Gender-Affirming Hormone Therapy for Transgender Youth: Telomere Homeostasis, Psychological Wellbeing, and Barriers to Research
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Executive Summary
There exists an ongoing mental health crisis amongst transgender youth in the United States. Children and young adults whose gender identity does not align with their sex assigned at birth face startlingly high rates of depression, anxiety, suicide, and psychological distress. In order to combat these life-threatening mental health concerns, trans youth often seek out gender-affirming hormone therapy (GAHT), which most typically involves the administration of puberty suppressants followed by hormone treatment with testosterone or estrogen. While there exists broad medical consensus regarding the efficacy of GAHT in curbing adverse mental health outcomes, the dearth of clinical research in this field of medicine is profound. There is only a limited number of studies investigating the impacts of GAHT on the psychological health of trans youth, and research looking into the biological health of GAHT recipients at the molecular level is virtually absent. Not only does this deficiency of research underscore the lack of unified approaches to studying healthcare for trans youth, but it also highlights the broader lack of institutional attention and funding given to research on transgender health. This inattention is deeply impactful, as a “lack of evidence” for the efficacy of GAHT for trans youth has been cited by numerous legislators across the US in an attempt to delegitimize the practice of gender-affirming medical care and illegalize GAHT for trans minors.

With the understanding that more comprehensive psychological and biological research on GAHT for trans youth is critical, particularly at the volatile sociopolitical juncture at which the trans youth of America are currently positioned, I collaborated with faculty at Princeton University and Rutgers–Robert Wood Johnson Medical School to initiate a large-scale, NIH-funded clinical study on the longitudinal impacts of GAHT on the psychological and biological wellbeing of trans youth. The subject pool consists of patients seeking GAHT throughout the Rutgers Health system, and the study is non-interventional, meaning there is no interruption to the patients’ standard care. We set out to quantify “biological wellbeing” by measuring the lengths of patients’ telomeres as they progress through their first year of GAHT. Telomeres are the structures at the ends of our chromosomes that protect our DNA from degradation, and they have been shown to shorten at higher than normal rates in children facing significant life stress, resulting in increased genomic instability and greater susceptibility to various heart conditions, cancers, and cognitive deficits. Thus, we planned to track telomere shortening in our patient cohort while also tracking their psychological health (using surveys, discussed below) to determine whether GAHT improves mental health in trans youth while also slowing the rate at which their telomeres shorten. This would provide novel evidence for the health benefits of GAHT for trans youth at both the molecular and macroscopic levels.

This thesis is a subset of the larger clinical study outlined above and has three core aims: (1) to determine a baseline effect of the hormones used in GAHT (testosterone and estrogen) on telomere shortening in a laboratory context; (2) to flesh out the infrastructure for the larger clinical study outlined above by establishing a patient cohort, validating our psychological measures, and creating a
biorepository for later analysis; and (3) to examine the procedural and systemic barriers to our clinical study and implement methodological reforms using a continuing quality improvement (CQI) approach.

Using PCR-based methods, we uncovered a baseline biological relationship between telomere homeostasis and testosterone/estrogen in normal human cells, thus complicating any results derived from our future analyses of telomere homeostasis in trans youth receiving testosterone or estrogen treatment. We then successfully formed an interdisciplinary research team for the clinical study, recruited a cohort of patients undergoing GAHT, validated the functionality of the surveys used for their psychological assessment, and initiated the formation of a biological repository to reliably track telomere shortening in the trans youth enrolled in our study. Finally, our CQI investigation revealed 7 major areas of concern in our patient recruitment strategies, allowing for the implementation of reforms that will not only improve our own methodologies but also provide insight into best practices for future clinical research on GAHT for trans youth.

**Background**

Recent estimates indicate that anywhere from 0.58-3.2% of adults in the United States identify as transgender (Meyer et al., 2017; Wilson & Kastanis, 2015), while trans youth are estimated to be around 0.73-1.3% of all children ages 13-17 (Meyer et al., 2017; Shields et al., 2013). While these data suggest that trans adolescents already number up to 300,000 in the United States, the percentage of individuals who identify as transgender has been increasing in recent years (Jones, 2021).

The growth and diversification of the transgender community over the past several years is important because trans individuals – particularly trans youth – are confronting a widespread mental health crisis. Nearly every study to date on transgender adolescents reports higher than average rates of anxiety, depression, suicidality, and body image dysphoria (Dhejne et al., 2016; Olson et al., 2015). **Recent studies have found that nearly 50% of transgender teens and young adults report suicidal ideation, while 35% report at least one suicide attempt** (Adams & Vincent, 2019; Johns, 2019). Moreover, transgender youth have been found to be 5.1 times more likely than cisgender adolescents to talk about suicide and 8.6 times more likely to attempt suicide (Aitken et al., 2016). While bullying, familial rejection, and social alienation are believed to be key stressors in the lives of transgender youth (Ahuja et al., 2015; Pariseau et al., 2019), the psychological trauma stemming from pubertal development plays a central role in bringing about such severe mental health outcomes in this demographic (Giordano, 2008; Lambrese, 2010).

Trans youth are often faced with feelings of intense distress during puberty that result from the incongruence between their physical/sexual development and their gender identity (Turban, 2020). In clinical settings, this distress is classified as “gender dysphoria,” and considering the impact that it can have on the wellbeing of transgender youth, medical organizations around the world have suggested a relatively common course of treatment to help avoid such deleterious mental health outcomes. The Endocrine Society, the American Medical Association Journal of Ethics, and the American Psychiatric Association recommend that transgender youth begin puberty blockers around age 10 or 11 (whose effects are reversible upon discontinuation of treatment) and continue with testosterone or estrogen treatment at age 15 or 16 (APA, 2020; Hembree et al., 2017; Lambrese, 2010).
The impacts of GAHT on the psychological wellbeing of transgender youth has become a subject of study only in the past decade. Foundational studies in the Netherlands (2014) and the UK (2015) uncovered preliminary improvements in psychosocial wellbeing after GAHT, but their sample sizes were too small to make definitive conclusions (Costa et al., 2015; de Vries et al., 2014). Recent studies have yielded stronger results, yet they are few in number and require more robust corroboration. A 2020 study found considerable reduction in body dissatisfaction among a cohort of 148 transgender youth (ages 9-18 years) (Kuper et al., 2020), while preliminary results from an ongoing longitudinal study revealed an overall decrease in depression and suicidal ideation and an increase in quality of life among 50 transgender youth as they progressed through hormone therapy (Achille et al., 2020). While these results suggest that gender-affirming care has an overall positive effect on the psychological health of trans youth, further study is needed.

Given that trans youth face are disproportionately exposed to social and psychological stressors, the biological impacts of such stress – and thus the biological impacts of gender-affirming medical care – require in-depth attention. Interestingly, recent investigation into telomeres, the small structures at the ends of our chromosomes, has uncovered an entirely new way of monitoring changes in stress at the molecular level.

Telomeres shorten at a baseline rate in all individuals and have thus been used as reliable markers of aging. Because telomeres are less effective at protecting our DNA as they shorten, this is a major theory behind why humans are more susceptible to disease and other health risks as we get older, as shorter telomeres are correlated with increased DNA instability and acquisition of heart diseases, cancers, and cognitive deficits (Blackburn, 1991; de Lange, 2005). Sadly, above average rates of telomere shortening has been well-documented in children facing extreme life stress. For example, the Fragile Families and Child Wellbeing (FFCW) Study found that children from disadvantaged backgrounds (e.g., low-income, single parenting, overly strict parenting, low level of parental education) had significantly shorter telomeres than did same-aged children raised in more advantaged environments (Mitchell et al., 2014). Children who have lost fathers, either through death, divorce, or incarceration, were also found to have above-average rates of telomere shortening (Mitchell et al., 2017). Other factors that are associated with reduced telomere length in children include prenatal stress (Entringer et al., 2015), childhood abuse (Kiecolt-Glaser et al., 2011), and psychological trauma resulting from maltreatment as a child (Tyrka et al., 2010).

No research has been conducted on whether telomere attrition can be used as a biological marker for stress in trans youth undergoing GAHT – and whether any improvements in mental health (ostensibly triggered by GAHT) would be accompanied by reductions in the pace of telomere shortening, thereby mitigating a child’s risk for genomic instability and acquisition of the various cancers, heart conditions, and cognitive deficits associated with advanced telomere shortening. Such research has the potential to provide novel insight into the impacts of GAHT both on the psychological wellbeing of trans youth and on their biological health at the molecular level.

While these unaddressed research questions are of critical importance to advancing our understanding of GAHT for trans youth, they also underscore the lack of biomedical research conducted in the field of gender-affirming medicine more broadly. This is relevant because a “lack of evidence” for the efficacy of gender-affirming care in curbing suicidality, depression, and other life-threatening mental health issues is often cited as a primary reason for anti-trans health policy, both in the United States and abroad (BBC News, 2020; Browning, 2021). In 2020 alone, a record 127 bills targeting transgender children and adolescents were proposed in United
**States legislatures**, most of which attempted to restrict access to puberty blockers and sex steroid hormones for individuals under eighteen years of age by categorizing the treatment as **medical malpractice, neglect, or child abuse** (López, 2021; Trotta, 2021). In April 2021, Arkansas became the first state to enact into law a complete ban on gender-affirming medical care for minors, issuing felony penalties and revocations of medical licenses to physicians who help administer GAHT to trans youth, regardless of parental consent or medical necessity (Trotta, 2021).

Despite there being consensus in the medical field regarding the best course of treatment for youth seeking gender-affirming care, there is an admitted dearth of peer-reviewed studies in this field. Since it is a relatively young area of medicine, there is still much to learn about the intricacies of GAHT, particularly through a biosocial lens, and thus, **there exists an imperative for more comprehensive research to establish a standard of care that is rooted in evidence-based medicine**. Many large research organizations have recognized the existing gap in research (and the sociopolitical ramifications of such a gap) and have issued plans to rectify it. In response to the 2021 bill in Arkansas banning GAHT for minors, for instance, the Society for Research in Child Development (SRCD) promised to continue “advancing developmental science” in order to more carefully investigate the ways in which GAHT impacts the health and wellbeing of transgender youth so that **the potential harm caused by restrictive anti-trans legislation could be made starkly evident** (SRCD, 2021).

By emphasizing a need for more focused attention to trans health research, organizations such as the SRCD are underscoring the paucity of validated methodologies and unified approaches to studying transgender healthcare. This lack of consensus on best practices for researching gender-affirming medical care has resulted in a notably limited number of studies on the psychological and biological wellbeing of trans youth undergoing GAHT.

Thus, this thesis sets out to not only initiate an investigation into the interactions between GAHT, telomere homeostasis, and psychological health in transgender youth, but also provide a robust analysis of the research methods and quality improvement tactics that future researchers can use in their efforts to contribute to the goals outlined by the SRCD and bolster biosocial research on GAHT for a population that is growing increasingly vulnerable in the healthcare domain.

**Aims & Methods**

**Aim 1: Laboratory Analysis**

Given our team’s ultimate goal to establish a psychobiological relationship between telomere homeostasis and mental health changes prompted by GAHT, it is critical to first identify any underlying biological relationship – irrespective of psychological effects – between the steroid hormones employed in GAHT (estrogen and testosterone) and telomere shortening. Thus, I first set out to determine whether estrogen or testosterone slows the rate of telomere shortening in cultured cells (as opposed to cells derived from live patients), allowing me to determine whether telomere shortening is being impacted by testosterone or estrogen chemically before our team assesses whether is being impacted by changes in our patients’ stress.

We cultured human cells in growing media and treated them with estrogen and testosterone. We then used PCR-based methods to measure the activity of the protein that lengthens telomeres, which can be used as a quantitative measure of telomere homeostasis.
Aim 2: Clinical Study
Because our clinical study is ongoing and data that would address our overarching hypotheses are still being collected, the second aim of this thesis is to establish a reliable infrastructure and methodological framework for longitudinally assessing the psychological health and telomere homeostasis of trans youth throughout their first year of GAHT.

First, we set out to establish a longitudinal cohort of ~100 transgender youth for psychological and biological evaluation during their first year of GAHT. This involved forming an interdisciplinary team of researchers, securing grant funding, creating a comprehensive budget, and forging institutional partnerships with nearby GAHT clinics that would grant us access to eligible patient populations. This also required establishing reliable controls for the psychological assessments, which include patients’ cisgender siblings, and for the telomere homeostasis measurements, which also include age-matched, unstressed controls from the Fragile Families and Child Wellbeing (FFCW) cohort, provided by Professor Daniel Notterman.

We then created a comprehensive set of surveys to assess mental health, gender dysphoria, body image, and distress in our patient cohort. We administered the surveys to our study subjects before treatment onset, 4 months into treatment, and 12 months into treatment. We conducted a semi-quantitative evaluation of the available responses, using score averages and T-scores as preliminary indicators of survey responses and subsequently using question completion rates as indicators of survey functionality.

Finally, we set out to collect and preserve DNA, RNA, and protein-based samples from patient blood draws. These samples, which are being collected at the same time points as the psychological assessments, will allow for future analysis of patients’ telomere shortening over their first year of GAHT.

Aim 3: Continuing Quality Improvement (CQI) Study
Despite the best efforts of our research team, we have been unable to achieve the recruitment goals established at the outset of the study. With the intention to enroll ~100 patients throughout the first year of the study’s recruitment period, we have been able to enroll only 8 patients. Due to (1) the curtailed enrollment observed over the first year of recruitment and (2) the scarcity of validated methodologies in the literature for conducting clinical research on the health of trans youth undergoing GAHT, I conducted a continuous quality improvement (CQI) study – a method of systematic quality assurance that has noted success in pediatric settings dealing with mental health care (Bickman & Noser, 1999) – to uncover potential insufficiencies in our study’s methodological design and recruitment strategies.

As per the narrative interview approach to continuous quality improvement research that has proven successful in the past (Greenhalgh et al., 2005; Sommerbakk et al., 2016), I conducted qualitative interviews with each member of the study team, as well as with a limited number of legal guardians of enrolled patients. Questions were aimed at assessing our team’s communication practices with potential subjects, outreach strategies to the trans community in New Jersey, expectations of individual responsibilities in the team, and institutional support from Rutgers and Princeton. Based on the interview responses, I identified possible areas of concern and used a semi-quantitative approach to label each an area of low, medium, or high concern. I then presented my findings to the study team and implemented several methodological reforms.
Key Findings

Laboratory Analysis
We found that there was indeed a baseline biological relationship between testosterone, estrogen, and telomere shortening. Both testosterone and estrogen treatment were found to decrease the rate at which telomere shortened. These results will make any clinical findings regarding the relationship between GAHT, telomere homeostasis, and psychological distress far more robust.

Clinical Study
I first formed a study team comprised of (1) an expert on telomere homeostasis in childhood adversity; (2) the Chief of Pediatric Endocrinology at Rutgers RWJ Medical School and the first pediatric endocrinologist in New Jersey to have systematically provided gender-affirming medical care to transgender youth; and (3) the foremost authority in the field of adolescent transgender psychology. Together, we established a comprehensive budget which covered the compensation for study subjects at each visit, the salaries of research coordinators and lab technicians working on the study, and the materials needed for biological and psychological assessment. We then secured year-long grant funding from the NIH and research approval from the Princeton University IRB. Finally, we forged institutional partnerships with the Rutgers Pediatric Clinical Research Center (PCRC) and the pediatric endocrinology clinic at Rutgers–Robert Wood Johnson Medical School, granting us access to a population of eligible study subjects and a more comprehensive team of physicians, nurse managers, and research coordinators to help carry out this study. This allowed us to successfully enroll eight patients over the first year of recruitment (April 2021 - April 2022), ages ranging from 11 to 17 years, 57.1% self-identifying as boys (all of whom were assigned female at birth), and 42.9% self-identifying as girls (all of whom were assigned male at birth).

After establishing this foundational research infrastructure, we then successfully validated the 12 surveys used to assess the psychological wellbeing of our patient cohort. The results from my analysis of the available survey responses tentatively confirmed my hypothesis that our psychological measures are functional with respect to the patient demographic we are interested in studying. Given that each survey asked respondents to “skip any questions [they] are not comfortable answering,” the consistently high question completion rates calculated from the survey responses (15 out of 18 measures showing >90% completion) provide proof of concept that this patient population understands and is generally receptive to the psychological measures we have developed. Moreover, the measures evaluated in this analysis can generally be considered trustworthy given that none of the response averages approached the absolute maximum or the absolute minimum.

Finally, having created a patient cohort of size n=8 thus far, we successfully established a growing biorepository containing the Time 1 blood samples (baseline, pre-treatment) of all 8 subjects and the Time 2 blood samples (4 months into treatment) of 5 subjects. These are stored at Princeton University and will be assessed for telomere length when the repository reaches a sufficient size over the coming months.

Continuing Quality Improvement (CQI) Study
After my interviews with each member of the study team and half of the families of patients enrolled in our study, I identified 7 principal areas of concern with respect to the enrollment protocols of our clinical study and labeled each an area of low concern, medium concern, and high concern. They are (1) mismatched expectations of responsibilities within the team, (2) insufficient written materials for
patients, (3) inconsistent/improper messaging with patients, (4) deficient in-person communication with patients, (5) deficient sources of eligible study subjects/insufficient community outreach, (6) unresponsiveness of patients after referral, and (7) burdensome travel/time commitments for patients. Major methodological reforms that we implemented (after a series of collaborative meetings involving all levels of the study team) included the following:

- The creation of a one-sheet handout for interested patients to outline all the details of the study in a more clear, concise manner.
- The creation of scripts for research staff and GAHT providers to use when introducing patients to the study to standardize communication with patients (as GAHT can be a very delicate subject for some patients).
- The creation of handouts (one more simple and one more complex) about telomere biology to allay fears about unwanted genetic testing.
- The encouragement of GAHT providers to resume in-person patient visits (as long as COVID-19 precautions are being taken) to bolster face-to-face communication.
- The addition of 6 new GAHT providers to the study team to increase the eligible patient population/the number of patients being referred to the study.
- The initiation of community partnerships with LGBTQ+ health organizations in the New Jersey area, including NJ Planned Parenthood, NJ PFLAG, and a NJ-based transgender support group.
- The promotion of the study throughout the Rutgers Health system and the NJ PROUD Center to better establish ourselves as a trusted research group in the trans community of New Jersey.
- The addition of a new research coordinator to solely handle enrollment logistics.
- The implementation of at-home phlebotomy to reduce the number of in-clinic visits that study subjects have to make.

Since implementing these reforms, the clinical study has seen a sharp increase in patient enrollment, and thus, I believe these results could prove helpful for future researchers planning to study GAHT in a clinical, pediatric context.

**Future Directions & Policy Recommendations**

This thesis addresses three major gaps in research, allowing for more comprehensive insights into (1) the biological relationships between GAHT and telomere shortening in a non-clinical, laboratory context; (2) the clinical research infrastructure needed to appropriately assess longitudinal changes in psychological wellbeing and telomere shortening in trans youth undergoing GAHT; and (3) the methodological tactics researchers can use to overcome the institutional and procedural barriers to studying GAHT in a clinical setting.

More broadly, this thesis underscores the need for more focused, better funded, and more comprehensive research on GAHT for trans youth – and it outlines the tactics that are effective (and ineffective) in carrying out this sort of research. The institutional and procedural barriers uncovered by our CQI study highlight the need for large, grant-giving institutions like the NIH and SRCD to actively encourage research on GAHT in the form of funding, and they emphasize the importance of prioritizing trans health research in large research hubs like universities and major medical centers like Rutgers Health. Moreover, they exhibit the value in creating longstanding partnerships with the trans community outside of a purely medical
context in order to establish credibility as a research group and mutual trust amongst the patient population.

Not only will the research frameworks offered by this thesis bolster our understanding of GAHT in a clinical sense (thus helping to establish a standard of care that is rooted in evidence-based medicine), but it will also help make abundantly clear that legislation attempting to ban GAHT for trans youth is counterproductive to the ongoing mental health crisis amongst one of this country’s most vulnerable populations. There must be more attention given to this field of research so that discriminatory and unscientific legislation cannot continue to be passed.

Excitingly, the research infrastructure established by this thesis could be applied to a much larger study assessing the longitudinal psychological health and telomere homeostasis of trans youth at GAHT clinics across the country. Because the study is non-interventional in nature, requires no additional phlebotomy that would not already be required by standard medical care, and utilizes surveys that are entirely electronic, the assessment of telomere length and psychological health would be easily employable from any location. With the appropriate funding, training for research staff, and laboratory resources, a more expansive study using the research framework established by this thesis would greatly contribute to the nascent efforts to bolster clinical research on GAHT for trans youth.
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