the safety, the toxicity, and efficacy of vismodegib in pediatric patients with recurrent or refractory MB. Ultimately, the study showed that this SMO inhibitor is safe and feasible in children, which is significant considering the lack of pediatric clinical trials with SMO inhibitors. A subsequent study administered a phase II dosage to three out of seven SHH MB patients from the first phase. Notably, antitumor activity was observed in 1 of these patients.[18] These clinical findings suggest vismodegib's robust safety and efficacy in SHH MB patients.

In 2 phase II studies conducted in 2015, 31 adult patients and 12 pediatric patients were treated with 150-300 mg/d of vismodegib. Responses were evaluated with neuroimaging and molecular tests. Protocol defined response outlined that a complete or partial response must be sustained for 8 weeks. A complete response was achieved when all lesions targeted disappeared, and a partial response was achieved when 30% reduction in the sum of the diameter of long lesions targeted. 3 adult patients and 1 pediatric patient with SHH-MB achieved the protocol-defined response. Progression-free survival was longer for patients with SHH-MB than non-SHH-MB. Prolonged disease stabilization was achieved in 41% of patients with SHH-MB. It was concluded that vismodegib acts against adult recurrent SHH-MB and not non-SHH-MB. For pediatric patients, there was no conclusion. It was also concluded that SMO inhibitors depend on the deviations in the genome of the tumor.[19] Taken together, vismodegib seems to work by specifically targeting the SHH pathway, which explains its inefficacy against non-SHH-MB tumors. While this clinical data is promising, vismodegib needs to be studied much more extensively in pediatric patients and its efficacy in mitigating mutated SHH MB tumors needs to be explored.

In a similar manner to vismodegib, sonidegib has also been clinically assessed as an inhibitor of the SHH pathway in MB patients.[20, 21] In 2014, a phase I clinical trial was conducted to assess the safety and efficacy of sonidegib taken orally in patients with MB and basal cell carcinoma (BCC). The prominent dose-limiting toxicity manifested as a 3/4 increase in creatine kinase in blood serum, which developed in 18% of patients. Nonetheless, sonidegib displayed an adequate safety profile and decreased expression of GLI1 mRNA in a dose-dependent manner.[20] Therefore, this clinical evidence suggests sonidegib's safety and efficacy in curtailing MB tumor growth by inhibiting progression of the SHH pathway. In a 2017 phase II trial, 60 pediatric patients and 16 adult patients with recurrent tumors received oral sonidegib treatment. Pediatric patients received a 680mg/m2 daily dose, while adults received 800mg. Out of all the pediatric patients, 39 had MB. A 5-gene Hh signature assay was conducted to determine the genetic driver of tumors in complete responders, partial responders, and non responders to sonidegib treatment. Notably, it was found that among the complete responders, 2 children and 2 adults, had SHH-driven tumors. This same outcome was found in the one partial responder.

Out of the 50 non-responders, none demonstrated an SHH-driven tumor.[21] This is significant because it indicates that sondigeib shows efficacy in mitigating MB tumor growth, specifically by inhibiting the SHH pathway. Overall, these clinical trials indicate that while both vismodegib and sonidegib show substantial promise as SHH inhibitors in MB, several limitations to their efficacy still need to be overcome. Thus, the clinical investigation of other potential SHH inhibitors is also warranted.

Temozolomide - another SHH small molecule inhibitor - is not yet approved, but there are ongoing studies about its use as a monotherapy or in combination with vismodegib. It functions by preventing DNA duplication in cells during proliferation, causing cell death.[22] Therefore, temozolomide works to disrupt division of tumor cells and consequently, hinders tumor growth. Though SHH MB seems amenable to temozolomide's mechanism of action, the most

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effective dosage has not yet been established. For instance, vismodegib and temozolomide monotherapies were studied in a patient with recurrent SHH MB, and were taken at a dose of 150 mg. The patient responded to both vismodegib and temozolomide over a significant period of time, though the efficacy of both treatments eventually waned due to drug resistance. Several mutations occurred to SMO: SMO-L412P, SMO-G477L, and PIK3CA-H1065L mutations, which indicates the importance of a treatment regimen that targets multiple aspects of the SHH pathway. Determining the optimal dosage of these SHH inhibitors in preclinical studies is critical before moving to clinical trials, where patient safety is at risk. Additionally, since the benefits of the pharmaceutics seem to dwindle over time, delineating time of intervention over the course of the disease progression is also essential to better treating patients.[23]

Lastly, GANT-61 is a GLI inhibitor. Inhibiting GLI in the SHH pathway may be an effective anti-cancer therapeutic. It has been studied in Daoy cells, a medulloblastoma cell line. The study found that GANT-61 succeeded at inhibiting GLI, a key transcription factor in the SHH pathway, downregulated the Bcl-2 target genes, and even made the tumor cells more sensitive to cisplatin (a chemotherapy drug).[24] This yielded a significant inhibition of cell proliferation, which would theoretically inhibit tumor growth in vivo.

4. Evaluation: Therapeutic Potency of SHH Inhibitors in SHH-MB

Targeted small molecule inhibitors of the SHH pathway have proven to be a promising therapeutic for SHH MB. Many studies and reviews indicate that these inhibitors are effective at halting proliferation.[24, 25] Overall, there are three main benefits of these inhibitors that contribute to their efficacy: their capacity to directly target the SHH pathway, their infrequent toxicities, and their intimate connection with the SHH pathway genes. However, significant limitations exist. Resistant mutations and the heterogeneity of the disease are two major limitations, and poor drug properties is a minor (but important) limitation. This section will address these benefits and limitations.

4.1 Precision Yet Resistance

Novel investigations evaluating the efficacy of targeted inhibitor therapeutics in SHH MB have demonstrated the capacity of these inhibitors to bind specifically to various factors in the SHH pathway, thereby preventing its progression. This makes targeted inhibitors an effective therapeutic because it attacks the cancer at the heart of its mechanism for proliferation. Poisoning the cells that carry the mechanism through chemotherapy, an alternative treatment, is not as precise. Sonidegib illustrates this. MB growth in mice decreased by 33% more than the control group following sonidegib treatment.[25] By targeting the SMO protein, sonidegib prevented SMO's translocation to the primary cilium, thereby halting the pathway. As a result, tumor progression significantly declined at a dose dependent rate, indicating its potential for treating SHH MB.[25] Another example is a 2016 study examining the efficacy of GANT-61, a GLI transcription factor inhibitor in SHH MB. The overexpression of this gene is associated with several cancers. In the study, varied concentrations of GANT-61 were administered to Doay cells, which serves as the in vitro model of MB. By inhibiting GLI, GANT-61 promoted apoptosis of the Daoy cells and downregulated the Bcl-2 target gene, substantially inhibiting cell proliferation.[24] Both GLI and Bcl-2 are components of the SHH pathway, illuminating how these inhibitors attack the MB cancer at the site of tumor initiation. In summary, the examples of sonidegib and GANT-61 illustrate that SHH targeted inhibitors are effective because they target the MB cancer at its source: the SHH pathway.

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Although the targeting nature of small molecule inhibitors is a significant benefit, mutations in the SHH pathway genes often lead to resistance. These mutations create proteins in the SHH pathway that interfere with the inhibitors' effects, rendering them ineffective at halting tumor growth. For example, multiple sources reveal that SMO point mutations result in proteins that do not allow for inhibitor binding.[1,6] Overall, patterns in current literature indicate that SMO, SUFU, GLI2, and MYCN are the genes that primarily experience inappropriate amplifications or mutations resulting in resistant effects. Patterns in these mutations and how each mutation results in resistance is still unclear, but multiple studies report that SUFU, GLI2, and MYCN are all downstream from SMO.[1, 19, 26] For example, one clinical trial found that patients with the downstream genes did not respond to SMO antagonists at all or initially responded to the antagonists but later experienced recurrence.[26] Conversely, this clinical trial and a separate clinical trial found that SHH MB patients with mutations in the upstream PTCH1 gene did respond to the inhibitors.[19, 26] These variable clinical responses are due to the heterogeneity of the disease and mutations that lead to resistance.[23, 26] Furthermore, a pattern in clinical trials has emerged where SHH MB patients' tumors will initially shrink, only to be followed with recurrent growth.[6, 26, 27] These patterns are supported by in vitro experimentation. Inhibitors such as sonidegib and novel Artemisinin derivatives significantly halted MB proliferation during early stages of experimentation but were unable to overcome resistant mutations that led to recurrent proliferation.[12, 28-30] It should be noted that not all of the sources agree on which gene mutations result in resistance. Most sources comment on SUFU, GLI2, and MYCN, but one review also discusses truncations of GLI1, amplifications of GLI2, cyclin D1, and upregulation of the ATP binding cassette transporter p-glycoprotein substrate. Given that the other sources did not mention these mutations, it is unclear whether they pose a significant limitation. Overall, resistant mutations pose a serious limitation to the efficacy of the inhibitors because they undo the exact mechanism used to halt proliferation of the MB cells. More research is needed to clarify which mutations most contribute to resistance and how they do so. However, the following additional benefits of SHH inhibitors still make these therapeutics a promising option.

4.2 Less Toxic Yet Less Stable

Notable advancements have revealed that targeted inhibitor therapeutics of SHH MB are much less invasive and toxic than the current standardized treatment. A 2010 investigation demonstrated that SHH inhibitors are an effective mode of treatment for other SHH-dependent cancers, such as Basal Cellular Carcinoma, Lung cancer, and Liver cancer.[31] Since 2015, SHH inhibitors have been a validated treatment option, specifically for treating metastatic or locally advanced non-resectable Basal Cellular Carcinoma.[25] Therefore, SHH inhibitors demonstrate rehabilitative potential in SHH MB, which is driven by aberrations in the SHH pathway. Administration of SHH inhibitors have been shown as safe with limited adverse effects, as indicated by its clinical approval for Basal Cell Carcinoma. Moreover, these inhibitors' low toxicity and limited invasiveness suggest its greater therapeutic capacity in comparison with current standardized treatment. For example, vismodegib and temozolomide have exhibited a promising safety index in recent investigations. One vismodegib clinical trial found that the patients revealed a low toxicity profile and that none of the patients withdrew from the clinical trial because of toxicity.[19] Another clinical trial found that a 16-year old patient suffering from SHH MB exhibited a steady response to vismodegib and temozolomide with limited adverse effects. Although the benefits eventually declined due to mutations, this study demonstrates that adverse events were not due to toxicity. [23] Nonetheless, surgical resection and subsequent radiation and

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chemotherapy are still the most prevalent treatment regimen for all MB subtypes. Given that the invasive nature of radiation and chemotherapy often generate chronic cytotoxic effects and tumor recurrence, inhibitors are a promising alternative.

Although this data indicates that inhibitors improve upon the standard treatment regimen for SHH MB in terms of toxicity and invasiveness, evidence suggests that developing inhibitors without these cytotoxic effects and optimal pharmacokinetic properties has posed a challenge. For example, two SHH pathway inhibitors that resulted in negative consequences are cyclopamine and HhAntag: cyclopamine generated cytotoxic effects in healthy cells and a preclinical study of HhAntag resulted in permanent developmental defects in the bones of the mouse models.[32, 33] Given that SHH is a pathway critical for development, the latter study has raised concerns about the use of SHH inhibitors in infants and young children. Cyclopamine also had poor pharmacokinetic properties, another limitation of SHH MB inhibitors. Adequate concentration and stability in circulation are two pharmacokinetic properties that have been commented on in the literature. For example, rat livers cleared away N-Phenylbenzamide too quickly, preventing adequate concentration. This ended its testing although it was initially promising.[34] Other examples are GANT-61 and vismodegib. GANT-61 has proven effective in vitro but is less stable than its GANT-58 alternative under physiological conditions.[35] Vismodegib has demonstrated encouraging pharmacokinetic characteristics in animal models, but its pharmacokinetic properties can also be improved upon. An in vivo study demonstrated that these properties can be further bolstered when hydrogen ions in the active sites are replaced with deuterium. This allowed for sustained benefits at lower doses.[36] Overall, although poor drug properties are important limitations, literature suggests that they are not as significant as genetic based limitations such as resistant mutations and heterogeneity. Most of the sources analyzed did not comment extensively on the cytotoxicity and pharmacokinetic properties, if at all. These challenges should be addressed, however, as the development of novel SHH inhibitors progresses.

4.3 Genetic Connections Yet Persistent Heterogeneity

Recent developments in the use of SHH inhibitors have also elucidated connections between the genetics of the disease and the efficacy of inhibitors. These connections have allowed researchers to craft treatment regimens that target specific genetic mutations, bolstering their efficacy. In 2015, a clinical study reported results from two Phase II trials that evaluated the safety and efficacy of vismodegib in patients with recurrent or refractory SHH MB.[19] It was found that the position of the genomic deviation in relation to SMO and PTCH1 were predictors of the response to SMO inhibitor activity. Both the transgressions of SMO and PTCH1 culminated in favorable outcomes with respect to attenuating tumor progression. This discovery is critical because it demonstrates that knowing the genetic basis of the patients' SHH MB tumor can predict whether or not they respond favorably to the inhibitor. Secondly, it was discovered that robust P53 diffuse staining in SHH MB was associated with a substantially less significant response to the inhibition of SMO. The mechanism underlying this finding is not yet understood because the relationship between P53 and SMO is unclear. However, results indicated that mutations of this protein generate chromothripsis, where thousands of chromosomal rearrangements occur, possibly upregulating the expression of SHH signaling oncogenes.[37] With this information, paired with the relationship between P53 and SMO that still requires further investigation, it is concluded that alterations to the P53 protein expression may be correlated with anomalous SMO activity.[19]

Moreover, inhibitors that can mitigate both P53 and SMO aberrancy, which are driven by genetic mutations, serve as a potential therapeutic strategy for SHH MB. This underscores the

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relationship between SHH MB's genetics and efficacy of inhibitors. A third key finding from this study was the delineation of the target population for vismodegib therapy, which was conducted via complete molecular profiling of SHH MBs. The evidence obtained demonstrated the importance of distinguishing SHH MBs that are driven by mutations downstream in the SHH pathway, which are not amenable to the inhibition of SMO. This serves as a significant milestone because it suggests the need for novel inhibitor therapeutics that target different proteins of the pathway while underscoring that inhibitor efficacy has genetic connections. This emphasizes the importance of further delineating the genetic basis of SHH MB in order to provide more effective individualized therapies.

Although there are useful connections between inhibitor efficacy and disease genetics, not all of the SHH MB genes have been discovered and important patterns in expression have yet to be identified. Much of the literature describes SHH MB as a heterogeneous disease, meaning that different genes are associated with tumor growth across patients. There is evidence for this in the variability of responses to inhibitors. For example, two adult males suffering from SHH MB had extremely different responses to vismodegib. One had a very favorable response while the other developed resistance multiple times. Furthermore, the second adult male even had a genetic profiling more similar to the childhood version of the disease, making it difficult to predict how he would respond to the treatment.[27] This highlights the complexity of the disease's genetic basis and suggests that there are subgroups of SHH MB based on combinations of affected genes. However, these nuances are still unclear because it is a rare cancer; more patients are required to detect patterns with certainty. This makes it difficult for researchers to develop effective inhibitors and assign patients to optimal clinical trials.[6] Furthermore, some genes might even be better suited for use as a diagnostic tool while other genes (such as GLI) have shown promising results as a therapeutic target.[24] Overall, the heterogeneity of SHH MB limits the efficacy of inhibitors in that they might not target the pathway optimally in each patient. Fortunately, studies have successfully identified preliminary patterns of tumor-inducing gene expression in SHH MB patients using transcriptome sequencing and whole genome analysis. These studies have found that there are patterns across age groups.[1,26] One study found that infants, children, and adults had instances of PTCH1 mutations, only infants had the SUFU mutations, and only adults had SMO mutations. Mutations in SUFU, GLI2, and MYCN genes have shown primary resistance to SMO inhibition.[26] This suggests that patients in clinical trials should be assigned inhibitors according to age, and that genetic sequencing of SHH MBs should be a part of the treatment planning process. Ultimately, a better understanding of the responsible genes is still needed.

Overall, the potential of SHH inhibitors makes them a promising therapeutic for SHH MB in the clinical setting, despite their limitations. Future research must address these limitations if the inhibitors are to be approved for clinical use. However, the promise of these inhibitors warrants the investment of such research. Given how they have improved upon the standard treatment regimen for SHH MB, small molecule inhibitors of the SHH pathway are likely the future of SHH MB treatment.

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Table 1: Milestone Studies Illuminating the Therapeutic Potential of SHH inhibitors in SHH MB

Type of study (preclinical/clinical)	Year	Type of inhibitor	Significant findings
Clinical	2014	SMO inhibitors	Genomic analysis of infant, children, and adult SHH MB demonstrate significant molecular heterogeneity. Among adult patients, 82% of those in the study exhibited PTCH1 or SMO mutations, indicating that these tumors would be amenable to SMO antagonist treatment.[25]
Clinical	2015	Vismodegib	Pediatric and adult recurrent SHH MB patients received 150-300mg of vismodegib daily. Compared to patients with non-SHH MB tumors, SHH MB patients demonstrated tumor amelioration to a greater extent. Vismodegib treatment induced genetic transgressions in the SMO gene, indicating anti-tumor capacity.[26]
Preclinical	2016	GANT61	In Daoy MB cell culture, GANT-61 administration attenuated tumor growth by hindering cell proliferation, migration, and infiltration. GANT-61 also stimulated apoptosis induced by mitochondria and the caspase-3 pathway. qPCR confirmed that GANT-61 effectively impeded GLI factor expression.[8]
Clinical	2018	Temozolomide, vismodegib, sonidegib	A SHH MB pediatric patient with the PTCH1 mutation received three different treatment regiments of Hh inhibitor. He first received vismodegib and demonstrated a partial metabolic response. However, vismodegib resistance eventually developed, as indicated by tumor recurrence. Temozolomide had a similar effect. Sonidegib resulted in exacerbated tumor growth and the need for emergency surgery.[19]
Clinical	2019	Vismodegib	Whole-genome analysis of two patients with SHH MB was conducted. While the tumors from both patients exhibited a mutation in PTCH1, only one of the patients was responsive to Vismodegib treatment. The expression of MYCN and GLI1, absence of PTEN, and depletion of P53 may explain the vismodegib resistance that manifested in the patient.[23]
Preclinical	2020	eHNP (nanoparticle)	Nanoparticles with SHH inhibitors loaded on can cross the blood-brain barrier (BBB). They can be used as a delivery carrier to cross the BBB and to the SHH MB.[39]

5. Further Research Needed

The current limitations of SHH inhibitors in SHH MB illuminate crucial areas of future research. First, optimal dosage and timing of intervention need to be established. In a case study, vismodegib and temozolomide were taken at a dose of 150mg. The therapeutic benefits of both inhibitors dwindled as mutations arose. The new tumors that formed varied genetically from the initial tumor. Following failure of those treatments, sonidegib was taken at a dose of 400mg, which resulted in the need for emergency surgery.[23] Therefore, optimizing dosage and timing of intervention are crucial for translating preclinical findings to clinical practice. Importantly, optimal timing and dosage of inhibitor treatment should be examined for both pediatric and adult patients, especially since clinical trials with pediatric SHH MB subjects are lacking.

Compared to the standard treatment regimen for SHH MB (radiation, chemotherapy, and surgery), SHH inhibitors demonstrate greater therapeutic promise. Nevertheless, future research should focus on improving the efficacy of these inhibitor therapeutics to combat resistant mutations. Resistance to both SMO antagonists and inhibitors of PTCH frequently develop in patients, which can exacerbate tumor growth and spur other adverse events. Moreover, more downstream inhibitors of the SHH pathway, such as GLI inhibitors, should be explored in studies. Investigation into various SHH inhibitors is also critical for treatment of SHH MB in pediatric patients, as SHH MB in the pediatric setting most often occurs due to genetic mutations downstream of SMO in the pathway.[26] It is also important to consider the use of combination inhibitor therapies for SHH MB. Since the efficacy of SHH inhibitors, such as vismodegib, may diminish over time due to drug resistance, synergistic inhibitor treatment with other therapies may elicit a more robust rehabilitative response. It may be beneficial to target both the SHH pathway and other pathways that interact with SHH signaling, such as P53, cAMP, Atoh1, Boc, CxCl12, CxCR4, and PI3K.[1,19] For example, though the interaction between P53 and the SHH pathway remains unclear, an in vivo study found that development of MB increased from 14% to more than 95% when PTCH loss was coupled with P53 loss.[38] Moreover, administration of SHH inhibitors with P53 mediators should be explored as a putative combination therapy for SHH MB. Additionally, cholesterol homeostasis may contribute to the overactivation of the SHH pathway and so, modulated inhibitor therapies that can also obstruct cholesterol regulation may serve as an important area of future research. Indeed, a recent milestone investigation not only demonstrated that lipid-based nanoparticles effectively crossed the blood brain barrier and delivered the therapeutic cargo to the tumor site, but also effluxed cholesterol from the cytosol of tumor cells.[39] Lower levels of cytoplasmic cholesterol

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generated cytotoxic effects in the SHH MB cells. Moreover, the use of lipid-based nanoparticles to carry targeted inhibitor therapeutics may bolster the efficacy of these pharmaceuticals and reduce the drug dosage needed to elicit a response.

Lastly, the pathological mechanisms of SHH MB are still largely unknown due to substantial molecular heterogeneity in these tumors. There are still a multitude of genes that drive this tumor that have not yet been identified, making it difficult to determine which inhibitor therapeutic would be most effective.[6] Among infants, children, and adult patients, the genetic basis of the tumor differs significantly. Future investigations should focus on further elucidating the genetic basis of SHH MB tumors to determine whether there is an age-dependent factor underlying the molecular disparities. Therefore, individualized targeted treatment aiming to alleviate the specific aberration in the SHH pathway is critical for formulating the most effective treatment of SHH MB in both pediatric and adult patients. All in all, extensive genetic profiling of SHH MB tumors serves as a pivotal area of future research.

6. Summary

In summary, in understanding the SHH pathway and inhibitors vismodegib, sonidegib, glasdegib, temozolomide, and GANT-61, benefits as well as limitations were exposed. Drug resistance due to SMO mutations, molecular heterogeneity, and poor drug properties were discussed. Gaps in knowledge for future research include establishing a more thorough understanding of the genetic basis of the disease, determining optimal dosage of the inhibitors, as well as time of intervention, pathological mechanisms, and synergistic treatments to improve efficacy. In conclusion, targeted inhibitors of the SHH pathway are a promising treatment method, though there are limitations that must be further explored to improve the efficacy and safety as a therapeutic treatment for Sonic Hedgehog Medulloblastoma.

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Refractory Epilepsy: Mechanisms of Pharmacoresistance

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Abstract

Refractory or drug-resistant epilepsy is a complex and debilitating disorder that impacts over one-third of people diagnosed with epilepsy. Many studies have suggested a variety of possible hypotheses for drug-resistant epilepsy, including the degeneration of neural networks, alterations of anti-epileptic drug (AED) targets, intrinsic severity/frequency of seizures, and genetic predisposition to pharmacoresistance. However, extensive research suggests that the overexpression of efflux protein transporters in brain tissue is the most viable hypothesis. Specifically, the overexpression of P-glycoproteins (P-gps) at the blood brain barrier proves the most compelling mechanism to discuss further. Studying the mechanisms of these transporters provides critical insight for new ways to combat pharmacoresistance. Thus, this review evaluates the co-administration of P-gp inhibitors with AEDs as a promising, yet relatively unexplored, treatment option for refractory epilepsy. This review specifically considers Tariquidar (TQD) the most promising P-gp inhibitor for refractory epilepsy treatment. This work aims to evaluate the role of P-gp overexpression in refractory epilepsy, consolidate current research about potential treatment options, and identify discrepancies or gaps in the literature related to P-gp inhibitory treatments for refractory epilepsy. It was concluded that, as a result of increased drug efflux processes at the blood brain barrier, overexpression of P-gp is the leading cause of pharmacoresistance. By inhibiting the activity of these proteins with the drug Tariquidar, an effective treatment for refractory epilepsy may become a reality.

Keywords: refractory epilepsy, pharmacoresistance, overexpression, P-glycoprotein, Tariquidar

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1. Introduction

Epilepsy is a chronic neurological disorder characterized by spontaneous, repeated, and unprovoked seizures resulting from abnormal electrical connections between neurons in the brain.[1] Characterized by spontaneous electrical disturbances in the brain, these seizures often result in physical manifestations such as convulsion, loss of consciousness, sensory disturbances, or uncontrolled movements.[2] Epilepsy encompasses many different types of seizure disorders that are classified according to relative regions of brain dysfunction, types of seizures present, and varying degrees of causal explanation. Clinical diagnosis of epilepsy requires at least two isolated seizures unrelated to brain injury, drug usage, metabolic disorders, or acute systemic dysfunction.[3]

Impacting 2.2 million people in the United States and over 65 million people worldwide, epilepsy is the most common neurological disorder in the world. It accounts for 0.5% of the global burden of disease, a measure that considers the number of years of life lost to premature death, illness, and disability. While this condition is prevalent across all age demographics, it disproportionately affects individuals in early childhood or late adulthood.[3] People with epilepsy are up to three times more likely to experience premature death compared to the general population. 1 in every 1,000 adults with epilepsy suffer from sudden unexpected death in epilepsy (SUDEP) every year, and those with drug-resistant forms are at increased risk of SUDEP. In the United States alone, over 150,000 new cases of epilepsy are diagnosed annually and one in 26 individuals are projected to develop epilepsy over the course of their lifetimes. Direct annual medical care costs of epilepsy in the United States total $9.6 billion and contribute significant financial burden to sufferers.[3] Along with the difficulties associated with seizures and other related symptoms, people living with epilepsy are often faced with challenges accessing high-quality healthcare, navigating treatment options, and dealing with public misunderstanding of the disease. In many areas across the globe, people with epilepsy also experience reduced access to educational and vocational opportunities as a result of stigma and discrimination associated with the disease.[2] This emphasizes the need to find treatments or cures for epilepsy. In doing so, the detrimental impacts of the condition and the socioeconomic disparities associated with it may be relieved.

While there are numerous therapeutic options available to treat epilepsy—such as antiepileptic drugs (AEDs), surgical intervention, nerve stimulation, or specialized diets—treatment methods are often ineffective. Over one-third of people diagnosed with epilepsy will develop refractory (drug-resistant) epilepsy as a result of pharmacoresistance, the inability to control seizure activity through AED therapy.[4] This form of epilepsy is clinically diagnosed after two trials of AEDs fail to manage or cease seizure activity. While about 50% of newly diagnosed epileptic patients obtain full seizure control within the first AED treatment and 13% after switching to a second AED treatment, 20-25% of total patients still do not see improvement after a single AED or a combination of AEDs.[5] Management of refractory epilepsy is particularly challenging because there is little conclusive evidence about the causes or mechanisms of pharmacoresistance, and individualized treatment plans are necessary due to different underlying disease mechanisms.[6] Some possible causes for drug-resistance may include environmental factors, including trauma and prior drug exposure, or genetic predispositions that impact the degree of absorption, metabolism, and uptake of AEDs at the blood brain barrier.

Most of the current research on the mechanisms of refractory epilepsy suggests that drug-resistance in epilepsy is caused by increased activity and expression of multidrug efflux transporter proteins in the ATP-binding cassette (ABC) protein transporter family.[7] There are three major types of ABC transporters that play a

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significant role in the failure of AEDs to control refractory epilepsy seizures: permeability-glycoproteins (p-glycoproteins or P-gps), multidrug resistant-associated proteins (MRPs), or breast cancer resistant proteins (BCRPs).[7] These active transporters use the energy of ATP through ATP hydrolysis to allow for the movement of molecules across a membrane, although ABC transporters specifically use an alternate access mechanism, which entails switching between "inward- and outward-facing states" at the membrane which, in turn, alternates whether the ligand-binding site is exposed inside or outside the membrane.[8] Many studies suggest this group of ABC transporters and their related efflux mechanisms to be the major cause of pharmacoresistance in refractory epilepsy, and the most compelling evidence suggests that the overexpression, or increased expression, of P-glycoprotein transporters most significantly reduces uptake of AEDs at the blood brain barrier.[7,9] Additional studies have shown that the inhibition of these particular P-gp transporters can reduce efflux transporter mechanisms and, subsequently, can potentially eliminate pharmacoresistance altogether.

In essence, the overexpression of P-glycoproteins in the brain leading to the reduced uptake of AEDs at the blood brain barrier is the most promising target for the treatment of refractory epilepsy.

2. The Transporter Hypothesis in Refractory Epilepsy

Many studies have worked to evaluate various theories regarding the potential mechanisms of pharmacoresistance in refractory epilepsy, although there is a general consensus that protein transporters have the most influence over refractoriness. This is especially noted in a comprehensive review focused on drug-resistant epilepsy that evaluated a wide variety of studies on refractory epilepsy and summarized the potential hypotheses for mechanisms of pharmacoresistance.[9] Tang et al., 2017 suggested that the drug-resistance of refractory epilepsy may come from seizure-induced neural damage that prevents anti-epilepsy drugs from accessing their neuronal targets. Another collection of studies included in the same review suggested that pharmacoresistance is inherent to the severity of epilepsy.[8] While many of these hypotheses are biologically plausible, there is a significant lack of evidence, association, consistency, and specificity in the literature to demonstrate their validity.

However, one hypothesis from the review stood out as the most promising mechanism for refractory epilepsy: the transporter hypothesis. The transporter hypothesis suggests that the overexpression of efflux transporter proteins in the brain tissue of epileptic patients causes drug resistance by decreasing the permeability of the blood brain barrier for AEDs.[10] There are different types of protein transporters that carry out this mechanism of pharmacoresistance: P-gps, MRPs, and BRCs, which are all part of the ABC transporter family.[7,9] Out of these transporters, the overexpression of P-gp is most prominently associated with pharmacoresistance, as increased expression of P-gp in the brain tissue of refractory epilepsy patients had a low AED response.[9] The overexpression of MRPs shows similar promise as a potential mechanism of refractory epilepsy, but they have not been extensively studied with controls to further prove their association. While BCRPs perform a similar role in pharmacoresistance, it has been suggested that these transporters have better therapeutic potential for cancer rather than refractory epilepsy.[9] Ultimately, based on the current findings and evidence, it can be deduced that the clearance of AEDs by P-gps shows the most promise as a mechanism of refractory epilepsy. Thus, resources should be allocated towards exploring these proteins and investigating them as a target for future treatment.

An earlier review from 2011, which elaborated on each type of transporter and their mechanisms at the BBB, detailed the inhibition of P-gp expression as a potential treatment option.[10] To build upon these analyses, this review focuses on P-gp overexpression, its role in

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refractory epilepsy, and potential treatments that target these mechanisms.

3. The Overexpression and Mechanisms of P-glycoproteins in Refractory Epilepsy

As previously mentioned, current data shows that about one-third of epilepsy patients do not respond to most types of AEDs, and it has been hypothesized in recent years that one potential mechanism for the resistance to AEDs is the overexpression of P-gp in the blood-brain barrier (BBB).[11] As ABC transporters, P-gps use ATP hydrolysis to allow for efflux of molecules using the aforementioned alternate access mechanism.[8,12] More specifically, P-gps are exporters, so they actively work towards moving molecules outside of the cell rather than inside, so it is clear that the overexpression of these exporters are bound to lead to decreased AED concentration.[8]

These protein transporters can exist in large numbers in a variety of locations in the body such as the intestines, liver, kidneys, and the BBB. The protein is likely expressed in these organs to serve as a defense mechanism against foreign substances.[12,13] For example, this protein exists in the intestinal epithelium, where it pumps toxins back into the intestinal lumen. P-gps can play an integral role in the effluxion, or clearance, of chronic disease drugs. Specifically, P-gp is a key efflux protein of the BBB that actively transports large amounts of lipophilic drugs, including AEDs, out of the BBB membrane and back into the bloodstream, therefore resulting in pharmacoresistance to therapeutic medications intended to target the brain.[11,12]

There have been many clinical studies that demonstrated how the decreased concentration of AEDs can result from P-gp overexpression. In one case study, surgery was conducted on a patient with partial refractory epilepsy to alleviate epileptic symptoms. Prior to this study, the patient was treated with many different AEDs and doses with no measured success in controlling the patient's symptoms.[5] Prior to surgery, researchers collected and measured AED blood levels in the patient's serum samples for 25 days before each administered dose of AEDs to observe the patient's AED concentration in the blood. It was found that all types of the AEDs that the patient received were observed in subtherapeutic levels in a significant portion of the serum samples collected. This indicates very low AED concentration throughout the blood. During the surgery, some samples of brain tissue were collected for immunohistochemical analysis to determine the cause of these subtherapeutic AED levels in the blood. From this analysis, it was found that the patient had high brain expression of P-gps in not only endothelial cells of the BBB but also in astrocytes and neurons. This overexpression of P-gps in regions where drug elimination actively takes place suggests that the protein plays an integral role in the efflux and clearance of AEDs from the brain.[5] This study is consistent with other case studies on patients with refractory epilepsy.

In a rat model of epilepsy, P-gp overexpression was assessed following the administration of an anti-seizure medication, phenobarbital. Immunohistochemical analysis showed a striking overexpression of P-gp in the limbic brain regions of the rats that did not respond to the medication.[13] In particular, overexpression of P-gp could be narrowed down to the brain capillary endothelial cells of the BBB.[13] These findings were consistent with the case study mentioned above, suggesting an association between P-gp overexpression and AED response. Other studies on BCRPs and MRPs have not been able to fully conclude these same associations with the AED response, suggesting that P-gps are the most promising candidates for a therapeutic approach.

Other experiments focused on looking at the specific mechanism of P-gps with specific substrates. For example, one study conducted the brain-to-plasma difference of 10-OHCBZ, an active metabolite, in refractory epilepsy patients who did not respond to the AED treatment Oxcarbazepine.[14] In this study, surgery was conducted to alleviate the epileptic condition of the patients, during which researchers intraoperatively measured 10-OHCBZ levels to

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determine if it is a substrate of P-gp and to understand the relationship between its brain-to-plasma concentration ratio and the levels of expression of P-gp. The results showed that 10-OHCBZ acts as a substrate for P-gp, and the concentration of 10-OHCBZ in the brain was not correlated to the plasma levels of the patients. However, a significant inverse linear relationship was found between the levels of expression of P-gp and the brain-to-plasma concentration ratio of 10-OHCBZ.[14] This inverse relationship showed that higher levels of P-gp resulted in lower levels of the active metabolite past the BBB, providing evidence of the hypothesized P-gp mechanisms. The conclusions of 10-OHCBZ apply to AEDs because the data showed that higher expressions of P-gp lead to lower expressions of 10-OHCBZ, and intracellular levels of the metabolite were increased when XR9576, a P-gp inhibitor, was introduced. However, these conclusions are not necessarily reliable due to the lack of control brain tissue from drug-responding patients. Furthermore, *in-vitro* studies do not necessarily confirm or reflect these mechanisms as would *in-vivo* models for refractory epilepsy.[13] The observational nature of the previous study is useful in developing a base for understanding the functions of P-gp and developing hypotheses, but in-vivo studies are needed to test and prove these hypotheses. Nevertheless, this pilot study still provided some fundamental insight into the mechanisms of P-gp overexpression in pharmacoresistance and demonstrated how substrates of P-gp, including AEDs, have difficulty crossing the BBB and reaching the brain if there is an overexpression of these transporters.

An *in-vivo* study by Van Vliet et al. measured the levels of phenytoin (PHT), a common AED used to control seizures, in different brain regions of both epileptic and nonepileptic rats.[15] The researchers wanted to compare PHT levels in regions of the brain with an overexpression of P-gp, such as the parahippocampal cortex and the temporal hippocampus, to PHT levels in brain regions with lower P-gp expression. The concentration of PHT in regions with overexpression of P-gp was significantly reduced compared to regions with lower P-gp expression. These results were, once again, consistent with the earlier findings, demonstrating the relationship between increased P-gp expression and decreased levels of AED in the brain.[15] Overall, it has been established, through studies using different AED substrates and refractory epilepsy models, that the overexpression of P-gp efflux transporters leads to decreased concentration of AEDs in the brain and, consequently, pharmacoresistance. Some of these studies have also suggested this mechanism as a potential therapeutic target, specifically with the use of P-gp inhibitors.[15,16] Together, the findings of these past works suggest promising research implications for P-gps as future treatment targets, which will be discussed in the next section.

4. Improving Standard of Care for Refractory Epilepsy

4.1 Significance of P-gp Inhibitors in Reducing Pharmacoresistance

Although overexpression of P-gps has been identified as a highly probable mechanism of drug resistance in epilepsy, treatment methods have yet to significantly account for this new knowledge. A promising yet relatively unexplored treatment option lies in therapeutics that target the activity of P-gps. P-gp inhibitors are a specialized class of substances that block or bypass the efflux activity of P-gps at the BBB.[16] In regards to AEDs specifically, these inhibitors work to prevent the efflux of AEDs from endothelial cells of the blood brain barrier (Figure 1), thus allowing for increased therapeutic effect. These inhibitors function to either block P-gp binding sites, reduce ATP hydrolysis by P-gp, or weaken cell membrane lipids.[16] The previously mentioned in-vitro study using 10-OHCBZ to observe the effect of P-gps on intracellular drug concentration briefly introduced the use of P-gp inhibitors, which resulted in increased intracellular drug concentration.[14] This previous work was able to provide the fundamental insight needed to suggest that concurrent administration of AED substrates with P-gp inhibitors may increase drug

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bioavailability or uptake at the BBB and result in an improved therapeutic effect.[14,16]

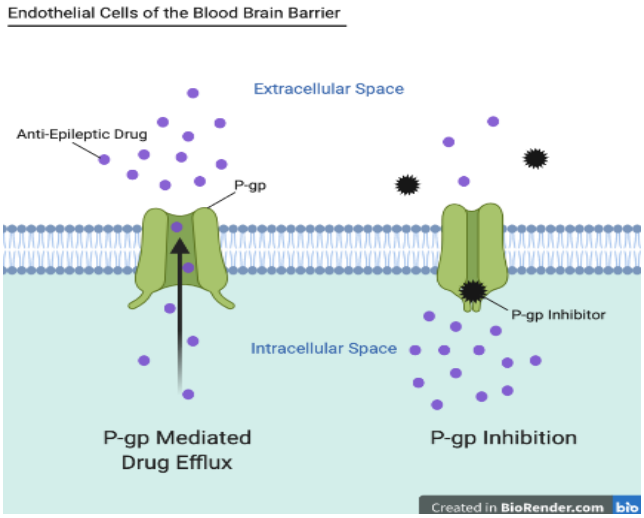

Figure 1. P-gp Inhibition to Prevent AED Efflux at the Blood Brain Barrier[1]

Table 1. Classification and Limitations of P-g Inhibitors

Generation	Specificity	Limitations	Advantages	Examples
First Generation	Low	-High toxicity -Must be administered in low doses -High serum concentrations -Wide range of substrate capabilities	-Most pharmacologically active -Significant research accomplished	Verapamil
Second Generation	Medium	-Inhibitory effect of unintended enzymes and other ABC transporters	-Greater affinity of P-gp	Valspodar
Third Generation	High	-Many still in developmental stage/not ready for clinical use	-Low toxicity -Can target specific locations	Tariquidar

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Three distinct generations of P-gp inhibitors that perform similar functions have been identified (Table 1). First-generation inhibitors are substances originally developed to treat other conditions that were found to also successfully inhibit P-gps. First generation inhibitors are the most pharmacologically active, but they pose potential limitations in dosage due to their latent toxicity, unusually high serum concentrations, and ability to substrate with a wide range of unintended, biologically beneficial transporters and enzymes.[16] Second-generation inhibitors do not hold the same range of pharmacological ability as the first-generation, but they do possess a greater affinity for P-gps, allowing for more specific targeting in the body. However, second-generation inhibitors are limited in clinical use by their tendency to inhibit certain enzymatic activities and other ABC transporters that lead to complicated drug-drug interactions.[16] Most third-generation inhibitors are still in the clinical development phase and aim to act with high P-gp specificity and low toxicity. While all three generations of P-gp inhibitors face potential restrictions in their use, they also offer promising properties as a clinical treatment for refractory epilepsy.

4.2 Tariquidar as a Promising Therapeutic Option

In identifying P-gp inhibition as a potential therapeutic option for refractory epilepsy, it is important to consider the specific functions and locations of these inhibitors. P-gp inhibitors may specifically target overexpression in the intestines, liver, brain, and kidneys or may only play a pharmacological role in increasing the uptake of certain drugs.[17] Two such P-gp inhibitors, Tariquidar (TQD) and Verapamil, have been identified as potential treatments for refractory epilepsy due to their high specificity in targeting the blood brain barrier.

Many studies have provided evidence to indicate that Tariquidar, or TQD, is a promising selective inhibitor of P-gp that may offer a solution to refractory epilepsy. As previously tested by Van Vliet, E. A. et al., TQD shows great potential in counteracting pharmacoresistance of AEDs resulting from the overexpression of P-gps.[15] TQD is a third-generation P-gp inhibitor known for its high selectivity, long active period, and advantageous oral administration and bioavailability.[17] Since its development, this exceptionally potent inhibitor has been considered one of the best ways to reduce the effects of P-gp overexpression. The oral intake of drugs such as TQD is often the most convenient and safest route of drug administration and, following the administration of TQD specifically, has shown to have a relatively strong bioavailability. TQD also acts as a non-competitive inhibitor to reduce the ATP hydrolyzing activity of P-gps. This activity facilitates the transport of substances out of cells; thus, TQD inhibits such behavior that is key to pharmacoresistance.[17] One study demonstrated the high potency of TQD as a P-gp inhibitor and discovered that the administration of one micromolar of TQD decreased the ATPase activity of P-gp by over 50%.[18] While TQD has mainly been studied as a modulator of reversing multidrug resistance in cancer, current evidence has also identified TQD as a potential modulator of pharmacoresistance in epilepsy.[12]

Many studies have demonstrated the inhibitory effect of TQD on P-gp overexpression that contributes to refractory epilepsy. Building upon their previous work measuring AED uptake in brain regions with P-gp overexpression, Van Vliet, E. A. et al. continued an investigation into the anticonvulsant effects of phenytoin (PHT) before and after administration of TQD. After co-administering PHT and TQD to rats with frequent daily seizures, researchers analyzed brain-to-plasma concentration ratio measurements of PHT.[19] The researchers also recorded the frequency of seizures to determine any observable changes in the epileptic activity of the rats when treated with TQD. The results showed that, when combined with TQD, PHT levels in the rats remained within the therapeutic range in the blood for an additional 8 hours compared to PHT alone.

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Additionally, overall seizure activity was reduced in rats treated with both drugs. In the control group, PHT treatment alone did not significantly decrease seizure activity until day 13. Conversely, the experimental group of PHT co-administered with TQD showed reduction in seizures during the first 3 days of treatment. This experiment concluded that the administration of TQD allowed for an increased uptake of PHT at the BBB, inhibiting the mechanism of P-gps in chronic epileptic rats and, thus, dramatically reducing the frequency of seizures. This analysis demonstrates TQD's ability to significantly reduce P-gp activity and provides a promising approach for improving the standard of care for refractory epilepsy.

A second major study showed consistent findings on the effects of TQD in reducing P-gp pharmacoresistance when co-administered with phenobarbital (PB), a drug widely used for its anti-epileptic effect.[20] In this study, researchers used EEG monitoring and blood sampling to analyze seizure activity and drug concentration in plasma. The study used a rat model of temporal lobe epilepsy with pre-established drug-resistance to phenobarbital. In one trial, co-administration of TQD and PB completely eliminated seizure activity in four nonrespondent rats and reduced seizures by over 90% in the remaining two nonrespondent rats compared to only a 24% reduction in the control group receiving PB alone. In a remarkable example, one rat went from experiencing an extensive number of seizures per day before treatment to becoming completely seizure free with the co-administration of TQD and PB. This study concluded that the co-administration of TQD with PB completely restored the anti-epileptic effect of the drug in pharmacoresistant rat models of temporal lobe epilepsy.[19] Another comparable study conducted using human subjects discovered a 2.5 fold increase in AED uptake at the BBB when co-administered with TQD.[21] Combined with the previous study, these results provide convincing evidence that the P-gp inhibitor, TQD, counteracts drug-resistance in epileptic rats and could act as an effective treatment method to improve the standard of care in refractory epilepsy.

In a single case study, a patient was administered Verapamil in conjunction with an AED proven to be a reliable treatment method.[22] With the combination of Verapamil and the AED, the patient was able to double the time between hospitalizations for seizures, demonstrating a significant improvement. Even so, researchers determined that it was impossible to isolate the cause for the improvement, as it could have resulted from Verapamil, the viable AED, the placebo effect, or any combination of the three. Only further research in a controlled setting would determine if Verapamil is a reliable P-gp inhibitor and treatment for refractory epilepsy. Moreover, even if researchers could determine that Verapamil was the cause for improvement, they could not determine the mechanism by which Verapamil acted. With only this one major study providing inconclusive evidence, Verapamil lacks the support needed to establish its efficacy as a P-gp inhibitor and positions Tariquidar as the better therapeutic option.[22]

4.3 Limitations of TQD

While many developments have been made in studying refractory epilepsy, significant uncertainty must be accounted for to better understand treatment options, especially in relation to TQD. Although TQD shows the most promise as a treatment for refractory epilepsy, there are limitations that should be considered. Recent TQD studies have demonstrated significant adverse events. The aforementioned study by Van Vliet E. A. et al. reported significant abdominal pain in the TQD treatment group, resulting in a dissolution of the trial. While examining the potential application of TQD for multidrug resistance in cancer, research was discontinued after 41% of subjects reported adverse effects to the drug.[23] Researchers in one study concluded that administration of

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TQD was not associated with enhanced toxicity or altered blood plasma pharmacokinetics as in the case of several other P-gp inhibitors, yet these results have not been replicated in similar studies nor demonstrate consistency with other findings.[20] Two studies revealed that, despite the significant improvement in seizure activity during the first few days of TQD and AED coadministration, TQD tolerance eventually developed through unknown mechanisms.[19, 21] These results implicate the need for further exploration of the pharmacology of TQD in order to reduce tolerance to the drug. TQD could potentially lead to toxicity in organs other than the brain, such as the intestines, liver, and kidneys by inhibiting the efflux effect of P-gps expressed in those organs as well.[17] More knowledge on the adverse events of TQD is necessary to ensure its safety in the clinical setting. Additionally, since current research predominantly considers the effects of TQD for two types of AEDs (phenobarbital and phenytoin), further investigation on whether these effects can be extrapolated to a broader range of AEDs would provide a more comprehensive treatment method for refractory epilepsy.

Conclusion

Refractory epilepsy is a complex, multifaceted disease that impacts millions of people worldwide. The drug-resistant nature of this condition poses unique challenges for physicians attempting to provide therapeutic care to patients and establishes an urgency for researchers to determine its mechanisms. While many hypotheses attempt to explain the mechanisms of refractory epilepsy, the overexpression of P-glycoprotein (P-gps) transporters in endothelial cells at the blood brain barrier best accounts for pharmacoresistance. Across the numerous studies examined within this review, a clear relationship was identified between the overexpression of P-glycoproteins in epileptic brain tissue and reduced uptake of antiepileptic drugs (AEDs) at the blood brain barrier. The consistent and replicated association between P-gp overexpression and reduced AED concentration demonstrates that, as a result of the drug efflux process, P-gp overexpression is the primary mechanism of decreased drug efficacy in refractory epilepsy patients.

With P-gp overexpression identified as the lead cause of refractory epilepsy, the focus shifts towards improving the standard of care for refractory epilepsy patients through the proposed treatment method of P-gp inhibition. This treatment method suggests that the co-administration of an AED and P-gp inhibitor reduces the efflux effects of P-gp and allows for increased AED uptake at the blood brain barrier, thereby increasing the therapeutic effect of the drug. Two P-gp inhibitors, Tariquidar (TQD) and Verapamil, have been identified as potential treatment options. However, due to the lack of evidence supporting Verapamil's efficacy, it has been determined that TQD poses the most promise as an effective treatment option for refractory epilepsy. Many studies examined within this review showed that when co-administered with an established AED, TQD led to increased AED concentration in brain tissue and, thus, greater drug efficacy. Despite these promising findings, Tariquidar still poses limitations that warrant further investigation. Many studies have shown that TQD may cause adverse events such as tolerance and toxicity. Additionally, very few studies using human subjects have been conducted on TQD inhibitors and their effect with various AEDs. With these limitations and gaps in knowledge, the need for more clinical trials specifically investigating the effects of TQD and other P-gp inhibitors such as Verapamil is clear. In addition, future studies should be conducted to determine the mechanisms of TQD tolerance and toxicity in order to improve the safety and efficacy of TQD. Such findings may affirm the ability of P-gp inhibitorto overcome pharmacoresistance and finally providean effective therapeutic strategy for refractory epilepsy.

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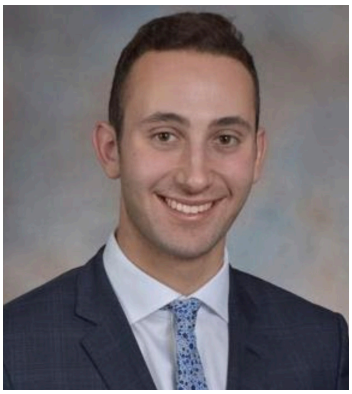

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