Turner Syndrome

Health and Wellness in the 21st Century:

The crossroads of health care delivery and health research

Edited by

Michael Silberbach
Charmian A. Quigley

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This book is dedicated to Dr. Carolyn A. Bondy, who devoted herself for over thirty years to advancing the health and well-being of those living with Turner syndrome.

About the Authors

Philippe F. Backeljauw, MD, is a Professor of Clinical Pediatrics at the University of Cincinnati College of Medicine, Cincinnati, OH. Dr. Backeljauw is engaged in clinical research related to growth and Turner syndrome. He is the medical director of the Turner Syndrome Center of Cincinnati, a multidisciplinary team that cares for more than 200 girls and women with Turner syndrome.

Carolyn Bondy, MD, is the former chief of the Developmental Endocrinology Branch and now Scientist Emeritus at National Institute of Child Health and Human Development, Bethesda, MD. Dr. Bondy has authored more than 120 publications on the topics of growth hormone therapy and well-being in girls and women with Turner syndrome. Dr. Bondy's "Turner Syndrome Genotype-Phenotype Study at the NICHD" was the largest multidisciplinary study of its kind in the world. Dr. Bondy promotes awareness of Turner syndrome and is recognized as a world-wide expert on the clinical and genetic features of the condition.

Steven D. Chernausek, MD, is Associate Chief of the Pediatric Diabetes and Endocrinology Section and holds the Edith Kinney Gaylord Endowed Chair in Pediatric Diabetes/Endocrinology at the University of Oklahoma Health Sciences Center, Oklahoma City, OK. Dr. Chernausek has extensive experience in investigation and management of children with various growth problems, including Turner syndrome, and is the past president of the Pediatric Endocrine Society (North America).

David Cole, PhD, is the former Vice President for External Affairs at the Whitehead Institute, Cambridge, MA, where he directed the Institute's philanthropic, communications, and public education programs. He currently serves as Executive Director of the Hagley Museum and Library in Wilmington, DE.

Melissa Crenshaw, MD, is a woman with Turner syndrome and a practicing clinical geneticist and assistant professor of Pediatrics at Johns Hopkins University School of Medicine. Dr. Crenshaw is the director of medical genetics services and Chief of the Division of Genetics at All Children's Hospital, Johns Hopkins Medicine, St. Petersburg, FL. Her research focuses on Turner syndrome and genetic aspects related to aortic aneurysms and dissection. Dr. Crenshaw has served on the Board of Directors of the Turner Syndrome Society of the United States and is a member of its Professional Advisory Board.

Marsha L. Davenport, MD, is a recently retired professor of pediatric endocrinology at the University of North Carolina at Chapel Hill, NC, where she cared for children with endocrinology problems and directed a large clinic for children with sex chromosome variations, particularly Turner syndrome. Her research has focused on early diagnosis for girls with Turner syndrome, routine screening for associated problems, and early brain development. She is principal investigator for a ten-year observational study of girls with Turner syndrome for which data collection was recently completed.

David S. Hong, MD, is an instructor at the Center for Interdisciplinary Brain Sciences Research and Division of Child and Adolescent Psychiatry, Stanford University, Stanford, CA. He is a child psychiatrist with a long-standing research interest in the cognitive aspects of neurogenetic syndromes and is actively involved in the Turner syndrome community.

Stephen Hooper, PhD, is Associate Dean and Chair of the Department of Allied Health Sciences at the University of North Carolina School of Medicine, Chapel Hill, NC. He has thirty years of clinical practice experience with various childhood conditions, and his research interests have focused on intellectual/developmental disabilities, genetic conditions, pediatric chronic illness, and interventional science.

Rebecca Knickmeyer, PhD, is an Assistant Professor in the Department of Psychiatry at the University of North Carolina, Chapel Hill, NC. She is a developmental neuroscientist with a long-standing research interest in sex hormone and sex chromosome effects on the brain.

Paul Kruszka, MD, is a clinical geneticist and staff clinician in the Medical Genetics Branch at the National Human Genome Research Institute at National Institutes of Health, Bethesda, MD. Along with Dr. Maximilian Muenke (Medical Genetics Branch Chief) and Dr. Nicole Banks (clinical geneticist), he is currently building on the genotype-phenotype study initiated by Dr. Carolyn Bondy, and is applying next generation sequencing technology to this cohort to determine the genetic and molecular underpinnings of congenital heart disease.

Nelly Mauras, MD, is a Professor of Pediatrics at the Mayo College of Medicine and a clinical endocrinologist at Nemours Children's Health Systems, Jacksonville, FL. Dr. Mauras is a nationally and internationally recognized speaker and is widely published in the field of endocrinology in general and Turner syndrome in particular, with over 160 papers in the peer reviewed literature.

Danny E. Miller is an MD/PhD student at the University of Kansas Medical Center, Kansas City, KS, currently working in the laboratory of R. Scott Hawley at the Stowers Institute for Medical Research, Kansas City, MO. His research interests include developing new methods to detect mosaicism in women with Turner syndrome.

David Page, MD, is the Director and a Professor of Biology at the Whitehead Institute, Howard Hughes Medical Institute, and Massachusetts Institute of Technology, Cambridge, MA. Dr. Page is the world's foremost authority on the Y chromosome.

Victoria Pemberton, RNC, MS, CCRC, is a Clinical Trials Specialist at the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD. She is the Project Officer for a number of pediatric clinical studies, including the Pediatric Heart Network, a collaboration between regional centers.

Siddharth Prakash, PhD, is an Assistant Professor in Medical Genetics at the University of Texas, Houston, TX. His research focuses on genetic causes of bicuspid aortic valves and related congenital anomalies of the left ventricular outflow tract and aorta. He is codirector of the Multidisciplinary Aortic and Vascular Disease Clinic at the University of Texas.

Charmian A. Quigley, MBBS, is an Associate Professor of Pediatric Endocrinology at Indiana University School of Medicine, Indianapolis, IN. Her studies of girls with Turner syndrome have focused on the understanding of growth and pubertal development in these girls through collaborations with groups at the National Institutes of Health, Thomas Jefferson University and the multicenter, long-term "Toddler Turner" study that ran from 1998 to 2015.

David E. Sandberg, PhD, is a Professor and the Director of the Division of Child Behavioral Health in the Department of Pediatrics at the University of Michigan, Ann Arbor, MI. As a pediatric psychologist, Dr. Sandberg provides psychoeducational and behavioral health services to children and adolescents with endocrine disorders, including Turner syndrome.

Nicole Sheanon, MD, is an Assistant Professor of Pediatrics in the division of Endocrinology at Cincinnati Children's Hospital Medical Center in Cincinnati, OH. She is part of the Turner Syndrome Center of Cincinnati, a multidisciplinary team that cares for more than 200 girls and women with Turner Syndrome. Dr. Sheanon is engaged in clinical research related to metabolic disturbances in Turner syndrome.

Michael Silberbach, MD, is a Professor of Pediatrics at Oregon Health & Science University, Portland, OR. He is a practicing pediatric cardiologist. He serves as the chairman of the Professional Advisory Board of the Turner Syndrome Society of the United States and is the chairman of the Turner Resource Network (TRN) project steering committee. His research focuses on the genetics, pathophysiology and imaging of aortic disease and the cause of congenital heart disease in Turner syndrome.

Cheri Svagerko, MOT, OTR/L, BA, holds a bachelor's degree in psychology and a master's degree in occupational therapy. After practicing occupational therapy for thirteen years, Cheri joined the Turner Syndrome Society of the United States as their Development Coordinator in order to advance the fundraising efforts of the Society.

Lournaris Torres-Santiago, MD, is a pediatric endocrinologist at Nemours Children's Health Systems in Jacksonville, FL, and Assistant Professor of Pediatrics at the Mayo College of Medicine. Dr. Torres-Santiago has a special interest in care of girls with Turner syndrome, authoring a pivotal paper on the biological effects of transdermal vs. oral 17 children and adolescentadolescents.

Jeanie B. Tryggestad, MD, is an Assistant Professor at the University of Oklahoma Health Sciences Center, Oklahoma City, OK. Her research interests include obesity, type 2 diabetes, and vascular function in these conditions. Dr. Tryggestad is the Director of the Turner Syndrome Clinic at the University of Oklahoma Health Sciences Center in Oklahoma City, OK.

Karin Weiss, MD, is a diplomate of the American Board of Medical Genetics and Genomics and is currently a research fellow in the laboratory of Dr. Maximilian Muenke in the National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

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Introduction

On July 13 and 14, 2014, the Turner Syndrome Society of the United States sponsored the inaugural meeting of the Turner Resource Network (TRN) in Jacksonville, Florida. In attendance were women living with Turner syndrome from all walks of life. They are mothers, daughters, lawyers, teachers, judges, administrators, clerks, pediatricians, internists, endocrinologists, clinical geneticists, students, and psychologists. These committed girls and women freely exchanged ideas with the more than thirty clinicians and scientists who were present at the meeting. A summary of their ideas has been published (1).

The overarching goal of the TRN symposium was to kick-start a national effort to improve the health and well-being of those living with Turner syndrome. The specific aims were to

1. identify the major health policy issues facing girls and women living with Turner syndrome;
2. review basic science and clinical questions that might be answered by either bench research, clinical trials, clinical datasets, or registries; and
3. discuss a strategy for how a national network of regional Turner syndrome resource centers (the TRN) will be organized.

The likelihood of premature death among those with Turner syndrome is significantly higher than the general population (2). And the probability of death from cardiovascular disease alone in those with Turner syndrome matches the risk of dying from all causes in the general population. Overall, mortality in women with Turner syndrome is threefold higher than in the general population at a given age, raised for almost all major causes of death, and elevated at all ages (2).

Apart from the major issue of the health and well-being of girls and women with Turner syndrome, the other major theme woven throughout these chapters is the fascinating and important question: What does increased susceptibility to common disorders caused by a missing or deficient second sex chromosome teach us about disease susceptibility in the general population? (3). Could knowledge of Turner syndrome reveal critical genetic differences between female and male individuals that may allow us to help humanity as a whole? In chapter five, David Page, director of the Whitehead Institute at the Massachusetts Institute of Technology, and Danny Miller of the Stowers Institute in Kansas City, Missouri, elegantly introduce this fundamental and underappreciated concept. Perhaps by addressing the pressing health needs of the Turner syndrome community, we can simultaneously make important discoveries about the pathophysiology of many common afflictions in the general population.

This book is a compilation of the presentations given at the 2014 Jacksonville meeting. The meeting agenda was divided into three areas, and these three areas represent the three major themes of the book: health policy, Turner syndrome science, and the Turner Resource Network road map. We hope this book will provide an overview of the significant health issues in the field and will engage others in helping to address currently unanswered questions. This work is the

product of the combined efforts of the stakeholders of the Turner syndrome community represented by the Turner Resource Network (the TRN). We hope it will be the first of many collaborations that will serve to improve the health and well-being of everyone whose lives are touched by Turner syndrome.

We are indebted to numerous organizations and their representatives who committed both financial support and time to make the symposium a success. These include the Turner Syndrome Society of the United States, the National Institute of Childhood Health and Human Development, the Office of Women's Health Research, the March of Dimes, the American Heart Association, and the Leaping Butterflies Ministry. Special thanks to the Turner Syndrome Global Alliance and the Turner Syndrome Foundation for their valuable insights and gifts of time.

In particular, the symposium and this book would not have been possible without the singular vision and constant support of Cindy Scurlock, the executive director of the Turner Syndrome Society of the United States.

Michael Silberbach, MD
Chairman, Turner Resource Network Project Steering Committee
Professor, Pediatrics Division, Pediatric Cardiology
Doernbecher Children's Hospital
Portland, Oregon
E-mail: silberbm@ohsu.edu

Charmian A. Quigley, MBBS
Pediatric Endocrinologist
Indiana University School of Medicine
Indianapolis, Indiana
E-mail: charmianq@gmail.com

Section 1

Health Care Policy and Health Care Delivery for Patients with Turner Syndrome

Chapter 1

The Future of Health Care in the USA: Patient Advocates, Caregivers, and Translational Research

Steven D. Chernausek, MD, and Jeanie B. Tryggestad, MD

The future of health care in the USA, what a big topic. Even more so is the number of individuals who have made greater contributions than me to the field of Turner syndrome. Barbara Lippe has been one of the leaders in the care of girls with Turner syndrome for many, many years, and she has led the way in galvanizing those that provide health care for patients with Turner syndrome. Carolyn Bondy gave up studying IGF binding proteins in rodent brains for Turner syndrome. And Judith Ross gets the merit badge for endurance in growth hormone studies.

In this chapter, we will look back to the beginnings of our dealings with Turner syndrome to see how things stood in the past and to get a sense of where we came from, which will lead us to some notion of where the field stands now and what the opportunities are for bridging basic research with clinical care for girls and women with Turner syndrome.

Henry Turner was an assistant professor at the University of Oklahoma when he first described Turner syndrome (1). And we all know that Turner syndrome is defined as monosomy X, or an X chromosome variant. The major health problems associated with Turner syndrome are primary hypogonadism, short stature, and cardiac anomalies. The prevalence of Turner syndrome is now 1 in 2,000 live female births.

We will now take a tour through the four phases of growth and development of Turner syndrome. We will start with phase one, which I have termed "childhood." This phase focuses on the early years of Turner syndrome management in the 1970s. The second phase, "adolescence," is like the growth spurt phase of Turner syndrome and focuses on the impact of therapies to improve linear growth in girls with Turner syndrome. The third phase, "young adult," is the now phase, and it focuses on the current focus of care and research in Turner syndrome. And the last phase, "mature adult," will focus on what the future directions in the care and management of Turner syndrome should be.

So in the early years, or "childhood," of Turner syndrome, the problems were the growth deficit, the cardiac anomalies, the replacement of estrogen, and the lack of how to deal with cognitive issues and social integration.

Growth hormone deficit was dealt with by giving patients oxandrolone at later ages. I would say it was a little too late, and it was not very effective. Typically, we would delay feminization to squeeze out a few more centimeters of growth, which was not very satisfying.

Cardiac anomalies, such as coarctation and bicuspid valves, were identified and corrected as appropriate by the cardiothoracic surgeon.

With regard to feminization, sex steroids were given. Conjugated estrogens were the treatment of choice. Again, doctors held off till the last possible moment to administer these in order to promote growth.

With regard to the cognitive deficits, we knew about them in the 1970s, and people reported them.

At the time, we recommended that patients receive the appropriate testing and connected them with resources to help them be successful in school.

And lastly, with regard to social integration, we tried to be helpful doctors who guided parents on how to support their girls. This was the 1970s, so it was a time prior to the support groups that are common today.

Now, on to the growth spurt, or "adolescence," of Turner syndrome. In 1980, Dan Rudman treated girls with Turner syndrome with growth hormone or oxandrolone, alone or in combination, and published his findings in the *Journal of Pediatrics* (2). This study was anathema to a bunch of pediatric endocrinologists. First, Rudman was an internist, and secondly, three months of growth for measuring was ridiculous. There were a lot of negative responses to his paper, but its publication coincided with the advent of recombinant growth hormone.

And so it was about this time that growth hormone was studied and proposed as an indication for Turner syndrome. You see, we were initially taught that growth hormone only worked for growth hormone deficiency, which was one of the complaints about Rudman's paper. The first studies were patterned after Rudman's trial of using growth hormone alone or in combination with oxandrolone. Turner syndrome was a perfect condition to try growth hormone for a non-growth hormone deficient state, as we had a good idea of how girls with Turner syndrome grew untreated. That diagnosis was straightforward—more straightforward than growth hormone deficiency—and the population was there.

So a multicenter trial was started using growth hormone with or without oxandrolone, but there was a lot of doubt about the efficacy, safety, and benefit of growth hormone. In 1996, many questions still persisted.

In the *Journal of Pediatrics* 1998 the controls were matched historical controls, and their growth was as expected. The second group, containing 17 subjects, received growth hormone alone and gained, on average, eight and a half centimeters at final height. And the group of 25 girls that received combination therapy, growth hormone plus oxandrolone, gained on average ten and a half centimeters (see figure 1).

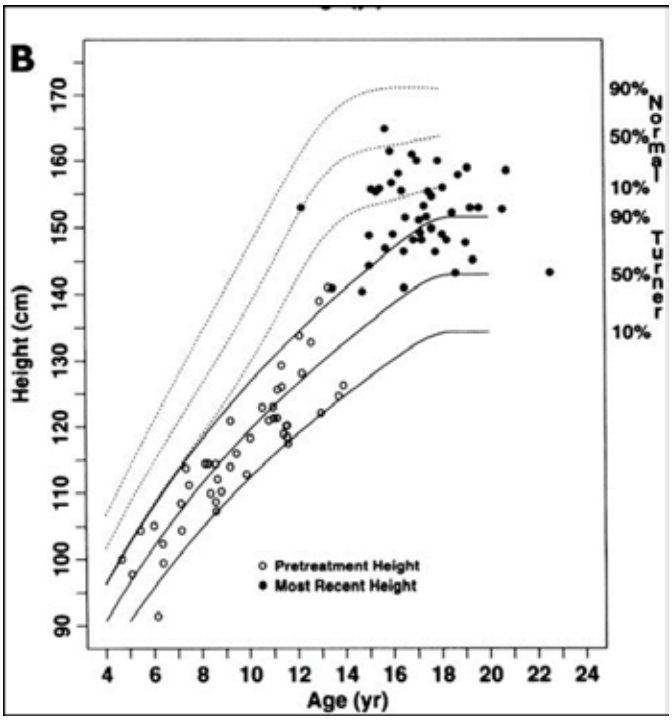


Figure 1. Height in centimeters after treatment with growth hormone and oxandrolone. Open circles indicate beginning height, and black circles indicate final height.

The open circles in figure 1 indicate the height at which the participants in the study started, and the closed circles indicate the height at which they ended. A good proportion of the participants got into the normal range for height, including participants who were started on growth hormone and oxandrolone very late.

Exploration of the oxandrolone and growth hormone combination and the delay of puberty have continued today. In a study by Gault and colleagues (4), girls were randomly given either oxandrolone or a placebo. They also received either growth hormone or feminization doses of ethinyl estradiol at two different ages. The results were analyzed in an interesting way (see figure 2).

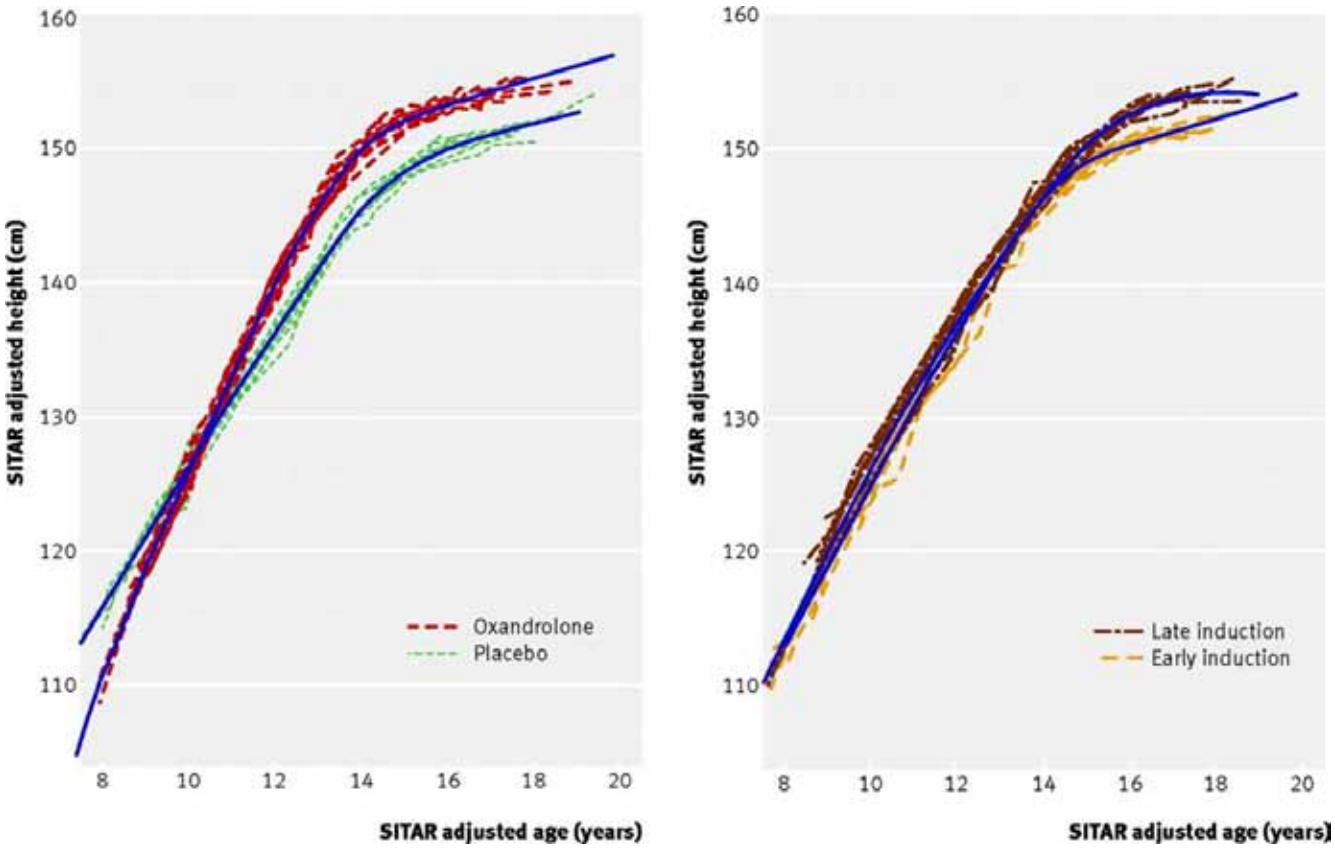


Figure 2. "Effect of oxandrolone and timing of pubertal induction on final height in Turner's syndrome" (4). The figure on the left demonstrates the effect of oxandrolone on height gain. The figure on the right demonstrates the impact of late pubertal induction on height gain.

In figure 2, each subject's growth curve is represented and then mathematically calculated to form a common growth curve. Then the two different variables, the effect of oxandrolone or the effect of late puberty, can be analyzed for the impact on growth. The graph on the left demonstrates the effect of oxandrolone. As you can see, the subjects seem to reach a greater final height at a faster pace. But the late induction of puberty, shown in the graph on the right, may provide more height gain. Interestingly, most pediatric endocrinologists continue to use growth hormone, but very few use it in combination with oxandrolone.

More importantly, the study shows that there is a benefit to final height when growth hormone is given to girls with Turner syndrome. We had found a way to treat the growth deficit safely. Other benefits came about as well. The multicenter trials morphed into post-marketing surveys, which got pediatric endocrinologists together. Turner syndrome support groups were born during this period. The Turner Syndrome Society, for instance, started in 1987. Along with these support groups, an increased awareness of Turner syndrome was born. Another benefit of this coordinated effort was that pharmaceutical companies wanted to license the product, which was a very, very good thing in the long run.

So during these middle years, or "adolescence," of Turner syndrome, we were treating girls with Turner syndrome to improve their stature. We were also starting to become more aware of the hazards of aortic root disease. We were learning how to identify it and considering what might be done about it.

We also made advancements in X chromosome biology in these middle years. We figured out how to identify which genes on the X chromosome are missing, and we figured out how these missing genes are actually translating into phenotype that might help people. One gene we identified was *SHOX*. This is the stature gene that contributes much of the height deficit in Turner syndrome. The deletion of *SHOX* is also found in other conditions where people have short stature without Turner syndrome.

In the "young adult" phase of Turner syndrome, which is the present, I believe the growth deficit is largely solved or solvable. A woman with Turner syndrome can reach a near-normal height if she is diagnosed and treated from a young age. The questions related to longterm- effects, optimal treatment, and benefit of using growth hormone and/or oxandrolone—well, those are all tougher questions, but I think answerable.

Now turning to sex steroid replacement. We know how to feminize. We are moving toward more of a fine tuning- phase, and we are taking into account the longterm- effects of the treatments.

We know egg donation works. We are seeing comprehensive disease, or condition oriented-, treatments with experienced providers. We are developing guidelines and position statements, which are things that help everyone. And, again, we are seeing patient advocate and support groups getting more involved.

I read the proposal again to see what the Turner Resource Network is supposed to do. It is supposed to coordinate excellent health care for girls and women with Turner syndrome, and it is supposed to conduct fundamental research through regional centers.

So now we come to the "mature adult" phase of Turner syndrome's growth and development. I want to focus on things that the Turner Resource Network might do or consider. what follows are my personal opinions. I will examine networks that work, and I will explain the potential causes of failure in those that did not work.

In view of the Turner Syndrome Network, what are we supposed to be doing? Now is the time when we can begin to incorporate new approaches to advancing clinical care, and I am going to provide examples of these new approaches.

First and foremost, Turner syndrome is a genetic disease. Modern genetic methodologies—applied in the correct way—could address meaningful health issues. The opportunity to link with their organizations is going to be very important too.

The Turner Syndrome Society, as a patient advocate group, informs the investigators about what is important to the girls and women with Turner syndrome. The society can partner with other individuals or societies to move things forward. The Pediatric Endocrine Society, for example, has a vested interest in this patient population.

So here are some questions for all of us to consider: Is there a one for- -all sex steroid replacement treatment? Or should we personalize the regimen based on biology, personal preference, or other issues that we should consider?

What is the true risk of pregnancy for a woman with aortic disease? How can the risk be reduced? American College of Obstetrics and Gynecology has a firm statement about women with aortic disease not having pregnancies.

What about "homologous fertility"? This means having your own progeny with your own set of chromosomes. Egg donation happens already, and in mosaic individuals, some have had cryopreservation of ovaries. These are all things we should explore further.

Here is an example of how one might approach a new method of looking at clinical conditions. There is something called a standardized clinical assessment and management plan (SCAMP). This plan was started by a cardiologist as a way to get more realtime- assessment of clinical conditions. SCAMPs are a way to guide clinical assessment and management to learn about conditions where there is limited available evidence. This is how it works: you get a bunch of expert clinicians together, and they try to figure out a plausible way of approaching some clinical aspect of medicine. It's sound practice. It's not guidelines. It is designed to improve quality. Now, there is an algorithm that you are supposed to follow, but you really don't have to. In fact, it's actually good if some people don't follow the algorithm because the data informs individuals of perhaps better alternatives.

So how would a SCAMP work? First, clinicians write a background position paper. They think of some plausible outcomes. Such as how a girl with Turner syndrome should be treated with estrogen by taking into account the size of her uterus as an adult. This relates somewhat to the ability to carry a pregnancy. Then the clinicians have a discussion about how one might best do this, but I'm not sure we know what the best method of doing this is, at this time.

That is how you start a SCAMP. You develop consensus. It is a realtime-, iterative process. Then periodically you collect data on your end points. You look at the people who have deviated, and you use that

data to inform the next steps. You examine your success rate, and you modify your protocol accordingly.

A SCAMP is not a randomized trial. It is not a guideline. It is a method of assessing outcomes in real time, real life, for a broad based- population. Everybody can participate in it. It is not something that has restrictions.

I am not saying that guidelines aren't good, but the issue with guidelines is that it is hard to keep up with the field sometimes. SCAMPs can be used to help with this. The use of a standardized clinical assessment and management plan is just another way to improve the quality of care.

The Pediatric Endocrine Society is starting one. The SCAMP is going to relate to estrogen therapy and Turner syndrome. It is being led by Bob Rosenfield. It is going to be available to all members of the Pediatric Endocrine Society, and it will look at the best way to use estrogen in patients with Turner syndrome.

Other opportunities could be in the area of genetic research. We stand at a place in time where our ability to look at gene function is unprecedented. For example, parental origin of X affects non-classical things like epigenetics and microRNAs. Figure 3 shows scans of the brains of individuals with Turner syndrome (5). The different colors mean that there is a difference between whether a patient has an X chromosome from her mom or her dad. In just the brains, you can see structural differences based on the parental origin of the X chromosome. A blue stripe means that patients received their X chromosome from their mom and that their brains are much more developed. This is just one example of many associated with the parental origin of X.

Another example is microRNAs. These are very small, non-coding RNAs that are made in the genome. They come out and affect the production of proteins by interacting with messenger RNAs. This is how it works: the microRNA (or miRNA) is made in the nucleus, then it comes out into the cytoplasm, where it is cleaved. Then the miRNA binds to the messenger RNA, affecting the production of proteins. It can also be secreted into the bloodstream and act against a different target. Each miRNA can impact potentially hundreds of other genes, causing decreased gene expression. So it is a way of coordinated regulation.

These miRNAs are implicated in kidney disease, heart disease, and cancer. There are about a thousand of them in the genome. Surprisingly, no one has published anything on miRNAs that are impacted by Turner syndrome. It cannot be because X chromosomes do not have any microRNA, because that isn't true. There is an abundance of microRNA on the X chromosome, just like on every other chromosome. It's only the poor little Y chromosome that seems underrepresented in terms of miRNA.

Let's turn our attention back to the current major issues for girls and women with Turner syndrome. In childhood, the focus is placed on optimization of growth, questions about side effects, and anticipatory guidance. In adolescence, the focus is placed on feminization schedules, social adaptation, comorbidities, and transition to adult care. And in adulthood, the focus is placed on comprehensive care, reproduction, aortic disease, and longterm- health.

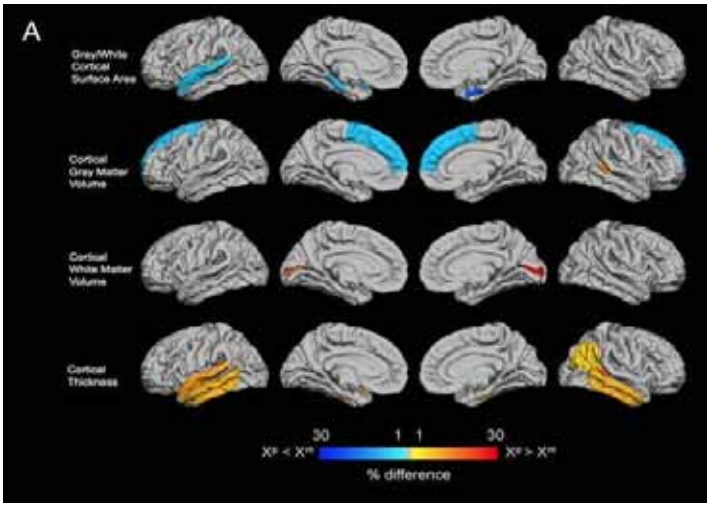


Figure 3. Brain morphology in Turner syndrome by parental origin of X. Cortical regions show significant differences between groups. *A*, Xp versus Xm. Colors show the percentage of difference between groups for each significant region. For comparisons with the control group, results are expressed in percentage change relative to controls. For comparisons between Xm and Xp, the comparison was made using Xm values as the baseline. Genomic imprinting effects are shown in *A*, where cold colors represent smaller values for individuals with Xp compared with Xm, and warm colors the opposite pattern. The rows of the figure correspond from top to bottom, to cortical surface area, gray matter volume, white matter volume, and cortical thickness.

Figure 4 shows the mortality related to Turner syndrome in excess of the general population (6). Mortality related to cardiovascular disease was 48 percent higher than the regular population. Diseases of the respiratory system were 13 percent above the general population, and digestive diseases were 8 percent higher. These issues become important considerations for adult women with Turner syndrome who are trying to successfully transition from pediatric care providers to adult clinicians. A transition can determine the quality of the women's future health care.

had at least one comorbidity. This study is an older one, and it was conducted in a country with universal health care. The study's findings show that this is an area that can definitely be improved upon.

Now, here are a few thoughts on the research network.

Guidelines and standards are helpful for the practicing clinician. They ground us, and they are a good thing to do.

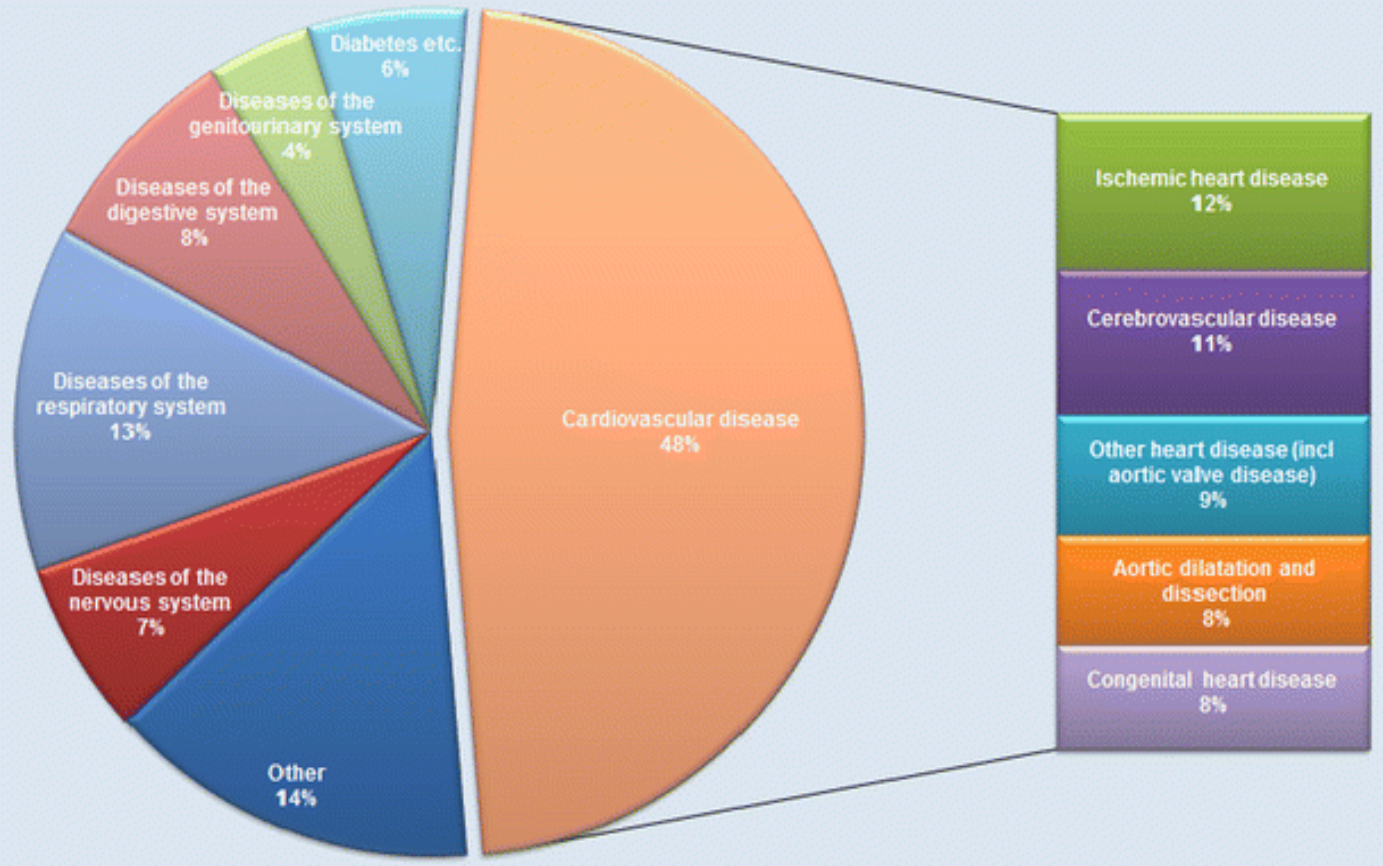


Figure 4. Excess mortality in Turner syndrome relative to the general population. Cardiovascular disease is the largest contributor to excess mortality in Turner syndrome.

To address the question of whether women with Turner syndrome are receiving the recommended screening for Turner syndrome–related conditions, Pedreira and colleagues examined thirtynine-individuals with an average age of thirty and found that only twothirds were having regular -follow-ups (7). They found that less than half had the recommended surveillance of comorbidities, but almost 90 percent

The network's push for multidisciplinary clinics would be validated if the network could actually prove that such clinics improve health care. The network says and believes that they do, but if the network can *prove* that these clinics improve health care, then the people who are paying for everything, such as insurance companies, may become more engaged in improvements, which could change the dynamics of

health care. The multidisciplinary clinics make sense to us because we see their benefits. If we could prove these benefits, then it would help a lot in terms of the reimbursement we'd get.

Increased awareness of Turner syndrome will facilitate research. The Study Network of Pediatric Endocrinologists (SNoPE) was one attempt at increasing awareness. SNoPE was something that the Pediatric Endocrine Society (PES) started maybe seven or eight years ago, and I was involved in it. SNoPE was based on a simple notion: can we study people with rare diseases by getting pediatricians together to do research that would not be possible at our individual institutions? Unfortunately, SNoPE was basically a bust. I can criticize it because I was part of it, and it died on my watch as president of PES.

Why did SNoPE fail? More importantly, how can we improve where SNoPE failed so the network won't? To make these projects worthwhile, you must have a meaningful goal and a clarity of purpose. In other words, what are you really there for? You must have principals that are really committed to the outcome. Even if you don't agree with everything, you still need to be committed. You must also have harmony between your objectives and the pathways to those objectives. And, finally, everyone involved in the network has to be on the same page; good communication is crucial.

For SNoPE, I would say we fell short in every one of these areas. SNoPE's goals and clarity of purpose were never stated. Individuals did not fully commit. We certainly weren't harmonious. And the communication? Well, there were breaks in it that hampered things.

For an example of a project that did succeed, we need look no further than the trials for growth. What made them successful? Well, Genentech and Lilly got involved, and they had a very clear, meaningful goal: they wanted to get growth hormone licensed. The purpose was very clear. The clinical trials needed to prove the efficacy. Everyone who joined was committed. We were harmonious. We had pretty decent communication. And, especially in the old days, we had fun. Various people, medical and nonmedical, helped plan the original studies, and as a result, the project succeeded.

Now, besides medical professionals, who would we ordinarily say we need for our research network? Parents? Yes, I think that is exactly right. We need information that only parents can provide when it comes to what is important for the health and well-being of their child.

What else do we need for a successful network? Money? Yes, money. It's a dog eat -dog- world when it comes to getting funding; however, I don't believe money is the first thing we need to start a successful network. The first thing, or things, we need is everything else that I've already mentioned. If you have a meaningful outcome, a clarity of purpose, and a will to persevere, then eventually the money will come. There may not be as much as you want, but it will come.

I think we're in a state now where there's going to be a lot of things on the table and a lot of questions. And some important decisions are going to need to be made. I've said all the hard stuff. I have to say, though, it was sort of thrilling to be involved in the initial trials of growth hormone, seeing how things moved forward and the outcome. But we're in a different place now. We don't have big drug companies giving us money. The questions are different. That doesn't mean they can't be just as exciting.

In conclusion, it goes back to our patients. You can see all sorts of fancy statistics, or you can just see the growth curve. Figure 5 is the growth curve of one of the individuals who was in the oxandrolone study.

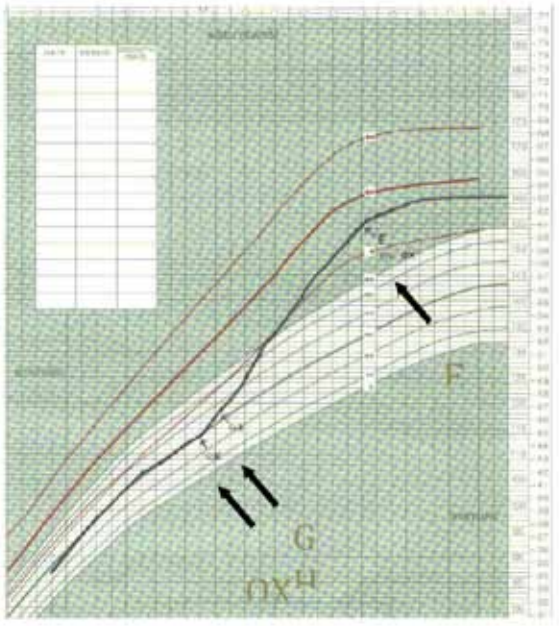


Figure 5. Representative growth chart. OX designates when oxandrolone was started. GH indicates when growth hormone was started. E indicates when estrogen was started. With growth promoting therapy, the subject reached a final height of 63 inches.

She was right on the fiftieth percentile for Turner syndrome at the start. She was put on oxandrolone for several years as part of the trial, then growth hormone was added, followed by estrogen, and she ended up five foot three. You know, this was an exciting time. And it was gratifying.

Chapter 2

State of Health Care for Patients with Turner Syndrome

Philippe F. Backeljauw, MD, and Nicole Sheanon, MD

Prior to and during the first meeting of the TS Resource Network, a panel of medical professionals addressed some of the unmet medical needs that patients with Turner syndrome (TS) encounter. The members of the professional panel are listed below in table 1. The goal of this professional panel was to discuss the need for and development of regional TS resource centers and how these centers should integrate to create the TS Resource Network (TRN). There were three main questions considered in this discussion: 1) What are the major health and well-being issues facing girls and women living with TS? 2) What are the barriers to better health and wellness for people living with TS? And 3) what is needed to ensure TS care through the transition period and into adulthood?

Table 1. Turner syndrome professional panel

Name	Association
Philippe Backeljauw, MD (chair)	Cincinnati Children's Hospital Medical Center
Joe Cernich, MD	Children's Mercy Hospital Kansas City
Melissa Crenshaw, MD	All Children's Hospital St Petersburg
Cydney Fenton, MD	Akron Children's Hospital
Angela Lin, MD	Massachusetts General Hospital
Laura Pickler, MD	University of Colorado
Judith Ross, MD	Jefferson University Hospital
Jeanie Tryggestad, MD	University of Oklahoma
Selma Witchel, MD	Children's Hospital of Pittsburgh
Katie Woods, MD	Oregon Health Sciences University

A survey on the status of the TS clinics in the United States (see table 2) revealed that approximately twenty to twenty-five health care institutions identified the existence of a subspecialty clinic for TS patients. The size of these clinics and the length of time these clinics have been in existence varies considerably. Some clinics have existed for a couple of months, while others have existed for more than ten years. The number of patients in the different clinics varies from less than 50 to over 300. The manner in which each clinic operates is also highly variable. For example, there are some institutions where the TS clinic operates once every three to four months and other institutions that have clinics open to patients six times per month. Only some institutions support a true TS center and are able to provide state-of-the-art medical care through the involvement of multiple specialties and the interaction with patient support groups. There are only six clinics that have an active website where they can advertise their clinical activities and provide information for patients. In addition, of the institutions surveyed, only five have had extramural funding to conduct research, most of which is clinical.

Table 2. Current status of US Turner syndrome clinics

Status	
Clinics	± 15
Length of Existence	< 1 to > 10 years
Number of Patients	< 50 to >300
Frequency of Clinics	6 times a month to 4 times a year
Patients	pediatric age (< 18 y) with few adults
Website	6 clinics with active website
Research funding	5 clinics
Transitioning Patients to Adult Care	most clinics are struggling with this

Most of the TS clinics see solely pediatric patients: children, adolescents, and few young adults. Only a small number of subspecialty clinics are also able to care for adults with TS. All the clinic directors and TS care providers identified that their biggest struggle was transitioning patients to adult care. With the initiation of the TRN, the aim is that more institutions with TS clinics will be identified. A list of all the TS clinics can be found on the website of the TS Society of the United States: http://www.turnersyndrome.org/#!clinics/cpkw.

The professional panel identified the major health and well-being issues facing girls and women living with TS (see table 3). One of the common concerns was the lack of access to appropriate developmental evaluation. Providers often struggle with how to provide families and patients with the knowledge they need on the learning problems and other developmental concerns that occur in patients with TS. Providers are also unsure if they are providing the right information on how to navigate the school systems (from pre-school through high school and college) and apply for individual education plans. The challenge of obtaining appropriate developmental evaluation and therapies also relates indirectly to TS patients' psychological and psychiatric needs and how and when these should be evaluated and treated.

Table 3. Major health and well-being issues facing girls and women living with TS

Access to appropriate developmental evaluation
Knowledge in applying IEP recommendations
Psychology/psychiatry evaluation and treatment
Self-esteem and intimacy issues
Insurance coverage for medical care, imaging, and treatments
Knowledge about adult TS co-morbidities
Preparedness for adult care/life
Reproductive issues/Parenting
Transitioning patients to adult care

Another major concern that health care providers had is inadequate insurance coverage. This is a problem observed throughout the United States, and it especially affects the ability to provide multidisciplinary and/or interdisciplinary care to patients with TS. The intent of providers is to apply the TS clinical guidelines in an optimal way, but sometimes insurance coverage prevents patients from getting the appropriate tests or seeing the appropriate subspecialists together in one setting.

A third concern is the lack of knowledge that medical professionals of adult patients with TS have about TS comorbidities. Pediatric endocrinologists and geneticists, who take care of most patients with TS, do a good job of educating patients and their families about the patients' health care needs. This includes recommending the needed screenings. Adult women with TS are not as well informed about their health care needs, especially about their risks for comorbidities and what screenings they should have because their providers lack such knowledge. Adult women with TS have even stated that *they* have to educate their adult providers about TS. There is a need for improved transition into adult care to allow continuation of adequate health care during adult life.

The final major health issue that women with TS have and that TS providers struggle to help them with is reproductive issues. Inadequate knowledge appears to be a major reason as to why eligible patients are not referred to reproductive endocrinologists or gynecologists. On the other hand, these specialists are also not always appropriately informed about the specific needs of TS patients.

In addition to identifying the major health issues that girls and women living with TS face, the professional panel identified several barriers to better health and wellness for patients with TS (see table 4). The lack of appropriate follow-up after a diagnosis has been made is prevalent in areas that are underserved by pediatric endocrinologists or other subspecialty services. Patients that live four or more hours away from a TS clinic have difficulty maintaining regular follow-up, as it can be a burden financially and logistically to do so (taking off work, missing school, traveling, coordinating appointments). There is also a lack of coordination between the subspecialists, who should be working together to provide TS care for optimal health supervision. To a certain degree, this could be because some subspecialists have limited knowledge of how their specialty relates to TS.

Table 4. Barriers to better health and wellness for girls and women living with TS

Lack of (loss of) follow-up
Lack of coordination between subspecialties
Lack of collaboration between TS clinics
Identifying a knowledgeable provider
Insurance coverage
Multiple billing instances in one day
Lack of uniformly described criteria for care
Ongoing development/vocational support system

Even when there is adequate insurance coverage to allow for patient care, an additional barrier is that some insurance companies will not reimburse for care that is provided in a multidisciplinary or interdisciplinary model.

Two more barriers to better care for girls and women with TS are a lack of uniformly described criteria for TS care and a lack of education about TS screening. The TS care guidelines were published in 2007, but not everyone applies these guidelines in a similar way. In addition, the guidelines are not always specific, and interpretation of these guidelines is not straightforward enough to help standardize medical care for patients with TS. And the transition from pediatric to adult TS care is a major barrier for adequate lifelong TS care (see table 5).

Table 5. Requirements to ensure TS care through the transition period and into adulthood

Clinic Coordinator
Knowledgeable provider to act as coordinator
Knowledgeable provider for adult TS patients
Network of adult providers
Training of adult providers
Educational programs focused on adult TS

There are several barriers that prevent a successful transition to adult care for young women with TS. For example, the lack of appropriate insurance coverage, the lack of a TS clinic coordinator, and the lack of a provider to act as a medical coordinator. Simply stated, there are not enough adult TS care providers. Providers in obstetrics and gynecology or reproductive endocrinology have been recommended to adult patients, but patients are often lost to follow up. A network of adult TS providers needs to be established and there needs to be better education in the various training programs for adult providers.

Considering all the major issues facing patients with TS and the barriers to care already mentioned, the professional panel aims to create a TS Resource Network (TRN) that can collaborate and provide better care to the TS population and work together for research purposes. The patient and patient's family should be at the center. While specialty care is important in the care of TS, excellent primary care is essential. In figures 1 and 2 below, we list the different members who should be involved in a TS clinic or resource center. This includes subspecialists, the primary care provider, the TS support group, and a care coordinator. The subspecialists needed vary based on a patient's age. For example, a cardiologist and an endocrinologist are often essential for younger patients, but in young adulthood, a reproductive endocrinologist or psychiatrist may become more important. The role of the TS care coordinator is to help the patient navigate the complexities of our health care system. A TS support group's connection to the TS clinic is also essential. Such a group can be a conduit for referrals and a source of information for patients' families.

Figure 1. Those who should be part of a TS resource center.

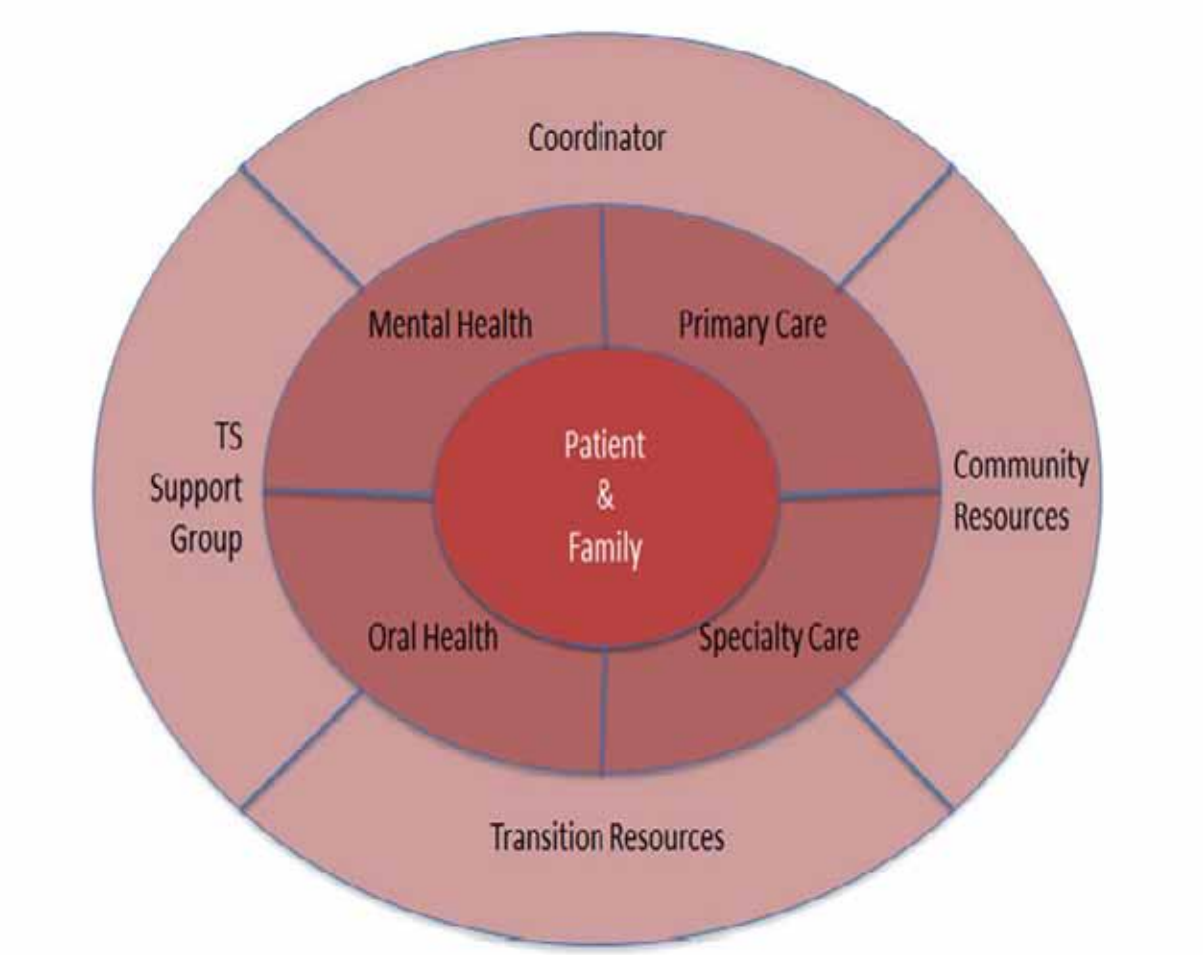


Figure 2. General concept of a TS resource center. Figure courtesy of Laura Pickler, MD.

Currently, care for TS patients is given in a number of centers as a one-stop approach (see figure 3). This means that there is one location for the clinic, and specialists try to see these TS patients if not in the same clinic setting then at least on the same day. This approach could be an overall approach or it could be focused—meaning that a patient could have cardiology issues addressed on one visit and on the next visit could have other health issues addressed. Care should begin prenatally or from birth and continue through adulthood.

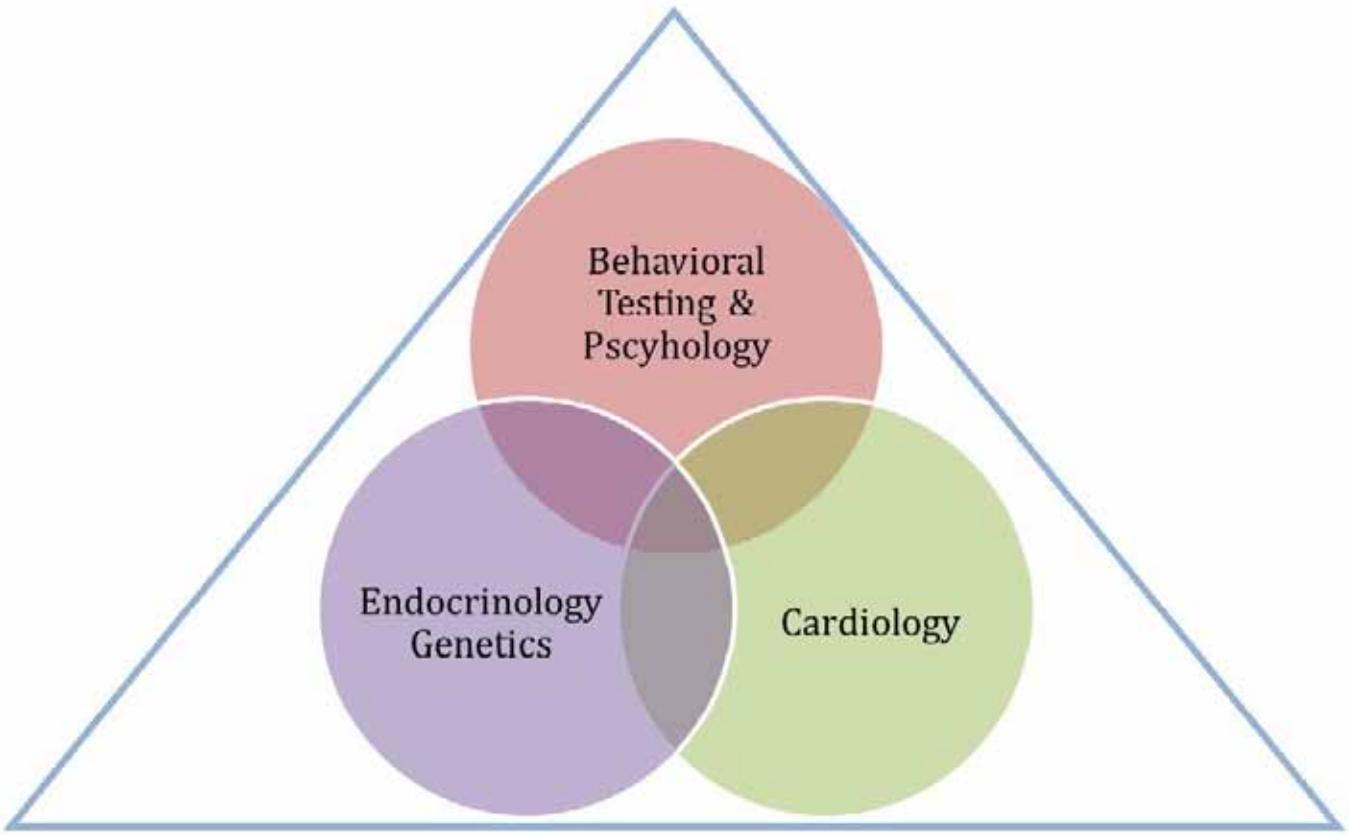


Figure 3. One-stop approach. Figure modified from one by Angela Lin, MD.

The alternative model is an interfacing approach (or interdisciplinary approach), which is the approach that a number of TS clinics operating in large university health care systems use. In this approach, the TS provider networks with a number of existing subspecialties (see figure 4). The key in this model is communication and the presence of a TS care coordinator.

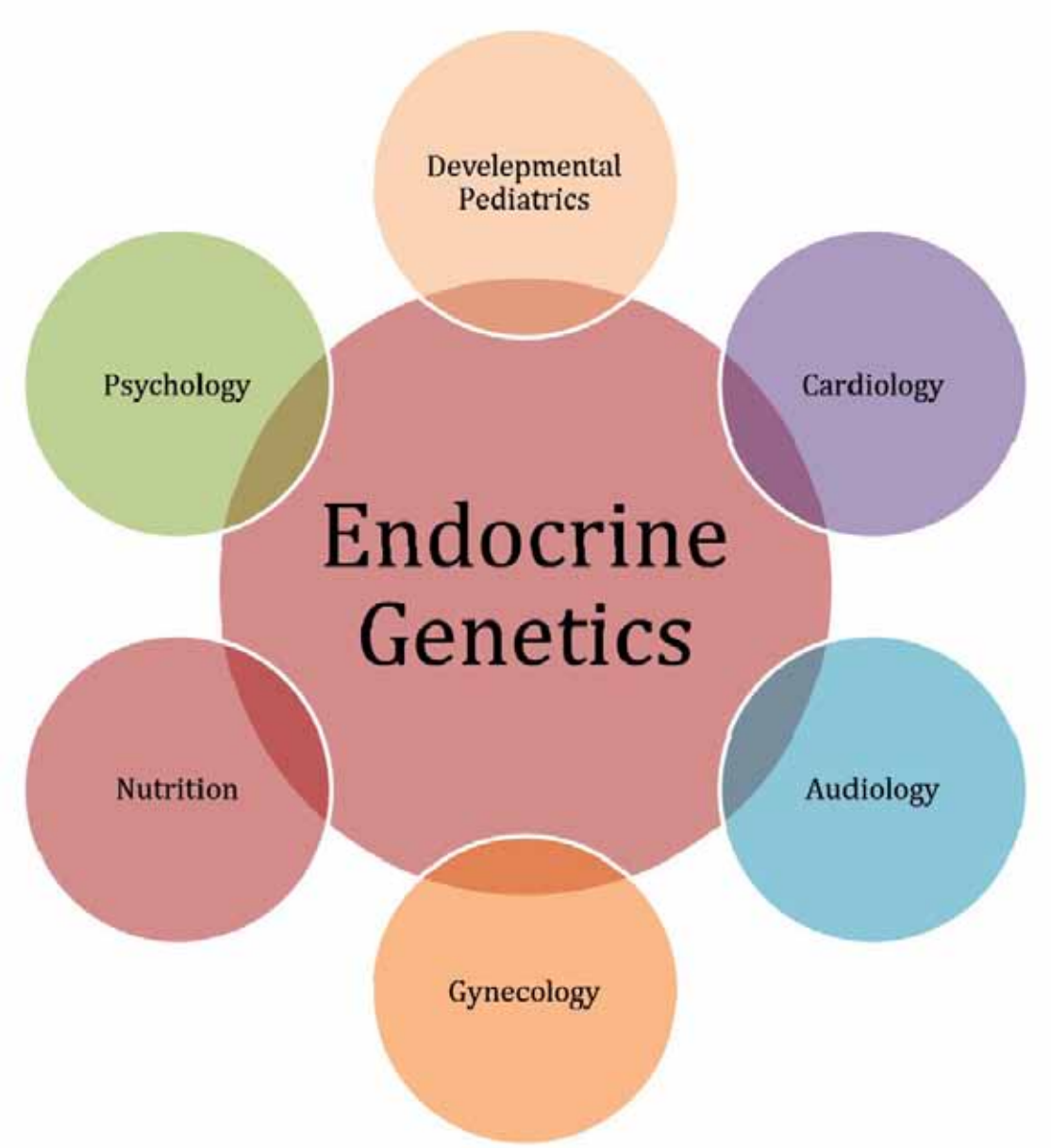


Figure 4. Interfacing approach**.** Figure modified from one by Angela Lin, MD.

What should a TS resource center look like in order to be successful (see table 6)? Well, Turner syndrome clinics could vary in size. For some institutions, a smaller clinic following an interfacing approach could be perfectly feasible and may be more realistic than trying to get different subspecialists to come together in a one-stop approach clinic. Places could be found for several large regional TS centers that could provide comprehensive TS subspecialty care. These centers could also work together with a regional TS support group, as well as with primary care physicians. These large centers could then be surrounded by smaller TS clinics that provide the same kind of clinical approach so that clinical care is integrated in regions of the country. Patients can be seen either through smaller clinics that operate multiple times per month or through larger clinics that operate less frequently but with more patients that come on one day. These larger clinics could also offer educational and collaborative efforts via TS support groups. In addition, these larger clinics could allow for better assessment of behavioral health issues, which would lead to better ways to address the issues.

Table 6. Elements of a TS Resource Center

TS clinics may vary in size
Regional comprehensive centers vs peripheral smaller clinics, integrate clinical care
Multiple small clinics meet often vs larger clinics operating less frequently
Educational component as a part of clinic
Group meetings with behavioral health focus
Multidisciplinary clinic model versus interdisciplinary collaborative effort
Centers of Excellence

The resources that will be needed to run these TS clinics are summarized below in table 7. To circumnavigate some of the barriers already mentioned, administrative support will be crucial. Turner syndrome clinics, whether small or large, will need support from their institution to enforce clinic activities dedicated to TS patient care. The providers that are seeing these patients will also need support from the division directors. The providers will need to be protected from being overloaded by other clinical duties during these clinics so that they can spend the time needed to provide comprehensive care to the TS patients. The presence of a TS clinic coordinator will be essential, as will the interaction of a local support group. And any type of extramural funding for the clinic and educational efforts will be a necessity.

Table 7. Resources needed to run a TS clinic

Support from administration for dedicated TS clinical activities
Support from division directors
TS clinic coordinator
Interaction with local TS support group
Extramural funding for clinic, education, and support activities

Given the existence of a well-designed and well-supported TS clinic, the next question is how research related to TS could become integrated through the proposed TRN (see table 8). Smaller clinics could feed their research into larger TS resource centers. The latter of which may need to develop specific criteria that the smaller clinics need to meet in order to achieve certain standards of care. These larger TS resource centers could then feed the research into a TRN-operated database, or many regional databases could combine and integrate all the research nationally.

Table 8. Integration of research through the TS Resource Network

Smaller clinics -> TS Resource Centers -> TRN
Nationwide registry or database
Regional databases to integrate nationally
Quality improvement projects
Seed money for pilot studies
Multicenter studies are key
Current TS clinics have too small sample sizes
National grants vs. smaller local funding

In conclusion, there have been rapid advances in genetics, cardiovascular imaging, and assisted reproductive technologies that present significant challenges for caregivers of the TS population. With these advances and the high morbidity and mortality rates in TS patients—underscoring the need for improved care in this underserved population—we think that the development of a TS Resource Network (TRN) is essential if we want to have any chance of improving clinical care and research for TS patients in the United States. A TRN would also provide a vehicle for novel research opportunities.

Chapter 3

Turner Syndrome Guidelines Revisited

Carolyn Bondy, MD, and Melissa Crenshaw, MD

In 2007, we last convened experts in cardiac, endocrine, developmental, and other areas of care for girls and women with Turner syndrome. Now, eight years later, it is essential for us to revisit the guidelines to determine which ones are still relevant today and which ones need to be updated. We also need to develop new methods for staying abreast of rapidly changing medical science.

I suggest a focused approach. There are ten or twelve topics that can form the basis for focus groups. Collaboration among these groups can then provide the groundwork for a larger conference that convenes leaders in each of these areas.

In the eight years since these guidelines were established, life, science, and technology have moved ahead rapidly. Most notably, significant advances in genetic technology and imaging have shown us aspects of Turner syndrome that are new or far more complex than we previously understood. This necessitates that we bring the most expert and experienced people together to evaluate, counsel, and make recommendations in accordance with these new findings.

One very controversial topic that many of us clinicians specializing in areas of endocrinology, genetics, and obstetrics often find ourselves faced with is the question of pregnancy in women with Turner syndrome. This question has become more frequent as IVF technology has advanced and become more accessible. But the data on pregnancy in Turner syndrome is widely variable. We often question this data as we consider our recommendations before counseling patients regarding this risk. These risks will be addressed further below.

There have been recent suggestions that both growth hormone and estrogen treatment should start in early childhood. New behavioral studies focusing on brain imaging supports these suggestions. While there has been a great deal of progress in the treatment of children with Turner syndrome, there has not been any substantial progress or new information in regard to adult women with Turner syndrome. There have been few controlled studies addressing their care. Thus, it is important that internists and adult specialists embark on more specialized analysis and become increasingly active in developing evidence-based guidelines for their care.

One of the rapidly expanding areas in the diagnosis of Turner syndrome is noninvasive prenatal testing or screening. Cell free- fetal DNA from dead cells from the placenta circulates in the maternal bloodstream by the tenth week of pregnancy. With noninvasive prenatal testing, the mother's blood is drawn, so the fetal DNA and maternal DNA can be sequenced and compared. This was initially done to look for trisomy 21, and subsequently for trisomies 13 and 18. Now companies have added in sex chromosome parameters so that they can screen for such things as Turner syndrome very early in a pregnancy.

At the present, the party line from the obstetrics community is that this type of testing should be reserved for high risk- mothers only. Ones who have

advanced maternal age, who have a prior history of or family history of aneuploidies, or who have an abnormal serum screen or fetal ultrasound. And the American Society of Obstetrics and Gynecology has presented this as a screen only. The society does not recommend basing decisions on the results of the screen. Diagnosis should still be made later in a pregnancy using the standardized and validated cytogenetic testing of the fetal tissues obtained from amniocentesis or chorionic villus sampling.

This maternal screening, however, is being widely used now in mothers who are not particularly high risk. Furthermore, some women are making decisions based on this screening test. This is quite concerning. Even more concerning are the many laboratories and different forms of testing, which are still rapidly evolving. Thus, it can be challenging for the clinician to remain abreast of the technology, assess its validity, and appropriately counsel patients regarding its use, benefits, and limitations.

The number of cases focused on Turner syndrome from this type of noninvasive prenatal testing is less than one hundred. The tests were all done with a cautious, conservative approach on women with high risk- pregnancies. So we have no data on the test's validity and accuracy in low risk, uncomplicated-pregnancies.

What's more, a false positive can occur due to low grade 45,X- mosaicism in the mother. There are three published cases of this happening. When screened the mothers had karyotypes consistent with Turner syndrome, but their unborn children were found to be normal. In particular, one woman had a mosaic karyotype with 13 percent 45,X cells. These types of results require clinicians with expertise to interpret and evaluate them. These experts can then provide guidance to colleagues, and to patients and their families regarding these results. It's important to note that this type of screening will diagnose a great number of children who never would have come to medical attention if they went through a full length-pregnancy.

In diagnosing Turner syndrome, there are new molecular methods that may soon replace standard karyotype/cytogenetic testing. Scott Rivkees and his colleagues developed a test that selectively targets sex chromosome polymorphisms and sequences them to determine the presence or number of different alleles in the sex chromosome (1). This is a reasonably sensitive screening test for Turner syndrome. It has also been validated in a substantial group using buccal cells via a cheek swab. This will provide a great advantage in screening studies for newborns, for example.

Whole genome SNPs and CNV arrays are also widely used. They are available in laboratories all across the country, like the Illumina chips and Affymetrix whole genome chips. These can be used to diagnosis Turner syndrome by determining copy number abundance and also loss of heterozygosity, as both SNPs and sequences are assessed. In collaboration with Dr. Diana Milewicz and Siddharth Prakash, I compared the use of an Illumina whole genome chip to karyotypes in fifty to seventy people with validated karyotypes from the NIH, and the accuracy was about 95 percent (2). Neither one of these tests can detect very low-level (<10 percent) mosaicism for 45,X cells. Nor can they detect balanced translocations, ring, or marker chromosomes that have noncoding DNA, as there are no SNP sites there. This raises the question of how clinically important this low-level mosaicism is.

Cardiovascular screening is an area that has progressed markedly since the last meeting. This progress has been driven by the more widespread use of MRI scans. As MRI technology has advanced, the screening can be done more quickly, and it can be done in young girls without sedation. A great deal of information can be gathered without contrast. In the past, we recommended that this screening should be done when girls were twelve or fourteen, now MRI screening could potentially be recommended for cardiovascular evaluation in girls as young as seven.

This data has been analyzed in great detail at the NIH in the years since the last guidelines. Through this analysis, it has become more and more evident that this imaging technology is essential to truly understanding the processes at work in the cardiovascular system of girls and women with Turner syndrome. An echocardiogram is really insufficient. It would be prudent to consider making this imaging technology the new primary modality for cardiac evaluation in patients with Turner syndrome. And it will be important to address this idea in more detail as we approach new cardiac guidelines. This idea

also underscores the importance of having pediatric cardiologists, and even adult cardiologists, who are familiar with congenital heart disease come and be involved with the Turner Society. They can share their expertise with us and help us develop new guidelines for care. Included in these guidelines should be more frequent cardiac follow up- with patients who have visible abnormalities, even if they are asymptomatic.

As we consider ongoing cardiac care, it is essential to address the question of risks in pregnancy for women with Turner syndrome. Further data has been gathered for the past ten years or so since oocyte donation became a successful and rather common way for women with Turner syndrome to become pregnant. This has been studied in detail by reproductive endocrinologists Megan Karnis and Richard Reindollar (3). They surveyed reproductive medicine departments across the US on the outcome of Turner oocyte donation -assisted- pregnancies. From their study, they estimated a death rate of 2 percent due to fatal aortic dissection. This data has been further analyzed and questioned over time.

In the NIH Turner syndrome study, we tried to approach this question of risks in pregnancy for women with Turner syndrome by reviewing every single publication since 1960 on both natural and assisted pregnancies in women with Turner syndrome and their outcomes. We included outcomes for both the mother and the fetus. Based on this published data, including several case reports and case series, we calculated a mortality rate for the mother of about 3 percent.

A French assisted-reproduction group has published experiences of their patients with assisted reproductive technology (4). Ninety-three- of the French women all had oocyte-donation (OD) pregnancies. The group noted two cases of fatal aortic dissection. According to the reports, none of the patients had had cardiology screening, aside from some of them having had an echo at some point in their lifespan. This was quite concerning and has given rise to a lot of caution and trepidation about women with Turner syndrome desiring to become pregnant.

This question of risks in pregnancy for women with Turner syndrome has since been explored through more population based- or epidemiological studies, which suggest that the situation might not be so bad. At NIH, we investigated all the NIH study participants with Turner syndrome who were twenty-five or older. We evaluated their obstetric history, including the pregnancy rate and the pregnancy outcome for both mother and child (5). The rate of spontaneous pregnancy was 2 percent, and the rate of assisted (ART) pregnancies was 2 percent. There were fourteen live births, and there were no serious maternal or fetal complications. While this was a small group, these were very well characterized- women. We had a direct and detailed history on all aspects of these women's health. Thus this data is quite reliable.

Anna Hagman from Sweden has been publishing on an essentially annual basis on pregnancy in women with Turner syndrome in Nordic countries. These countries have a great deal of data because they have national health registries and population control. With these registries, they are able to track each pregnancy and they know what the karyotype is of each pregnant woman. They are also able to couple this with the cause of death in cases of a maternal demise. After reviewing this data, the pregnancy outcomes in northern Europe seem to be favorable.

In one of Hagman's studies, she reported that 115 women with Turner syndrome had 210 live births (6). There was one nonfatal aortic dissection. This is the famous case published by Kerstin Landen -Wilhelmsen in which a woman had a partial Y chromosome, two spontaneous pregnancies, and an aortic dissection that was surgically cured during her second pregnancy.

Another one of Hagman's studies focused on oocyte-donation pregnancies in just Denmark, Norway, and Sweden (7). In this study, 106 women delivered 131 live babies. There was one nonfatal dissection. There was also one woman who had HELLP[1] syndrome, and one who had a postpartum hemorrhage. There were no fatalities.

Finally, in another study in Sweden, Hagman addressed the question of what happens a year or two after a pregnancy (8). She looked at an epidemiological study that included 124 women with Turner syndrome who had had one or more live births. Then she compared those women to 378 women with Turner syndrome of the same age who had had no pregnancies. She followed the women from one to

[1]. HELLP stands for hemolysis, elevated liver enzymes, low platelet count.

thirty years after the pregnancy and found there was no increased mortality or morbidity in the women who had had pregnancies. But aortic dissection was common among women with Turner syndrome who had not had any pregnancies.

This is something we always have to remember. We must compare the rate of aortic dissection in women with Turner syndrome who are pregnant to women with Turner syndrome who are not pregnant—instead of to women in the general population who are not pregnant. This will be important in rebalancing our recommendations as we reassess our guidelines regarding pregnancy in women with Turner syndrome.

An important aspect of treating girls with Turner syndrome is growth hormone treatment. There is reassuring and good quality data on the growth promoting effect of growth hormone and the safety of growth hormone treatment in girls with Turner syndrome. But this is based on an average start date of age seven and an average duration of treatment of about five years. The question of treating girls prior to age seven still remains, even though many pediatric endocrinologists currently begin this treatment well before that age.

The Lilly sponsored toddler study investigated growth hormone effects on growth in very young girls who had not yet fallen behind on the growth charts (9). The study showed that you could promote staying with the normal curve by starting treatment at an early age.

This additional data raises several questions: If we begin growth hormone treatment earlier, does that mean that we have to treat for ten years instead of five? If so, would that be as safe as only treating for five years? What is the cost effectiveness of treating for a more extended period? Finally, is there a positive health benefit to being taller as a result of growth hormone? This last one is a question that should be explored, at the very least.

In addition to growth, patients and parents continue to be very, very interested in the concept of spontaneous puberty and fertility. We have improved tools to predict when spontaneous puberty will happen now. These tools have been promoted by Outi Hovatta of the Karolinska Institutet and her faculty as they continue to take an interest in this subject matter.

Being able to predict when spontaneous puberty will occur has important implications for the fertility of women with Turner syndrome. There is the question of whether, when a young girl with Turner syndrome has follicles, there is actual potential for saving or freezing that ovarian tissue.

There is also treatment with estrogen therapy. Dr. Judith Ross has shown us that you can start estrogen therapy very early in combination with growth hormone without inhibiting growth, possibly even enhancing the final adult height (10, 11). We do not yet have data to establish its safety over the long term, though. We do know that girls have a decent estrogen level when they are very young, but girls with Turner syndrome have lower than normal levels.

Dr. Ross has been interested in the neurological and neurocognitive effects of the sex steroids, specifically whether replacing them at a physiological level at a young age may have important neurocognitive and neurological effects. But this raises the concern that automatic estrogen treatment at a young age will mask endogenous puberty. There needs to be some way to evaluate that. In terms of the formulation and route of delivery, this will be covered more fully elsewhere in this book.

Along with puberty comes the transition to adult care. There was an interesting paper from a Belgian group in 2011 (12). They looked at the frequency of important diagnoses from a group of eighty to eighty-five adults who were admitted to a new multidisciplinary clinic in Belgium. These adults had been followed in their childhoods for Turner syndrome. When they were admitted to this new multidisciplinary clinic, the clinic captured a detailed list of new diagnoses. Approximately 30 percent of these new diagnoses were of bicuspid valve, coarctation of the aorta, or aortic dilation, and another 30 percent were new cases of hypothyroidism, hearing loss, lipid problems, and hypertension. So the problems captured by this multidisciplinary adult clinic were excellent. Unfortunately, we do not have anything like this clinic in the United States.

Given this lack of adult follow-up, we still have little information about optimal hormone replacement therapy (HRT) for adults with Turner syndrome. We know that HRT can prevent osteoporosis, but we do not have solid safety data or best practice data on its

use in adults with Turner syndrome yet. And we have just been shown that there's excess morbidity and mortality at a relatively early age from cardiovascular disease (13). This includes atherosclerosis.

We have been pursuing the idea that some of the excess atherosclerosis in Turner syndrome could be due to the expression of a differential, exclusive expression of a maternal X chromosome.

So we followed up on a study we did in the past where we looked at body composition, visceral adiposity, and lipids in Xm versus Xp adults. The adults were young women. The Xm group had an atherosclerotic profile, visceral adiposity, and high triglycerides and LDL cholesterol, but they were much too young for us to expect to find coronary calcifications or myocardial infarctions. Given their age, we could not do radiologic imaging for screening purposes. So we had to wait until they got older or we could find older women. This was a very hard task. It took many years to get enough women in their forties and fifties who still had living parents who could contribute material that we could genotype to find out if there was actually more coronary disease in women with a single maternal X chromosome. We have done that, and we now have data that the women with the maternal X have significant coronary disease where the women with the paternal X do not.

In addition to our data on adult onset cardiac issues, we now have new behavioral observations related to advanced functional MRIs. This will be addressed in more detail by the Stanford group (14). We also have new large studies on behavioral outcomes in girls and women with Turner syndrome. This includes a study of 250 adults from the NIH who had a battery of tests in behavioral outcome measurements. Furthermore, it includes a population based- epidemiological study using the national registries of who is employed, who is unemployed, who is married, and who has Turner syndrome (15).

The education levels of people with Turner syndrome in both Denmark (16) and the NIH program are significantly better than population norms. Even the average of postgraduate work was significantly better.

Employment levels were found to be similar to or greater than that of the general population. There was no increased rate of disability or dependency, which, you know, is observed through the disability rolls in Denmark and through the occupational health department here in the US. Approximately 50 percent of the people with Turner syndrome are married.

In general, we found the behavioral outcomes of people with Turner syndrome in Denmark and the United States to be pretty good. There is the question, however, of whether the people who participated in the NIH study were a biased population, and thus more highly educated, motivated, and competent. But we found that we had a diverse population, including many participants who had significant challenges. The fact that our occupational health and psychological psychometrics team felt that the people with Turner syndrome had better compensation than the 400 women with phenotypically normal premature ovarian failure gives additional weight to this as a comparison.

This data suggests a focus for studies in the future on cognitive or neurocognitive function. It will be important to examine coping skills, as it seems evident that there is a tendency toward extremely robust coping skills in a good number of people with Turner syndrome.

Here ends Dr. Bondy's presentation. An extensive question and answer period ensued. This included questions regarding the estrogen status of women in the NIH study, some of whom were treated with transdermal patch and some with oral contraceptive pills. The use of the transdermal patch and contraceptive pills was taken into account when assessing cardiac status and atherosclerosis in these women. There were also several questions regarding cardiac care. Dr. Bondy reiterated the importance of obtaining an MRI for older girls and women with Turner syndrome to assess for occult cardiac anomalies. She also recommended a medic alert bracelet for those with increased aortic size and surgical intervention for those with an aortic index greater than or equal to 2.5.

Of additional discussion was the use of MRIs in neurocognitive assessment. The point was made that MRIs themselves are not behavioral. Then there was the discussion that while employment among women with Turner syndrome was studied, the function within the employment situation was not.

Chapter 4

Barriers to Health Care Access for Patients with Turner Syndrome

David E. Sandberg, PhD

Turner syndrome (TS) has been extraordinarily well described from a clinical standpoint (1). And clinical practice guidelines provide strong guidance regarding details of screening for problems at the time of diagnosis and the need for ongoing monitoring (1). This clarity notwithstanding, studies of the physical health and health-related quality of life (HRQoL) of girls and women with TS suggest that these guidelines, in some respects, have served as an aspirational road map rather than an established standard of care (2, 3). The distinction between what is recommended and what is achieved is nowhere more apparent than in the coordination of the multiple specialty services involved in caring for those affected with TS and their families.

TS is a complex condition with multiple targets for clinical management, yet delivery of health care in complex systems fails to adequately address the issue that "health" is ultimately judged by the person's "complete physical, mental, and social well-being" (4). Accordingly, we need to consider how generalists and specialists, alike, define health for girls and women with TS and what the barriers (intra-, extra-, and cross-institutional) are that impede delivery of comprehensive and integrated care.

TS clinical practice guidelines call for "a multidisciplinary approach to treatment" (1). This need is easily understood in terms of the complexities of TS, such as the biological systems that are impacted and the broad range of functions that could potentially be affected (see figure 1). Despite the common misapplication of the term "multidisciplinary" for teams that function in an "interdisciplinary" fashion (5), there are several examples that can serve as models for comprehensive and integrated care in TS (6–8).

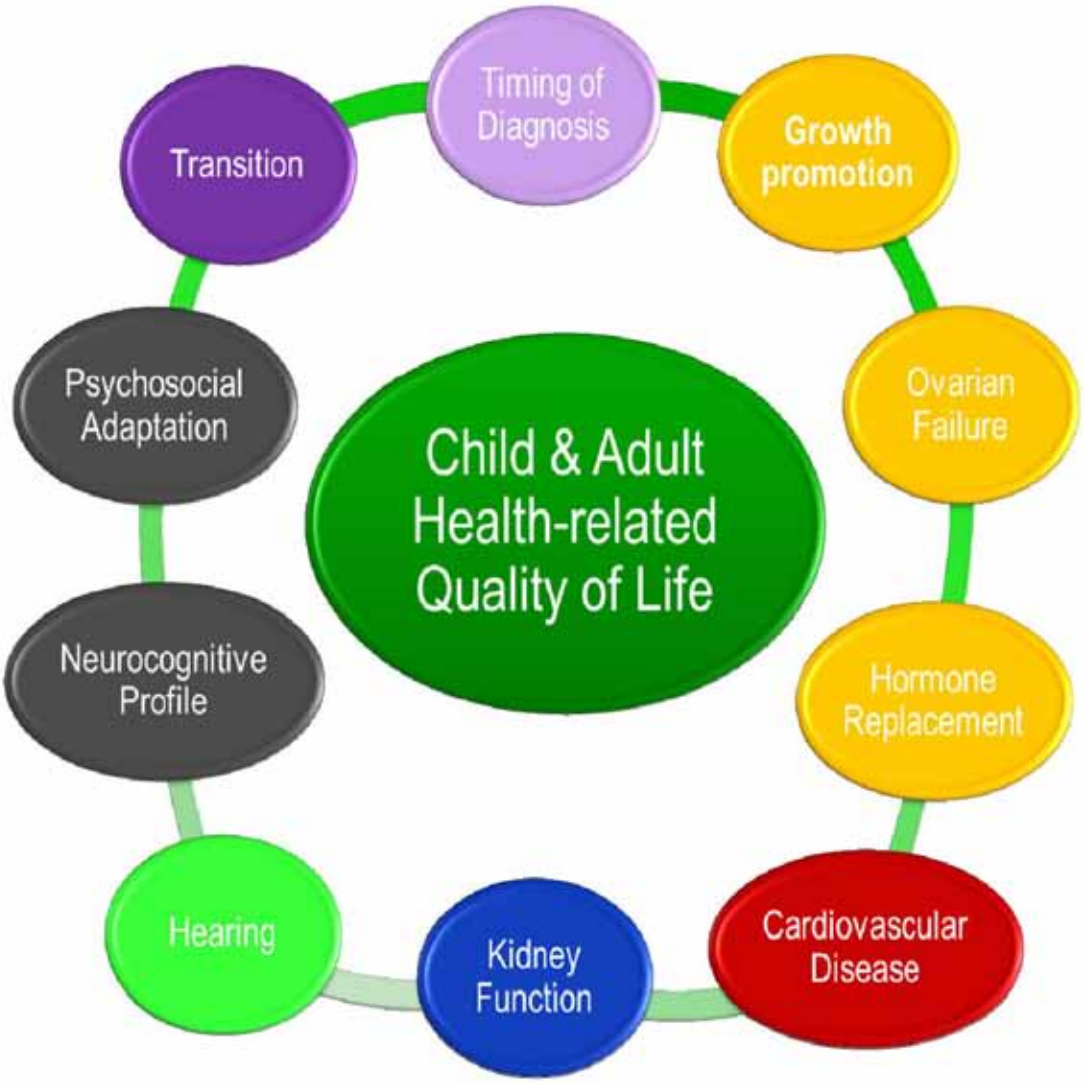


Figure 1. Multifactorial contributions to patient health-related quality of life.

When you examine the domains listed in figure 1, it quickly becomes apparent that achieving optimal HRQoL across stages of development requires simultaneous consideration of the interrelatedness of all factors and coordination of services. Yet our health care system is best designed to attend to individual targets. For example, one could aim to maximize height in the woman with TS without necessarily attending to the child's psychosocial adaptation, heart function, or hearing (9). The barriers to delivering evidence-based comprehensive and integrated health care services can be classified as intra-institutional, extra-institutional, and cross-institutional (see table 1).

Table 1. Selected barriers to full implementation of Turner syndrome clinical practice guidelines

Internal	External	Cross-Institutional
• integration / coordination	• location	• standardization
• "team" care	• cost / insurance	• practice guidelines
• behavioral health	• transition	

At the intra-institutional level, integration, coordination, and continuity of care loom large as barriers to optimal health outcomes. The terms "multidisciplinary" and "interdisciplinary" are often used interchangeably; however, meaningful distinctions need to be emphasized. Because of the complexity of TS and the need for involvement of a wide range of specialists, health care for TS requires greater integration than typically achieved with a multidisciplinary approach. While the multidisciplinary approach ensures that the assessment and recommendations are comprehensive, it does not guarantee that the team is functioning synergistically or harmoniously (3). Instead, it is discipline oriented, with all providers working in parallel, with clear roles, specified tasks, and hierarchical lines of authority.

In contrast, providers in an interdisciplinary team meet regularly in order to discuss and collaboratively set treatment goals for the patients. Then the providers jointly carry out the treatment plans. Ideally, they are on the same hierarchical level, with a high degree of communication and cooperation among the team members (10, 11). A by-product of this model is that team members learn how the goals of their own discipline may require modulation when taking into account considerations of the other specialties and the agreed upon goal for the patient.

An additional barrier to interdisciplinary care is the lack of availability of particular specialties, in particular behavioral health—either because of scarcity of this resource at the institution or because of limited reimbursement that makes participation available on a consultation basis only. The latter of which shares many of the drawbacks of conventional multidisciplinary teams.

Extra-institutional barriers include factors such as the geographic location of interdisciplinary health care teams for TS, which are generally found in tertiary care centers. Even assuming that there is a regional center providing optimal comprehensive care to girls and women with TS, geographic distance can impose substantial burdens on families (e.g., costs of travel, missed work, etc.) and, in some cases, can ultimately prevent access to integrated care. Insurance coverage and its cost represent additional potential barriers to interdisciplinary team care. Although the Affordable Care Act has expanded health care coverage and prohibited the practice of excluding coverage to children with preexisting conditions, payers may still restrict delivery of services to preferred, in-network providers and facilities that are unable to assemble the expertise required for optimal care of girls with TS. Further, multiple co-pays and high deductibles impose unequal burdens across families. Finally, effective implementation of the transition of health care services from pediatric to adult providers remains elusive (12, 13). The contrast between the quality of care in the pediatric and adult health care environments can be stark, with substantial negative consequences (2, 14).

Now, in this context, cross-institutional barriers refer to the limited standardization in the process of diagnosis, in the description of the TS phenotype, and in the clinical management practices across health care centers. While authoritative clinical practice guidelines for TS are available (1), there is no enforcement except that which is self-imposed. Electronic health records offer the opportunity to enhance the process of standardization and reduce variability in practice by incorporating clinical decision support tools that follow trustworthy clinical practice guidelines (15). Movement in this direction not only promises to improve the quality of care and the outcomes for the individual patient, but the standardization that would flow from such initiatives could also serve as a scalable platform for clinical research involving a national network of TS clinical teams.

SECTION 2

Turner Syndrome Science—From Genotype to Phenotype

Chapter 5

Turner Syndrome as a Model for Understanding Sex Biases in Human Disease

David Page, MD, and Danny E. Miller

Introduction

In this age of genetics and genomics, progress in understanding Turner syndrome promises to contribute to a larger understanding of sex biases in disease in the general population. The journey began in 1959 when Turner syndrome was equated with monosomy X (or more formally 45,X), which was a great advance in understanding the syndrome (1). This happened the same year that two other syndromes were equated with chromosomal anomalies: Klinefelter syndrome was equated with XXY (2), and Down syndrome was equated with trisomy 21 (3, 4). Ironically, the association of Turner syndrome with the 45,X karyotype also became the biggest obstacle to progress, because it led to an oversimplification. When the syndrome was initially reported by Henry Turner and Otto Ullrich in the 1930s, they described a complex, multisystem clinical phenotype, not a chromosomal anomaly (5, 6). Yet, starting in 1959, the very definition of Turner syndrome was reduced to "monosomy X." This simplistic view overlooked the nuances of clinical phenotype in Turner syndrome and their underlying molecular causes. We now know that Turner phenotypes can arise from a myriad of sex chromosome anomalies, including deletions and rearrangements of both the X and Y chromosomes; and many, if not most, cases involve mosaicism (7). In light of modern genetics and genomics, we can now revisit the definition of Turner syndrome as a prelude to exploring how research into the syndrome will help us understand sex biases in disease in the general population.

Human Genome 101

To think about Turner syndrome at the most basic level, we need to start with the fundamentals of human genetics. Cells are the basic building blocks that make up all the tissues in our bodies. A human body is comprised of about 10 trillion cells (8). At the center of each cell is a nucleus that is surrounded by cytoplasm, and within each nucleus, there are two copies of the genome—one that is maternally inherited and one that is paternally inherited. The human genome contains roughly 20,000 protein-coding genes and is organized into twenty-three pairs of chromosomes: twenty-two matched pairs of autosomes and one pair of sex chromosomes. Females have matched X chromosomes, while males have a differentiated X and Y pair. So, for the twenty-two autosomes and the matched X chromosomes in females, there are two copies of every gene in each cell. In cases of chromosomal aneuploidies, meaning when an entire chromosome is missing or present in extra copies (i.e., 45,X or trisomy 21), this balance of gene dosage is disrupted on a large scale. Deleterious dosage imbalances can also result from deletions or duplications of chromosomal regions that encompass one or more genes. In any given cell or tissue type, only a subset of the genome's 20,000 genes are expressed, so different body systems are affected by gene disruptions to varying degrees.

Sex Chromosome Biology and Sex Differences

Because Turner syndrome involves the loss of all or part of a sex chromosome, we can now make connections between Turner syndrome and other studies of the human X and Y chromosomes, including the roles that these chromosomes may play in sex differences in disease incidence and severity in the general population. By reviewing the findings in these related areas, we are led to ask a fundamental question: Does sex chromosome constitution (XX vs. XY) influence male and female biology beyond sex determination and reproduction? Understanding Turner syndrome and sex differences in disease thus begins with a comprehensive knowledge of the X and Y chromosomes, and we will consider each separately.

Historically, the human X chromosome is the most intensely studied chromosome in all of genetics, but it has fallen out of favor in recent years. *Online Mendelian Inheritance in Man* (OMIM[2]) summarizes all the human traits that have been mapped to a chromosome or to mitochondrial DNA. Even though the X chromosome contains less than 5 percent of the genes in the human genome, roughly 8 percent of the traits catalogued in OMIM have been mapped to the X chromosome. Around 2006, genome-wide association studies (GWAS) became the dominant mode of study in human genetics. These studies involve genotyping many people with a particular phenotype or disease and asking what genetic polymorphisms occur in this particular sample more often than they occur in the whole population. Remarkably, only 1.5 percent of published GWAS have found associations that map to the X chromosome. This discrepancy is not a biological phenomenon, but a technical phenomenon. GWAS usually exclude the X chromosome because of the inconvenient fact that half of the population typically has only one X chromosome, and this complicates analysis (9). The wholesale dismissal of the X chromosome by the human genetics community not only hinders studies of Turner syndrome but countless other diseases and complex traits.

Studies of the Y chromosome have lagged even further behind. Many physicians and scientists believe that the Y chromosome is only important within the reproductive tract. There are two reasons why they believe this. First, the Y-linked gene *SRY* triggers the cascade of events that induce the undifferentiated gonad to develop into a testis. Second, the Y chromosome encodes a number of genes that play an important role in sperm production. These two reasons have led scientists and physicians to believe that, outside of the reproductive tract, the Y chromosome has no biological function, and that the genomes of males and females are functionally equivalent.

Indeed, the Human Genome Project and the attendant initiatives in personalized medicine are based on all humans being 99.9 percent identical. Bill Clinton used this idea to try to bring the country together in his 2000 State of the Union address when he said that "we are all, regardless of race, genetically 99.9 percent the same." But that is only true if the people you are comparing are all male *or* all female. When you compare the genomes of a 46,XY male and a 46,XX female individual, they are only 98.5 percent identical. Therefore the genetic difference between a male and a female individual is fifteen times the genetic difference between two male individuals or between two female individuals. To put this in perspective, the genetic similarity between a man and a male chimpanzee is also 98.5 percent, so a male human is about as closely related to a female human as he is to a male chimp.

The human genetics revolution has largely overlooked the uniqueness of the sexes, opting instead for a unisex model for disease. The genomic differences between males and females, however, are critical to understanding sex differences in disease, which are widespread and profound. For example, rheumatoid arthritis is two to three times more common in women than in men (10), and seven to fifteen times more women suffer from lupus compared to men (11, 12). Conversely, some conditions, such as autism spectrum disorder, are four times more likely to occur in boys than in girls (13). None of these are disorders of the reproductive tract.

For many disorders that affect males and females with equal frequency, the severity or consequence of the condition can significantly differ between the sexes. A great example of this is dilated cardiomyopathy, which is where a very specific genetic defect causes

[2]. *Online Mendelian Inheritance in Man* can be found at this website: http://www.omim.org.

dangerous thinning and ballooning of the wall of the heart. Women with this disorder tend to die around age sixty-eight, but men who carry the same genetic defect tend to die at a much younger age—about ten years earlier on average (14). Historically, this difference has been attributed to sex hormones produced by the reproductive organs, but this has not been demonstrated to be the root cause.

Unfortunately, we really do not understand why one sex is more commonly or more severely affected by certain disorders. Although we can explain why disease risks and severity may differ between two males or between two females, we cannot explain why they differ between the sexes. But we do know that males who tend to get diseases such as autism or dilated cardiomyopathy are XY, and females who tend to get lupus or rheumatoid arthritis are XX. This is a fundamental difference that suggests being XX or XY is medically important—not only in the reproductive tract, but in many other tissues.

Several research groups are beginning to explore where and how, beyond the reproductive tract, Y chromosome genes are expressed in male bodies. Recent studies have shown that the Y chromosome operates throughout the body—in the pancreas, the heart, the ear, the skin—and that at a deep level, all these tissues know that they are either XX or XY, or even 45,X (15). But do these differences actually matter outside of the reproductive tract? Most researchers working with human cells today do not know whether they are working with XX or XY cells. This means that in many cases researchers who are trying to discover the underlying causes of diseases or new treatments do not distinguish between XX females and XY males at the cellular level. Going forward, it is imperative that we find a better way to study disease and treatments.

Turner Syndrome and Studies of Sex Chromosome Evolution

Turner syndrome provides a model for understanding the biology of the X and Y chromosomes and sex differences in disease. Understanding the evolution of sex chromosomes has revealed this connection, and in turn it has offered insights into Turner syndrome. To provide context for this argument, let's begin by returning to the last common ancestor of humans and birds, which lived about 300 million years ago (16). In the mammalian descendants, males are XY and females are XX. But in birds, females have two different sex chromosomes, Z and W, while males carry two copies of the Z chromosome (17). The W chromosome in birds is a female-specific chromosome, just like the Y chromosome in mammals is a male-specific chromosome.

The common ancestor of birds and mammals that lived 300 million years ago had separate sexes—females made eggs and males made sperm—but did not have sex chromosomes. This is similar to present-day crocodiles and alligators, where the sex of a developing embryo is determined not by genetics but by the temperature at which the egg incubates (18). Comparative genomics has revealed that bird and mammalian sex chromosomes evolved from distinct sets of autosomes that were once identical in males and females. The mammalian X chromosome is similar to chicken chromosomes 1 and 4 (19, 20), and the avian Z chromosome is similar to human chromosomes 5, 9, and 18 (21).

By examining the X chromosome gene content of multiple mammals and chicken autosomes 1 and 4, Bellott and colleagues (15) estimated that the ancestral autosomes that gave rise to our X and Y chromosomes carried 639 genes, and most of those genes persist today on the human X chromosome. Surprisingly, on the Y chromosome, only 17 of the 639 ancestral genes have survived, all of which are also on the X chromosome; you can see this in figure 1 (15). Since the Y chromosome is the only differentiator between XY males and Turner females, it is likely that some of the genes that contribute to Turner phenotypes are found among the small subset of genes on the X whose counterparts on the Y survived. The Y chromosome has therefore, at least in the context of Turner syndrome, helped to whittle down the list of genes that may be responsible for this disorder.

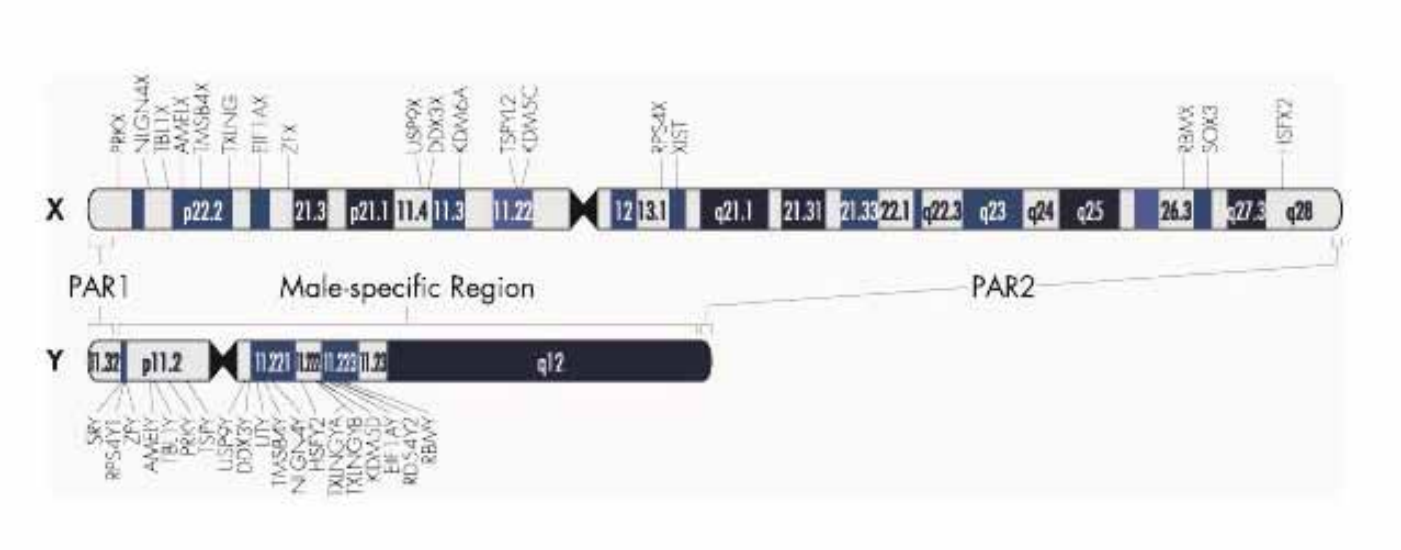


Figure 1. Ideograms of the X and Y chromosomes. Both chromosomes have a long (denoted q) and short (denoted p—for petite) arm. The centromere of both chromosomes is represented by the tapered region in the middle. Several X and Y gene pairs are noted in the figure (15). Additional X and Y chromosome genes are also noted. The two pseudoautosomal regions shared by the X and Y chromosomes are noted as PAR1 and PAR2. Of note, *SHOX*, a gene implicated in the short stature commonly seen in Turner syndrome, is located in PAR1.

Twelve of the 17 surviving X and Y gene pairs tend to be involved in regulating the activity of other genes throughout the entire genome. They are broadly expressed across human tissues and across developmental time, and they are dosage sensitive and haploinsufficient (two copies are required for normal function). Even in the cells of an XX female, where one of the X chromosomes is typically inactivated, the 12 broadly expressed genes with Y chromosome counterparts escape X inactivation and are expressed in all cells. In Turner syndrome, we know that short stature is due in part to haploinsufficiency of the pseudoautosomal *SHOX* gene (22), and it is likely that other somatic features of Turner syndrome are due to haploinsufficiency for one or more of the 12 broadly expressed surviving ancestral X and Y gene pairs.

Intriguingly, the X and Y genes encode distinct protein isoforms (non-identical proteins that may exhibit functional differences). In an XX or 45,X female, only the X isoform of these proteins is expressed, but an XY male can express both the X and Y isoforms. So having one X chromosome, two Xs, or an X plus a Y could have profound functional consequences. This is potentially relevant to understanding not only Turner syndrome but also sex differences in disease susceptibility between XX females and XY males.

Q & A

DR. SANDBERG: During his introduction, Scott [Hawley] talked about the need for biologists and other researchers to pay attention to the clinical phenomena to guide their work. And I'm wondering, what would be the explanation for why? Despite knowledge of sex ratios being really lopsided in so many diseases, it's taken Francis Collins [the director of the National Institutes of Health] until just recently to say in *Nature* (23) that we have to study sex differences in disease. Are scientists oblivious to phenomena in the real world?

DR. PAGE: It's a great, great question.

As Barb Lippe just suggested—not so under her breath—I think the question would be perceived differently by men and women. In some sense, in the world of medical research, it's been seen as normative to be male for a long time. In a great deal of neurobiological research, for example, animal studies are limited to males—and why is that? Because researchers don't want to deal with the estrous cycle as a variable.

All kinds of excuses have accumulated. It's slightly different for this field from that. But, to be fair, I would say in the case of human genetics that there is one category of disease where enormous differences between the sexes have always been embraced. Those are the X-linked recessive disorders. They are much more common in males than in females, and we have an easy explanation for that.

Apart from X-linked recessive diseases, the field of human and molecular genetics has largely steered away from the issue of sex differences. This is because we have not had the research toolkit with which to approach the question of sex differences. My laboratory is devoting more of its energies to building a scientific toolkit that would allow us to tackle this important issue.

But I think the question has many, many answers. To be honest, I was not entirely satisfied with Francis Collins's recent framing of the question. I think he framed it as a compliance issue for researchers. It would be yet another checkbox on grant applications for investigators to say that they had included males *and* females. This approach doesn't embrace sex differences in health and disease as a rich scientific question to be explored in its own right.

DR. SILBERBACH: The purpose of this meeting is to try to figure out how we can use the resources that we have available to us to get more research focused on Turner syndrome. Our greatest asset is the people with Turner syndrome themselves. And it's the reason why I've asked all the speakers today—we're talking about basic science—to tell us how we could organize a study that would use our resource centers to ask fundamental questions.

Do you have any thoughts on how we might do that?

DR. PAGE: Well, I don't have any fully baked ideas, but here are my thoughts: In my laboratory and a number of others, we're beginning to assemble a catalog of the molecular differences between XX female and XY male cells and tissues. This stage is reminiscent of the time when the first anatomists were discovering the structure of the human body. You know how this all started? The cadavers of criminals were dissected in various anatomic halls in Europe. And guess what the sex of those early cadavers was? Well, they were all males. At some point someone dissected the body of a female and was shocked to find that the innards of a male and a female were different.

Now, imagine if that anatomic discovery of the female body had never been done. That's where we are at the molecular level today. Today we need to have a molecular inventory that is the moral equivalent of that anatomic differentiation of the male and the female.

So [my laboratory] and others are beginning a type of molecular catalog of the differences between XX and XY cells and tissues. I would strongly advocate that we add to that catalog the differences between 45,X as well as XXY and XYY cells and tissues. I believe that such a catalog can provide enormous insight with regard to Turner syndrome and Klinefelter syndrome, and more broadly account for the basic differences between XX females and XY males.

Chapter 6

Genetic and Epigenetic Mechanisms Underlying Early Brain Development, Cognition, and Behavior in Turner Syndrome

David S. Hong, MD

Introduction

Defining cognition and behavior is a challenging task due to the complexity and heterogeneity of the many components comprising these domains. But the importance of doing so is paramount, as neurocognitive behaviors often have an outsized influence on quality of life and adaptive functioning. A promising means of addressing this issue has been to identify a stable intermediate phenotype that would more directly relate underlying genetics of Turner syndrome to observable phenotypes. Indeed, advances in MRI technology have given us some insight into potential neuroanatomical or neurofunctional measures that might act as biomarkers, linking genetic variation in Turner syndrome to known risks in neurocognitive phenotype. Further correlation of these measures to clinical outcomes and adaptive functioning remains one of the ultimate goals of clinical care for patients with Turner syndrome.

To date, the literature has produced robust descriptions of cognitive profiles in Turner syndrome. Firstly, it is well established that overall intelligence is in the normal range, though global scores may be slightly lower compared to siblings (1, 2). This difference is most likely driven by a very specific split between verbal abilities, which tend to be preserved or enhanced in Turner syndrome, and nonverbal cognition (i.e., performance IQ), which is often impaired (3). Within nonverbal cognition, subdomains such as visualspatial- skills, arithmetic, and executive function have been most frequently reported as being affected by independent studies throughout the literature (4–6).

In the last two decades, consistent neuroanatomical patterns have also been reported (7, 8), and while there are some discrepancies, a set of highly consistent findings has emerged across a number of independent cohorts (9–11). While neuroanatomical variation in and of itself does not confirm pathological processes, the consistency of these differences across studies provides solid evidence for centralized neurodevelopmental processes that are affected in Turner syndrome. This progress is certainly encouraging; however, there continue to be significant gaps in current knowledge about neurocognitive development in Turner syndrome, most notably about variation across the developmental lifespan and a more explicit understanding about which genetic differences are driving these brain and behavior findings. The sooner these relationships are elucidated, the more quickly a unified genetic-neuroanatomical approach can be utilized to improve clinical care.

Developmental Trajectories

The vast majority of early neuroimaging studies in Turner syndrome were comprised of adult women, but recent work has increasingly utilized a developmental approach in the study of neurocognition. Over the past decade, numerous studies have been published on school-aged children with Turner syndrome who have not received estrogen replacement treatment. The studies collectively demonstrate consistent patterns of decreased gray matter volume in the occipital and parietal lobes of these children compared to their typically developing peers, who had increased volume

in the temporal lobe, insular regions, and the amygdala-hippocampal complex (9, 12).

While these findings confirm certain consistencies in brain anatomy across children and adults with Turner syndrome, there also appear to be notable differences between these life stages, particularly during dynamic periods such as adolescence. In fact, when comparing prepubertal girls with Turner syndrome to adolescents who had received estrogen treatment, Lepage and others found that changes in white-matter volume and cortical thickness in the parahippocampus and orbitofrontal cortex varied compared to controls in the same age cohorts, suggesting a modulating influence of estrogen on brain structure in girls with Turner syndrome as they enter the pubertal period (12). Similarly, in the first systematic longitudinal neuroimaging study of girls with Turner syndrome, Green and others found that the rate of expected changes in the superior parietal region varied over time between girls with Turner syndrome and typically developing controls (13). More importantly, changes in these regions occurred in parallel with performance on visuospatial tasks, a cognitive domain highly associated with this anatomical region.

Together, these studies provide some of the first concrete evidence to support long-standing theories that brain development occurs in a nonlinear fashion over the lifespan. Further information is needed to better understand the trajectory of these changes, particularly during the most dynamic ranges: the perinatal period, adolescence, and late age.

It is important to better understand the extent at which neurodevelopment—and associated neurocognitive functions—"catches up" or equilibrates during early development and how it is affected in older adult women with TS. From a clinical perspective, this will provide significant insights into the discordant findings that younger individuals with Turner syndrome often present with challenges in cognitive and social function during childhood, whereas epidemiological surveys of adult women with Turner syndrome often demonstrate equivalent self-ratings of quality of life compared to the general female population (14–18). An improved understanding of this subject will thereby result in more specific interventions in clinical settings and allow for better prognoses over the entire lifetime of an individual with Turner syndrome.

Encouragingly, important work is currently underway in delineating these processes in early development (see chapter by Knickmeyer et al.), particularly in the first few years of life, but there continues to be a remarkable dearth of knowledge about the later stages of the lifespan spectrum, namely around menopause and later life. These issues are further complicated by the fact that there are likely significant cohort effects over the past decades, as the availability of clinical treatments has changed drastically with regard to access and changing recommendations initiation and dosing of hormone treatments. The interactive effects of these changes during dynamic developmental periods of brain development should be considered when evaluating the totality of the Turner syndrome literature.

Genetic and Epigenetic Mechanisms

A clearer elucidation of the genotype-phenotype correlation in Turner syndrome is also critical. As described above, significant progress has been made in better understanding the mechanisms underlying the observed neurocognitive differences in Turner syndrome; however, a fine-grained understanding of which genetic variations are driving these changes is still needed. From a global perspective, genotypic variation has been grossly accounted for by using standard karyotyping methods, with early studies including a broad range of karyotypes and more recent work focusing more narrowly on individuals with X monosomy. But a greater degree of understanding is needed of the underlying genetic variation in Turner syndrome, as exemplified by recent findings on cryptic mosaicism, which is emerging as a potentially fundamental mechanism during the earliest stages of fetal development (19). Indeed, even within individuals with a monosomic karyotype, there is substantial individual variability in regard to cognitive behavioral performance and neuroanatomy, which highlights the fact that chromosome number alone does not adequately explain why some individuals with Turner syndrome have neurocognitive impairments while others do not.

An area that holds particular promise for decoding this complex domain is epigenetics, which looks beyond the genome. Epigenetics delineates when and how genetic information is translated throughout development. A specific example—and of particular

importance in Turner syndrome—is the concept of imprinting, which posits that a chromosome inherited from the father may be translated differently than a chromosome inherited from the mother. One early study suggested that social cognition abilities were heavily influenced by imprinting, such that individuals with a paternally inherited X chromosome had improved performance on verbal skills, executive function, and social cognition measures compared to individuals with a maternally inherited X chromosome (20). But a more recent paper, one that examined social function in the largest study to examine imprinting effects to date, showed no significant differences between prepubertal girls who had a maternally inherited X chromosome or a paternally inherited X chromosome on specific measures of social cognition. The study did, however, find differences on the performance IQ index in favor of individuals with a maternally inherited X chromosome (21). Interestingly, there were also neuroanatomical differences between these groups. Girls with a paternally inherited X chromosome tended to have increased cortical thickness in the temporal lobe regions, an anatomical finding that correlated with cognitive performance, which suggests a putative imprinting effect where girls with a paternally inherited X chromosome are more likely to demonstrate cognitive and neuroanatomical findings compared to their counterparts with a maternal X chromosome (22).

Greater specificity in understanding the role of the genes themselves on the X chromosome is also needed. There is increasing evidence that genetic effects have a direct influence on sex-specific development—independent of hormones. This is best seen in animal models, which show direct genetic effects on domains of aggression, pain perception, neural tube defects, and autoimmune functions (23). But the task of separating out the effects of genetics from sex hormones is much more challenging in human research, and particularly in individuals with Turner syndrome, as most individuals often demonstrate signs of ovarian or estrogen deficiency early in development, which significantly overlaps with the developmental period of genetic influence.

An alternate way to investigate how the X chromosome impacts phenotype is to comparatively study other individuals with an abnormal number of sex chromosomes, such as boys with Klinefelter syndrome who carry a 47,XXY karyotype. Given that Klinefelter boys typically do not demonstrate sex-hormone deficiency until adolescence, they, along with prepubertal girls with Turner syndrome, can be assessed to allow inferences to be made about the "dose effect" of genes on the X chromosome. This concept of X-chromosome gene dosage has been established in physiological domains, such as height, which is partially determined by the number of active copies of the *SHOX* gene located on the distal end of the X chromosome (24). Indeed, girls with Turner syndrome who have only one copy of the gene are typically shorter, whereas boys with Klinefelter syndrome who have three copies of the gene are often taller than male peers. The question remains, does this dosage relationship also exist in neurocognitive domains?

A weighted mean analysis of cognitive measures in Turner and Klinefelter syndromes showed interesting profiles between these two groups. Full-scale IQ fell within a similar range, while the underlying cognitive profiles demonstrated inverse relationships in regard to strengths and vulnerabilities in verbal compared to performance IQ (25). More detailed genetic studies suggest that these neurocognitive differences may arise from inverse transcription patterns from a region on the X chromosome not far from the *SHOX* gene, where overexpression of the genes correlates to verbal IQ deficits in Klinefelter syndrome and deletion of a similar region in Turner syndrome correlates to characteristic features associated with the Turner syndrome phenotype (26, 27).

Additionally, an investigation of neuroanatomy demonstrated similarly inverse patterns in regions associated with these cognitive domains. A cross comparison between girls with Turner syndrome and boys with Klinefelter syndrome revealed inverse volume differences in temporal and parietal lobe regions. The girls with Turner syndrome had relatively larger temporal lobes and smaller parietal lobes, and the boys with Klinefelter syndrome had larger parietal lobes and smaller temporal lobes compared to sex-matched peers (28).

Together, all these findings support a framework by which genetic and epigenetic mechanisms mediate neuroanatomical and cognitive-behavioral functions in Turner syndrome, and potentially sex-chromosome aneuploidies at large.

Conclusions

As insights into the role of genetics and sex-hormone function continue to expand, the need for further research of greater breadth and depth has become apparent in order to bridge the final link to where these findings can be translated into specific interventions for individuals with Turner syndrome. Future work should take advantage of methodological advances that allow for fine-grained reading of the genome, which will provide a high-resolution examination of genetic variation associated with neurocognitive symptoms. Similarly, a broader understanding of Turner syndrome neurocognitive features as they vary over the lifespan is also needed to provide a better understanding of which interventions would be most appropriate at different stages of development. An important push to move these initiatives forward would be through infrastructure provided by the Turner Research Network, which provides a unique opportunity to standardize and broaden the reach of research efforts across all the stakeholders involved—patients, families, clinicians, and researchers. Specifically, larger sample sizes involved in this process will allow greater understanding of cognition and behavior in Turner syndrome and expedite the goal of using evidence-based research to inform best practices in clinical care.

Chapter 7

Early Abnormalities in Social, Attentional, and Working-Memory Circuits in Infants with Turner Syndrome

Rebecca Knickmeyer, PhD; Stephen Hooper, PhD; and Marsha L. Davenport, MD

Introduction

There is an extensive body of research that reveals a particular pattern of cognitive strengths and cognitive challenges faced by women, teens, and school-age- girls with Turner syndrome. Areas of challenge often include higher-order visual-spatial functions, arithmetical abilities, executive function, specific aspects of language (e.g., verbal fluency, complex syntactic knowledge, and articulation), and sociocognitive processes, which include emotion recognition, memory for faces, identification of gaze direction, and theory of mind. Areas of strength often include rote memory, specific aspects of language (e.g., word knowledge, receptive and expressive abilities, and verbal memory), and social motivation (1). It is assumed that this cognitive pattern reflects changes in underlying neuroanatomy and function. Studies in adults and older children demonstrate that females with TS differ from females without TS in key components of the neural circuits controlling social cognition, attention, and working memory. In particular, structural magnetic resonance imaging (MRI) studies consistently reveal enlargement of the amygdala and orbitofrontal cortex (2–5) and reductions in somatosensory and inferior parietal cortex (6, 4, 5). Diffusion tensor imaging (DTI) has revealed widespread reductions in fractional anisotropy (FA), an indicator of reduced structural integrity (7). Functional magnetic resonance imaging (fMRI) studies have shown abnormal activation of amygdala during affect recognition (8) and disrupted frontoparietal circuitry (9–11).

What remains unknown is when these differences in neuroanatomy and function arise during development. Studies comparing pediatric, pre-estrogen girls with monosomic Turner syndrome to adolescent, post-estrogen girls with Turner syndrome suggest that the neuroanatomical phenotype is extremely stable (5). In addition, a recent longitudinal study reported that abnormalities in the parietal cortex were constant during childhood, with the potential exception of the left superior parietal cortex (12). This strongly suggests that the prenatal and early postnatal period is critical in the development of the cognitive and neuroanatomical profile observed in TS. But until recently no studies had performed detailed cognitive testing or neuroimaging on infants and toddlers with TS. This is an important gap as infancy is the most dynamic phase of postnatal brain development. In this period there is extensive synaptogenesis (13), exuberant dendritic (14) and axonal growth (15), and rapid myelination (16). These developments are reflected in significant increases in gray and white matter volumes assessed by structural MRI (17, 18) and changes in anatomical (19) and functional connectivity (20), assessed by DTI and fMRI respectively. The rapid pace of structural brain development is matched by an equally rapid development of cognitive functions, including social cognition (21) and working memory (22). The developmental timing of these cognitive advances likely reflects the enormous spatiotemporal complexity of brain development in this period (23, 24).

In this chapter, we will describe the preliminary results from the first neuroimaging study of infants with Turner syndrome. Ultimately, through detailed

assessments of social and cognitive development from birth to four years, we will be able to generate a strong base of knowledge to inform medical professionals and families about what to expect. This knowledge, combined with a better understanding of altered brain circuitry, will also inspire new options for treatment and intervention.

BodyWith support from an NIMH-sponsored K01, we recruited a unique cohort of 48 infants and young girls with Turner syndrome. We saw 18 of the children as neonates, 27 of them at six months, 32 at one year, 24 at two years, and 7 at four years. All participating children had behavioral assessments, and many of them successfully completed brain imaging with conventional MRI, DTI, and resting state fMRI. A significant subset attended multiple times, allowing us to collect longitudinal data.

At the TRN symposium, we presented preliminary neuroimaging data on 23 of the children: 19 were scanned at one year of age and 12 were scanned at two years of age (8 children were scanned at both one and two years of age). Girls with TS were matched two-to-one- to a set of female controls that were similar in terms of their age of birth, age at scan, and birth weight. We focused on key components of the neural circuits controlling social processes and working memory (see figure 1 and table 1).

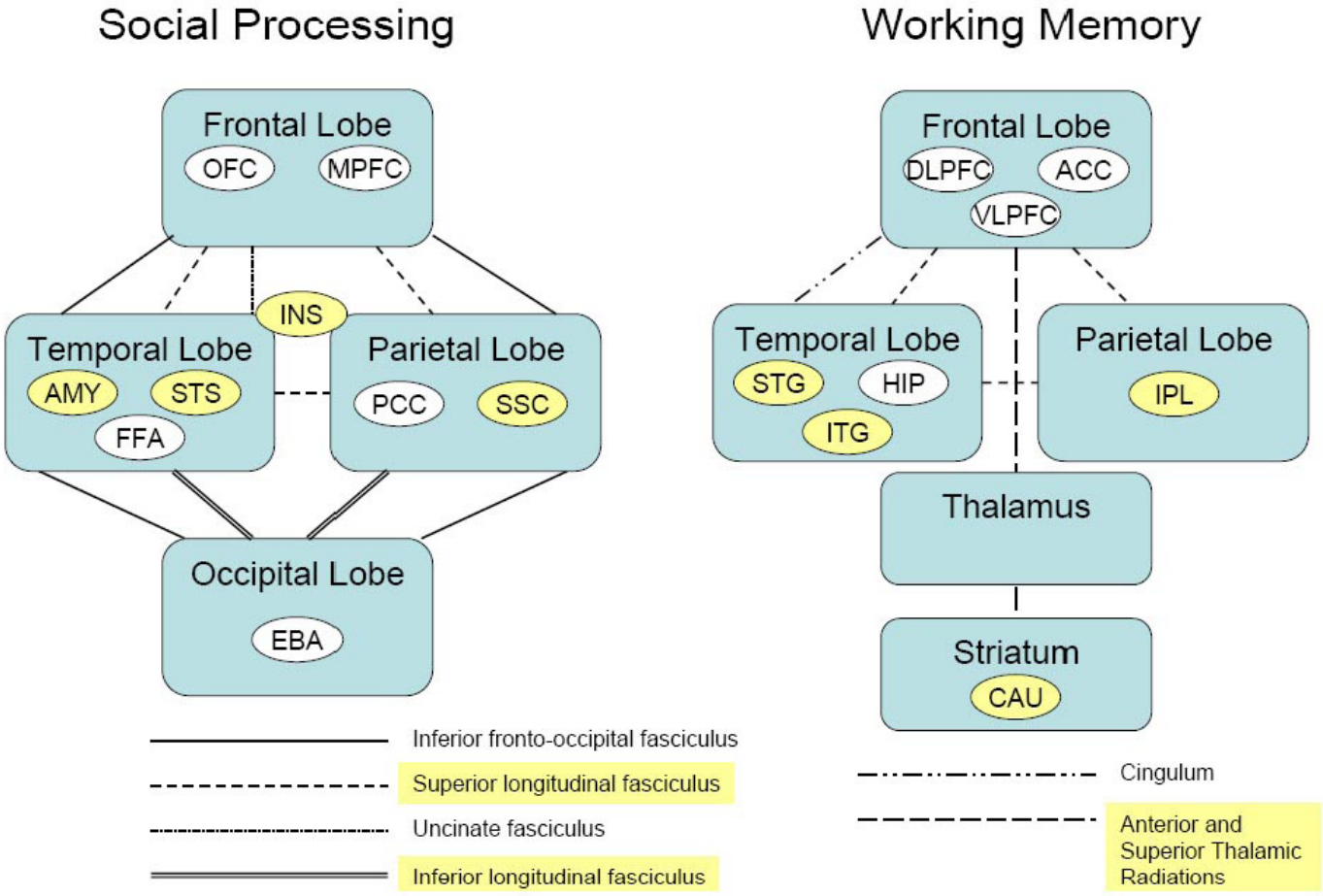


Figure 1. Brain regions and white matter association tracts in the neural circuits for social processes and working memory. Regions in yellow were the primary focus of the current study due to their well-replicated alterations in older individuals with TS. OFC (orbitofrontal cortex), MPFC (medial prefrontal cortex), INS (insula), AMY (amygdala), STS (superior temporal sulcus), FFA (fusiform face area), PCC (posterior cingulate cortex), SSC (somatosensory cortex), EBA (extrastriate body area), DLPFC (dorsolateral prefrontal cortex), ACC (anterior cingulate cortex), VLPFC (ventrolateral prefrontal cortex), STG (superior temporal gyrus), HIP (hippocampus), ITG (inferior temporal gyrus), IPL (inferior parietal lobule), CAU (caudate).

Table 1. Key nodes in circuits for social processes and working memory with corresponding anatomical regions in the AAL Atlas (25).

Primary Regions of Interest	**Corresponding Regions in AAL Atlas**
Insula	Insula
Somatosensory Cortex	Postcentral gyrus
Amygdala	Amygdala
Superior Temporal Sulcus	Superior temporal gyrus
	Middle temporal gyrus
Inferior Temporal Gyrus	Inferior temporal gyrus
Inferior Parietal Lobule	Inferior parietal
	Angular gyrus
	Supramarginal gyrus
Caudate	Caudate
Secondary Regions of Interest	
Orbitofrontal Cortex	Superior frontal gyrus, orbital part
	Superior frontal gyrus, medial orbital
	Middle frontal gyrus, orbital part
	Inferior frontal gyrus, orbital part
Medial Prefrontal cortex	Superior frontal gyrus medial
Ventrolateral Prefrontal Cortex	Inferior frontal gyrus, opercular part
	Inferior frontal gyrus, triangular part
Posterior Cingulate	Posterior cingulate
Fusiform Gyrus	Fusiform gyrus
Extrastriate body area	Middle occipital gyrus
Dorsolateral Prefrontal Cortex	Superior frontal gyrus, dorsolateral
	Middle frontal gyrus
Anterior Cingulate	Anterior cingulate and paracingulate gyri
Hippocampus	Hippocampus

Regarding conventional structural MRI, we performed tissue segmentation as described by and others (8) . Regional tissue volumes were computed via atlas-based structural segmentation employing diffeomorphic registration (26), including a 90 regional parcellation—like in the AAL atlas (25). After quality control, data was available for 17 children with TS at age one and 11 children with TS at age two. Results indicate that one- and two-year-olds with TS already have significantly altered brain volumes in several key nodes of the social cognition and working-memory circuits (see table 2).

Table 2.

ROI	CONTROLS Mean (SD)	TURNER SYNDROME Mean (SD)	Percent Difference	P-Value
Smaller in TS at 1 Yr				
Postcentral_L_GM	10601(1236)	9692(1247)	8.57%	0.0350
Postcentral_R_GM	9334 (1283)	7807 (844)	16.4%	<0.0001
Parietal_Inf_L_GM	8867 (948)	8216 (978)	7.34%	0.0350
Parietal_Inf_R_GM	4899 (795)	4149 (778)	15.3%	0.0044
SupraMarginal_L_GM	4890 (668)	4453 (607)	8.94%	0.0259
SupraMarginal_R_GM	6485 (1186)	5636 (845)	13.1%	0.0132
Temporal_Sup_R_GM	9327 (1166)	8610 (638)	7.69%	0.0333
Larger in TS at 1 Yr				
Insula_L_GM	5189 (766)	5699 (816)	9.82%	0.0221
Insula_R_GM	5644 (701)	6203 (861)	9.90%	0.0115
Amygdala_L	635 (110)	701 (117)	10.4%	0.0342
Caudate_L	2655 (381)	2931 (398)	10.4%	0.0309
Smaller in TS at 2 Yr				
Postcentral_R_GM	10658 (1602)	8692 (1383)	18.4%	0.0044
Parietal_Inf_L_GM	10360 (1246)	9366 (774)	9.59%	0.0281
Parietal_Inf_R_GM	5447 (731)	4676 (939)	14.7%	0.0056
SupraMarginal_R_GM	7296 (1216)	6357 (953)	12.9%	0.0340
Larger in TS at 2 Yr				
Insula_L_GM	6337 (744)	7025 (897)	10.9%	0.0492
Insula_R_GM	6814 (785)	7922 (934)	16.3%	0.0024

Note: Volume in cubic millimeters. P-values are from a two-sided non-parametric Wilcoxon test. In cortex only GM was tested. Subcortical structures include both GM and WM.

Regarding DTI, we used unbiased tract-based spatial statistics (uTBSS) and deterministic fiber tracking to compare infants with TS to matched controls. High-quality DTI data was available for 16 children with TS at age one and 12 children with TS at age two. We did not observe extensive reductions in FA as reported by Yamagata and others (7). Instead, we observed focal reductions in the bilateral anterior corona radiata, the left inferior frontooccipital-fasciculus, and the splenium of the corpus callosum, suggesting that global reductions in FA arise after two years of age. The latter two findings are of particular interest, as the inferior fronto-occipital fasciculus plays a critical role in determining where an object is in space and the splenium of the corpus callosum carries fibers that connect left and right parietal and occipital lobes, areas important in visuospatial processing. We also observed increased FA in several regions, including the bilateral external capsule, and right superior temporal and left inferior temporal WM, which could explain the preserved or enhanced language skills in girls with TS.

We investigated functional connectivity using seed regions in the networks mediating social cognition and working memory. High-quality DTI data was available for 9 children with TS at age one and 9 children with TS at age two. For this particular analysis, children with TS were matched to a single-age, gender-matched control. Given time constraints, we only discussed results for the two regions with the greatest number of voxels

exhibiting significantly changed connectivity strength ($p < 0.05$, FDR corrected) at each age. At age one, these regions were the left supramarginal gyrus and the right inferior parietal lobule. Functional connectivity maps revealed reduced connectivity between supramarginal gyrus and frontal regions in infants with TS, and a lack of typical connectivity with the temporoparietal junction. Infants with TS also showed increased connectivity with the insula (see figure 2). A similar pattern of results was observed for the right inferior parietal lobule.

We have only recently begun to explore the cognitive data collected in this cohort. In our TRN presentation, we shared early results from the visual reception scale of the Mullen behavior scales (27) social scores from the communication and symbolic behavior scales (CSBS) (28) and the behavior rating inventory of executive function (BRIEF-P) (29). Regarding visual reception, most girls with TS were in the normal range, but there did appear to be worsening over time, which would be in keeping with studies of visuospatial- function in older girls with TS. This suggests that behavioral

Figure 3.

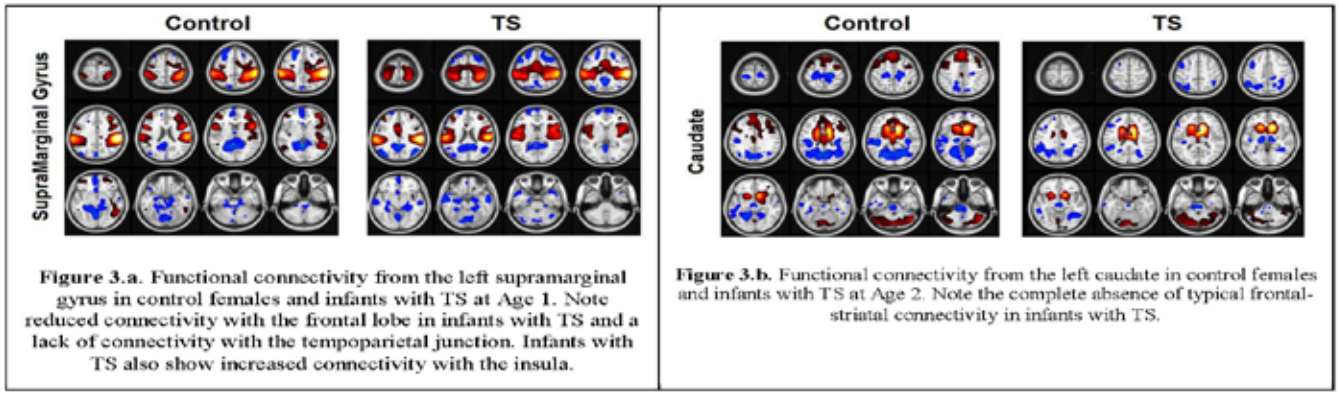


Figure 3.a. Functional connectivity from the left supramarginal gyrus in control females and infants with TS at Age 1. Note reduced connectivity with the frontal lobe in infants with TS and a lack of connectivity with the tempoparietal junction. Infants with TS also show increased connectivity with the insula.

Figure 3.b. Functional connectivity from the left caudate in control females and infants with TS at Age 2. Note the complete absence of typical frontal-striatal connectivity in infants with TS.

At age two, the regions with the greatest number of voxels exhibiting significantly changed connectivity strength were the left caudate and the left middle temporal gyrus. Functional connectivity maps suggested an absence of typical connectivity between caudate and frontal lobe in infants with TS. Regarding middle temporal gyrus, we found that extensive anticorrelations, including inferior frontal cortex, insula, and motor cortex, were present in infants with TS, but were not present in controls. In addition, we found that the controls' left middle temporal gyrus showed little connectivity with its right hemisphere counterpart. In contrast, girls with TS were markedly less lateralized.

To summarize, early results for our neuroimaging data strongly suggest that infants with TS show altered development of social processing and working-memory circuits at ages one and two. It is important to note that differences in brain structure or function should not automatically be perceived as a negative. There is great diversity in brain development, and group differences can be associated with positive or negative cognitive outcomes, or they could be neutral. So the crucial question is, how do these differences relate to cognitive function in the short and long term?

interventions in this period could be of tremendous value. Regarding CSBS social scores, we saw strong performance with possible improvement over age. But it is important to keep in mind that differences in brain structure may appear functionally neutral until they are challenged by particular situations. The transition to school age and adolescence are likely vulnerable periods for girls with TS as regards social cognition. Regarding the BRIEF-P, girls with TS had slightly elevated scores (denoting poorer performance) on scales measuring inhibition and working memory, as well as planning and organization.

Discussion

Additional studies are needed to examine detailed longitudinal changes in brain structure and function in infants with TS to determine whether individual variation in neuroimaging phenotypes predicts cognitive outcomes and how clinical variables (such as genetic and hormonal variation) relate to brain development. As regards the latter, we discussed the relative lack of

information about estrogen levels in girls with TS in the first year of life. Infants with TS do show elevated FSH levels, which suggest estrogen deficiency (30), but this has not been directly demonstrated. In addition, what remains unknown is how much individual variation is present and whether estrogen deficiency in infancy impacts neurodevelopment. If so, low-dose estrogen supplementation in infancy could potentially normalize developmental trajectories and improve cognitive outcomes. In addition, as there is heterogeneity in the expression of social and cognitive problems in TS, early MRI scans could play a role in identifying children at risk for specific problems later, allowing for intervention targeted to the individual child's needs. At present, there are no specific interventions for girls with TS in this age range, but behavioral interventions originally designed for other groups of children could prove valuable. For example, very low birth weight preschoolers benefit from a computerized working-memory training program (31), and there are numerous social skills interventions developed for use in children with autism spectrum disorders (32, 33).

Chapter 8

Estrogen Replacement in Adolescents with Turner Syndrome

Lournaris Torres-Santiago, MD, and Nelly Mauras, MD

The biological effects of estrogen are ubiquitous and necessary for normal physiologic function in women. Estrogen is critical for the growth and differentiation of mammary tissue, the growth of reproductive organs, and the regional fat distribution in women—with more fat around the hips than men. Estrogen is also indispensable for the maintenance of bone mass and bone mineral density accrual, among many other actions. The principal ovarian-derived estrogen in females, 17β-estradiol (E_2), is produced in women of reproductive age at a daily rate of 60 to 150 μg, depending on the phase of the menstrual cycle. There are a plethora of available estrogen preparations that have been used, yet the best time, type, dose, and route of estrogen replacement in hypogonadal girls has not been completely characterized.

Timing of Estrogen Replacement Initiation

There is general consensus in the pediatric endocrine community that we should no longer postpone the introduction of estrogen and the feminization of young women with hypogonadism due to Turner syndrome or other etiologies simply to promote linear growth. Since the last guidelines for the care of girls with Turner syndrome, we have been beginning estrogen therapy for feminization at the anticipated time of puberty, that is, no later than eleven or twelve years old (1). Ross and others (2) at the NIH investigated this issue of timing further by examining whether estrogen could be safely started even earlier and whether it could promote linear growth. Girls with Turner syndrome between five and twelve years old were randomly assigned to either estrogen/placebo; growth hormone (GH)/placebo; GH/estrogen; or placebo/placebo. Then Ross and others followed the girls longitudinally for an average of 7.2 years. The estrogen used was given orally in the form of ethinyl estradiol with a complex titration scheme in starting doses as low as 0.5 μg/day (~25 ng/kg/d). There was a net gain of about 2.1 cm in those that used the ethinyl estradiol along with GH, whereas those that took estrogen alone and those that took placebo had a net loss in height SD score over the course of the study. While this is provocative data, the net height gain of the estrogen plus GH was quite modest and there was no height gain when using the ethinyl estradiol alone. In addition, since the long-term safety of such early introduction of oral estrogens remains to be established, this approach cannot be recommended for routine clinical use at this time. Further studies need to be conducted.

Type of Estrogen

Many forms of estrogen are available. Examples of some of the most commonly used ones are summarized in table 1. As recently as 2006, the vast majority (80 percent) of practicing pediatric endocrinologists surveyed by the Pediatric Endocrine Society in the US reported using conjugated equine estrogens (CEE), like Premarin, to feminize hypogonadal girls. The study also showed that 9.8 percent used ethinyl estradiol, 7.3 percent used a combination of estrogen and progestin (oral birth control pills), and another 7.3 percent used estradiol transdermal patches (3). CEE, which comes from urine from mares, has more than one hundred forms of estrogen of different potencies, many of which

have different biological activities depending on the target tissue. Ethinyl estradiol has also been used for induction of feminization and is a principal component of birth control pills; however, it is no longer available in the United States as a single agent. Considering that estradiol (E_2) is readily available and affordable, the use of these other estrogen types should no longer be necessary for estrogen replacement in girls. Estradiol, or 17β-estradiol, is identical to the product of the ovaries and is available in oral, parenteral, and transdermal formulations. In addition, it can be accurately measured in contemporary assays. Given its ready availability, it should be the preferred product for estrogen replacement in young girls.

Table 1

Examples of Commonly Used Estrogens			
Oral Estrogen Tablets	**Brand Name®**	**Concentrations**	**Comments**
Conjugated Estrogen	Premarin, Cenestin	0.3 (Cenestin), 0.325 (Premarin), 0.45, 0.625, 0.9, 1.25 mg tabs	Premarin-equine origin, Cenestin-synthetic; multiple estrogenic forms included.
Estradiol	Estrace Gynodiol	0.5, 1.0, 1.5 (Gynodiol), 2.0 mg tabs	Micronized estradiol. This is identical to the product of the intact gonad. Can be measured in commercial estradiol assays.
Ethinyl Estradiol	Birth Control Pills	20µg, 50µg	Only available in combination with progestins. As of 2004 ethinyl estradiol preparations alone have been discontinued in the U.S.
Parenteral Estrogen			
Conjugated Estrogen	Premarin IV	25mg/5ml	Used for hemorrhagic bleeding
Estradiol	Depot-Estradiol, Depogen DepoGynogen	5mg/ml (cypionate)	Intramuscular given monthly 1-5 mg doses. Used in Turner syndrome in doses starting at 0.2 mg up to 3 mg monthly
Transdermal Estrogen			
Estradiol Skin Patch	Vivelle, Vivelle Dot, Estraderm, Alora, Climara, Menostar	0.014, 0.025, 0.037, 0.05, 0.75, 0.10µg patches	Twice weekly or weekly applications
Estradiol Gel	EstroGel	1.25 g (0.75 mg estradiol)	Daily applications

Route of Delivery

Previous studies have addressed the issue of the differential effects of the estrogen delivery route on body composition, lipids, and bone metabolism, but most of those studies have compared different types of estrogen and different routes of delivery based on inadequate bioequivalence data (4-9). We critically examined the issue of the metabolic effects of oral versus transdermal estrogen using the exact same type of 17β estradiol on a group of girls with Turner syndrome who had been treated with growth hormone. We performed complex physiological studies using stable label tracers of the essential amino acid leucine (13C-leucine) to measure whole body protein synthesis rates, as well as d5-glycerol infusions to measure rates of whole body lipolysis in these girls (10). We also performed dual energy X-ray absorptiometry (DXA); we measured substrates and hormones as well as indirect calorimetry before and after six weeks of randomized administration of either oral or transdermal 17β estradiol with a washout period in between. Overall, the rates of whole body protein synthesis, lipid oxidation, carbohydrate oxidation, resting energy expenditure, plasma lipids, fibrinogen, and fat free mass were not any different between the oral versus the transdermal group when the exact same type of 17β estradiol was used (10).

Subsequently, we conducted a follow-up study on the metabolic effects of oral versus transdermal 17β estradiol in a group of forty girls with Turner syndrome, the principal study outcome being the differences in body composition and lipid oxidation rates between the groups. Patients were randomly assigned to oral or transdermal 17β-E2, and the estradiol doses were titrated monthly to maintain estradiol concentrations in plasma comparable to those of the normally menstruating adolescents, whom we recruited to establish normative data using the same LC/MS/MS assays. Patients were well-matched for age, height SD score, BMI, and years of prior growth hormone treatment. We were able to achieve similar estradiol concentrations between the groups of girls with Turner syndrome, and comparable concentrations to those of normally menstruating adolescents. The concentrations of estrone were markedly higher, however, after oral estradiol, as were the concentrations of estrone sulfate (11). We also used a recombinant cell bioassay (12) to measure total bioestrogen activity in plasma, and these concentrations were also markedly higher after oral dosing when compared to transdermal use and to the controls (see figure 1).

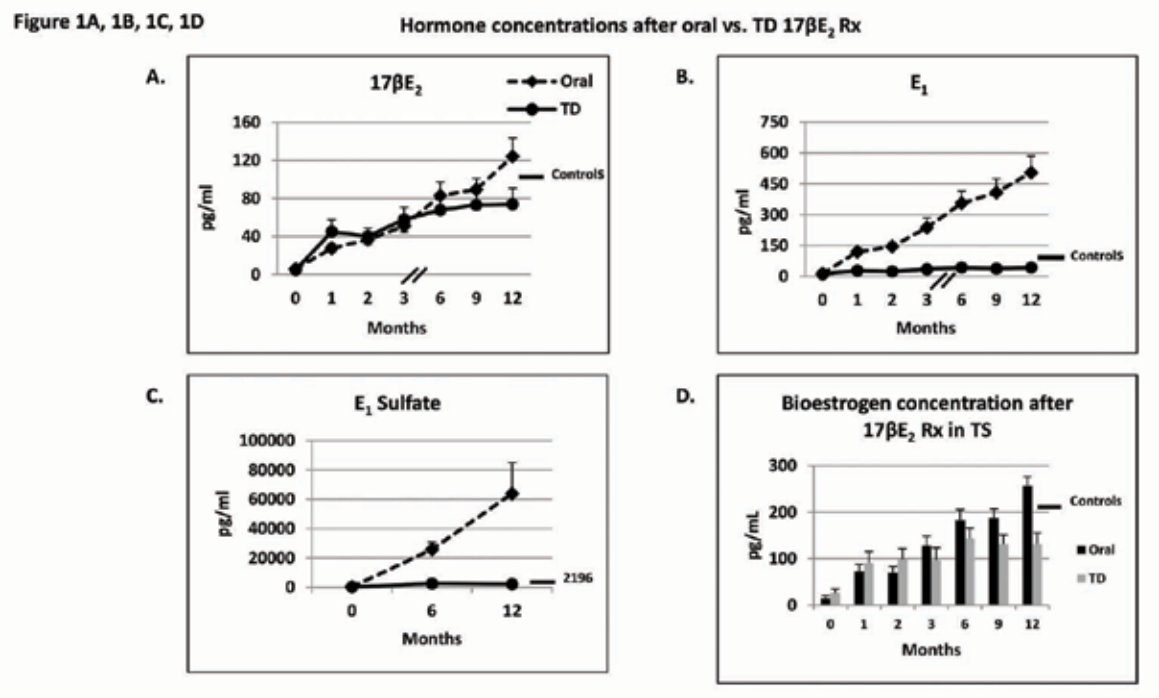
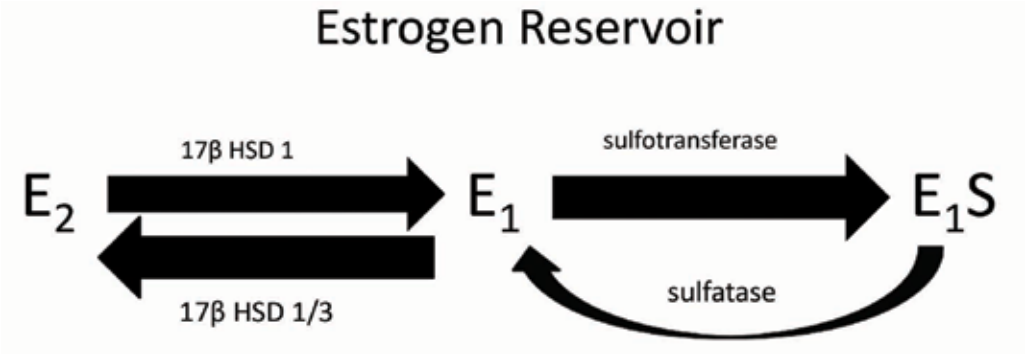


Figure 1. Hormone concentrations after oral vs. transdermal (TD). Mean ± SE plasma concentrations of 17β-E2 (A), E1 (B), E1S (C), and Bioestrogen (D), after oral or transdermal estradiol treatment (Rx) over twelve months. Black bars represent controls.

Adipose tissue can serve as a reservoir in situ for estrogenic activity as estradiol gets converted to estrone through 17β hydroxysteroid dehydrogenase 1 and estrone gets converted further to estrone sulfate through a sulfotransferase. Estrone sulfate in turn gets converted back to estrone, and through 17β hydroxysteroid dehydrogenase 1/3 back to estradiol (13), creating a pool of readily available estrogen stores (see figure 2).

Figure 2.



Other metrics of bioactivity, such as gonadotropin concentrations, were suppressed comparably using oral versus transdermal E2. The principal study outcome, body composition, was comparable between the groups after one year, with similar weight-gain rates, BMI, and percentage of total fat mass and abdominal fat (see figure 3). The rate of accrual of bone mass was comparable at the whole body and lumbar level, whether using oral or transdermal 17β-E2. IGF 1 concentrations tended to be higher after transdermal use, but they were still within the normal range. Lipid concentrations were the same between groups and, as expected, sex hormone–binding globulin was much higher in the oral versus the transdermal group. Using indirect calorimetry, we also measured lipid oxidation rates and resting energy expenditure, and there were no differences between the oral and transdermal groups over the twelve months of the study.

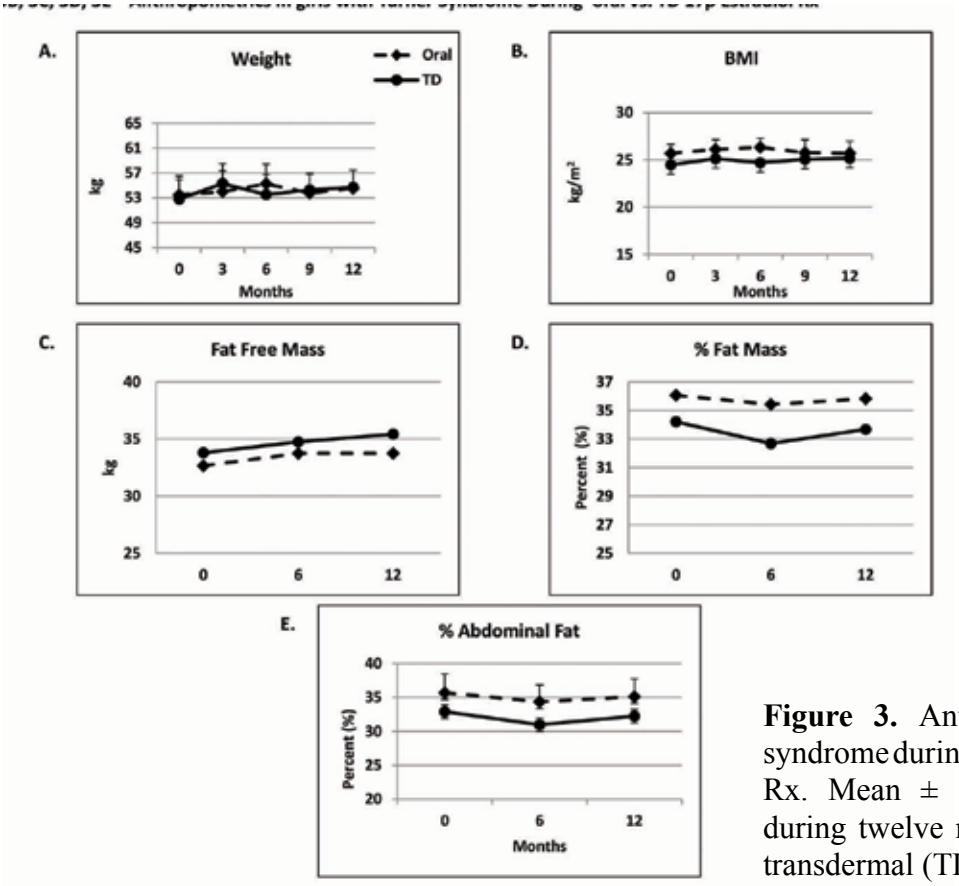


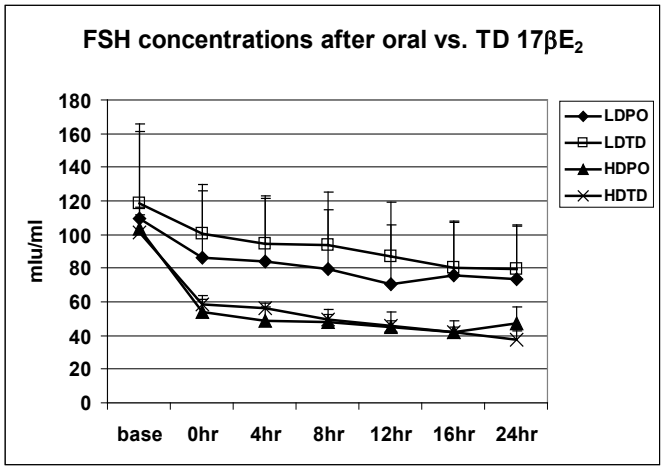
Figure 3. Anthropometrics in girls with Turner syndrome during oral versus transdermal 17β-estradiol Rx. Mean ± SE measures of body composition during twelve months of 17β-E_2 treatment, oral and transdermal (TD) in girls with Turner syndrome.

So, based on our study, when using the same form of estrogen and when E_2 concentrations are titrated to those of normally menstruating girls, there is no difference in body composition between oral and transdermal estradiol use; however, the total estrogen exposure and the accumulation of unphysiologic estrogens were much greater in the oral group. These findings are concerning, due to the potential increase in thromboembolic risk and the possible breast cancer risk. Estrone sulfatase is particularly increased in breast cancer tumors (14). In a meta-analysis, the odds ratio for thromboembolic events using oral estrogen was 2.5 times greater than the general population (15). When examining the risk of stroke, investigators followed > 870,000 postmenopausal women and found that oral estrogen increases the risk of stroke while the transdermal route does not (16). In the study of a million women in the UK, the relative risk of stroke was 1.4 times higher in oral and transdermal (17). Considering that premature mortality in Turner syndrome is higher than the general population (18, 19), mostly due to increased cardiovascular risk, the issue of proper selection and dosing of the estrogenic compounds is of compelling relevance in women with this condition. The data thus far suggests that when the same type of estrogen (17β-estradiol) is used orally and transdermally the metabolic effects are overall similar; at the same time, the data raises questions about potential biological effects of other estrogen metabolites.

Which dose to use?

Much of the data on the bioequivalency of the different forms of estrogen comes from work published in 1986 by Chetkowski and others (20) in which bioequivalency was based on the degree of suppression of LH concentrations (which have a marked ultradian and monthly rhythm) and vaginal cytology (with inherent variability in sampling and measurement), neither of which gives a true measure of biologic potency. At the time, these surrogate markers were used as the different forms of estrogen could not be measured in the same assays. Because of the ligand-dependent and ligand-independent receptor activation caused by estradiol, as well as non-nuclear actions through cell-surface receptors of estradiol, comparisons of bioequivalency are difficult, at best, to make in the absence of an adequate assay. Contemporary studies strongly suggest that mass spectrometry assays (LC/MS/MS or GC/MS/MS) are superior to both indirect and direct RIAs for measurement of the very low concentrations of estradiol (21).

We recently studied the pharmacokinetics and pharmacodynamics of oral versus transdermal 17β-estradiol in ten girls with Turner syndrome and compared them to twenty normally menstruating adolescents (22). We employed a sensitive LC/MS/MS assay (23), as well as a recombinant cell bioassay. Using a yeast transfected with the estrogen receptor gene, we were able to determine even minuscule estrogenic activity in the blood samples (12). The girls were randomly assigned to either a lower dose group of 17β-E_2 (oral 0.5 mg/day and transdermal 0.0375 mg/day) or to a higher dose group of 17β-E_2 (oral 2 mg/day and transdermal 0.075 mg/day). Each group was given their respective dosage for two weeks. This was followed by a twenty-four-hour study where the participants' blood was taken frequently and sampled in the clinical research center. A two-week washout period followed. Both low- and high-dose oral estradiol resulted in significantly higher estrone concentrations compared to the concentrations achieved after transdermal estradiol was given to the girls with Turner syndrome and to controls. Bioestrogen concentrations were the highest and closest to normal in the high-dose transdermal group. We also observed maximum overall reduction in gonadotropins in the high-dose transdermal group (see figure 4). As mentioned above, we have demonstrated the ability to individually titrate estradiol doses to normally menstruating adolescents using serum levels measured by LC/MS/MS.



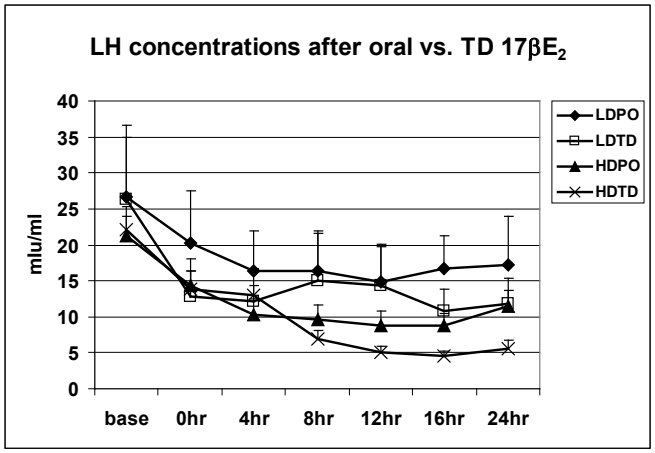


Figure 4. Pharmacodynamics of E_2 administration on LH and FSH measured over twenty-four hours, two weeks after initiation of treatment.

Summary

The timing, type, dose, and route of estrogen replacement in hypogonadal girls have now been better characterized. Micronized 17β-E_2 should be considered the first choice, as it is the most physiological. Doses can range between 37.5 μg and 100 μg daily or lower (if there are concerns regarding growth) and they can be titrated individually using concentration-based dosing by means of contemporary assays (LC/MS/MS).

Despite similar estradiol concentrations, total estrone, estrone sulfate concentrations, and bioestrogen concentrations are much higher after oral therapy, suggesting that transdermal 17β-estradiol results in a more physiologic estrogen milieu than oral administration. Long-term studies are needed to better assess cardiovascular and breast cancer risk in this population.

Chapter 9

The Genetics of Bicuspid Aortic Valve in Turner Syndrome

Siddharth Prakash, PhD

Thanks to Michael [Silberbach], again, for this terrific opportunity to present our work. I am an imaging cardiologist. I work with Dianna Milewicz in the John Ritter Research Center for Aortic and Vascular Diseases at UT Houston. Our clinic is focused on families and individuals with congenital thoracic aneurysms and aortic dissections, as well as other genetically determined vascular disorders and bicuspid aortic valves.

I will first provide an overview of bicuspid aortic valve genetics, explain its relevance to Turner syndrome, summarize our genomewide- association results and our protocol for X chromosome mapping, and conclude with a road map for further research opportunities.

A normal aortic valve looks somewhat like a Mercedes Benz logo as you look down at it, with three equal-sized cusps. The disorder that I chose to investigate, bicuspid aortic valve, or BAV, occurs when two of the three cusps fail to separate during development. This can occur in a variety of patterns, depending on which combination of cusps is involved. The most common configuration is caused by the fusion of the right and left cusps, which creates a "horizontal" or nine o'clock–three o'clock pattern. The clock face is one way to view a BAV, as if you were looking down at the valve, but there are, of course, many different ways that we can describe valve anatomy.

Bicuspid aortic valves are the most common congenital heart defect and are present in approximately 1 percent of the population. BAVs are more common than all other adult congenital heart defects combined. By most estimates, BAVs are responsible for about half of all aortic valve replacements in patients over fifty years of age in the United States.

We know, primarily from animal studies, that migratory neural crest cells are critically involved in the formation of the aortic valve, the entire aortic arch, and the blood vessels that arise from the aortic arch to supply the head and neck. All these structures are remodeled by invading neural crest cells in mid to late embryonic development. Without neural crest involvement, the heart valves and the outflow tract of the heart, which becomes the aortic arch, fail to develop properly. Work by Jonathan Epstein and many other people using genetic techniques in mice have shown that ablation of the neural crest results in developmental defects involving the aortic valve, pulmonic valve, and aortic arch. The spectrum of abnormalities in mutant mice with these genetic defects include an increased prevalence of BAVs.

BAVs do not always occur in isolation; they are more common in genetic syndromes that involve the left ventricular outflow tract and aorta. BAVs are enriched in patients with patent ductus arteriosus, coarctation of the aorta, supravalvular aortic

stenosis, and even hypoplastic left heart syndrome. These structures share a common developmental origin and they are all remodeled by neural crest cells.

Echocardiography provides a dynamic view of BAV structure and function. This image[3] shows an adult heart with a BAV, as well as the left ventricle, the aorta, and the left atrium. When the aortic valve is partially open, please notice its unusually curved appearance. Most aortic valves open and close like trapdoors: straight up and straight down. This curved or doming motion is a telltale indication that a congenital malformation of the valve is present.

This BAV[4] is slightly more thickened and calcified, but the principle is the same. When the valve is partially open, the orifice is restricted into a football shape. This is also very abnormal and it suggests that one or more of the cusps is fused together. This image shows the most typical horizontal configuration of BAVs, related to the fusion of the left and right coronary cusps. The opening and excursion of this BAV are restricted, because the cusps are tethered along the length of partially fused commissures. The commissures are the points where the valves are attached to the aortic annulus. In this second case, note that the valve is vertically oriented due to fusion of the right and non-coronary cusps. Once again, the partially open cusps are restricted into a football shape, but they are oriented almost exactly perpendicular to the previous example. These images illustrate the two most common configurations of BAVs that we see in our laboratory.

While there are many ways to describe BAVs, the International Bicuspid Aortic Valve Consortium, a research group that is investigating the genetic causes of BAVs, has endeavored to institute a universal classification system that will allow us to combine worldwide observations in a more systematic approach. The scoring system that we use is designed to help researchers speak the same language when describing BAV anatomy. In scientific literature, BAVs have been described in so many different ways that the observations are often very difficult to compare. Our classification system includes four characteristics that not only reflect the anatomic configuration of the valve but also provide an estimate of its function: cusp orientation, cusp thickening, the degree of calcification, and mobility. We also record the "clock-face" orientation of the valve as I mentioned previously. The sum of these scores has been shown by my colleagues at Mayo Clinic to predict the longterm- outcome of BAV patients, such as valve degeneration requiring surgical replacement.

When we performed an autopsy study of BAV cases at UT Houston, we uncovered a remarkable spectrum of disease. The individuals on this slide[5] were all around fifty years of age at autopsy, but in each case there were dramatic differences in the thickness and calcification of similarly oriented BAVs. This valve is encased by so much calcium that the cusps are almost invisible, whereas this valve is virtually devoid of any calcium.

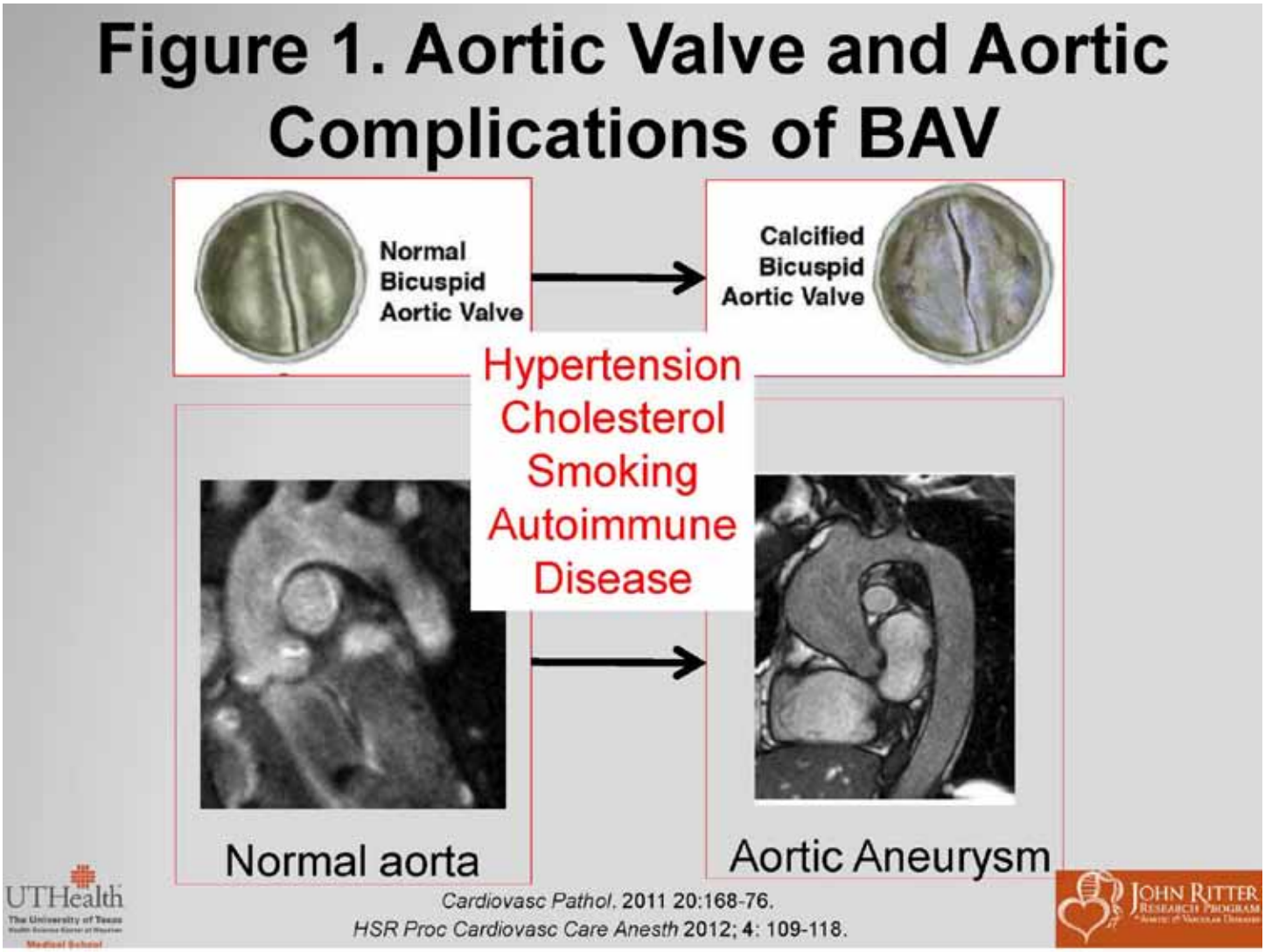
Why are BAVs so diverse in orientation and appearance? What happens to make some valves calcify, thicken, and fail, requiring replacement, while others remain relatively normal? We know that bicuspid aortic valves are associated with other aortic disorders, but they do not occur in all patients. For instance, only 20 percent of BAV patients develop clinically significant aortic aneurysms. How can we explain the pleomorphic outcomes of BAV?

Observational studies have defined some factors that tend to accelerate aortic disease in BAV patients, including the same CV risk factors that drive most cardiovascular disorders (see figure 1).

3. This echocardiogram was shown at the symposium and could not be reproduced in book format.

4. The BAV being discussed here was also shown using an echocardiogram and could not be reproduced here.

5. Slide not shown.

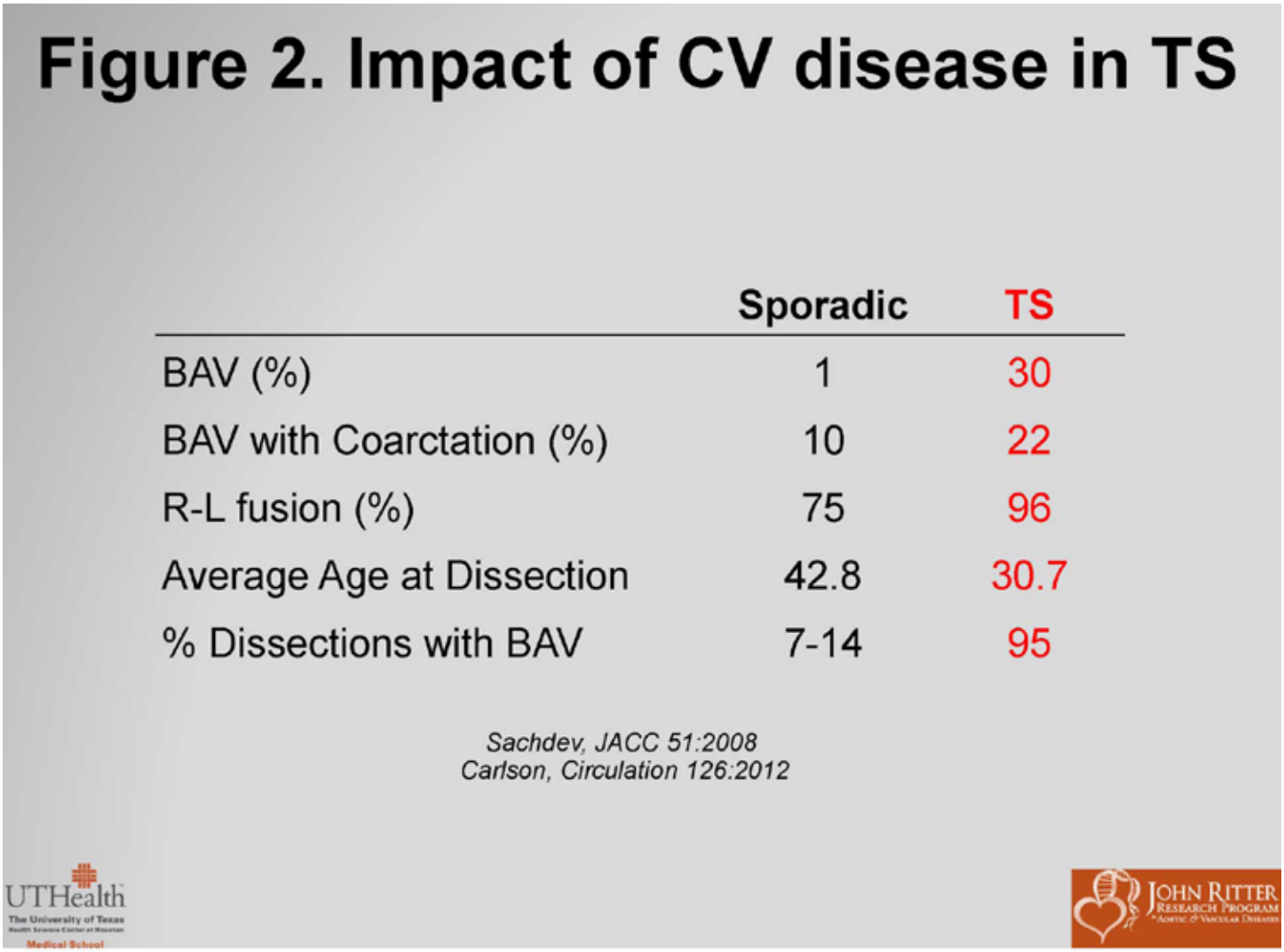


Older age, hypertension, high cholesterol, and smoking are clearly associated with the acceleration of valve disease and aortic aneurysm growth in BAV patients. Some autoimmune disorders may also promote more rapid aortic enlargement and valve calcification. In BAV patients without Turner syndrome, the incidence of aortic complications is approximately 1 percent per year. This includes elective coarctation and aortic root repairs, acute aortic dissections, and aortic valve replacements. We do not yet know if the morphology or anatomic orientation of BAVs is associated with outcomes, because these observational studies included small numbers of BAV cases and did not use the rigorous classification system for BAV configurations that we proposed.

BAV includes an entire spectrum of valve disease, ranging from an isolated, normally functioning valve with an "innocent murmur" to an aortopathy with multiple congenital defects, including coarctation and aortic aneurysm development. Depending on the burden of the associated defects, BAV patients may be clinically recognized at an advanced age with incidental findings or at a very young age with multiple complications. We think that Turner syndrome is at the severe end of the BAV spectrum and, therefore, is a model for the kind of approach that we want to adopt for all forms of BAV disease in order to really understand the connections between these phenomena.

It is remarkably easy to sum up the current state of BAV genetics. In fact, it only takes one sentence: "To date, only the transcriptional regulator *NOTCH1* gene at chromosome 9q34.3 has been linked to the development and progression of nonsyndrome-associated BAV in humans, [and] in a limited number of familial cases," (1) which is perhaps 4 percent of all cases of BAV. Therefore we know next to nothing about BAV genetics in humans. Most of what we know comes from animal models that involve a variety of genes in the Notch signaling pathway, but none of these have been replicated in humans to date.

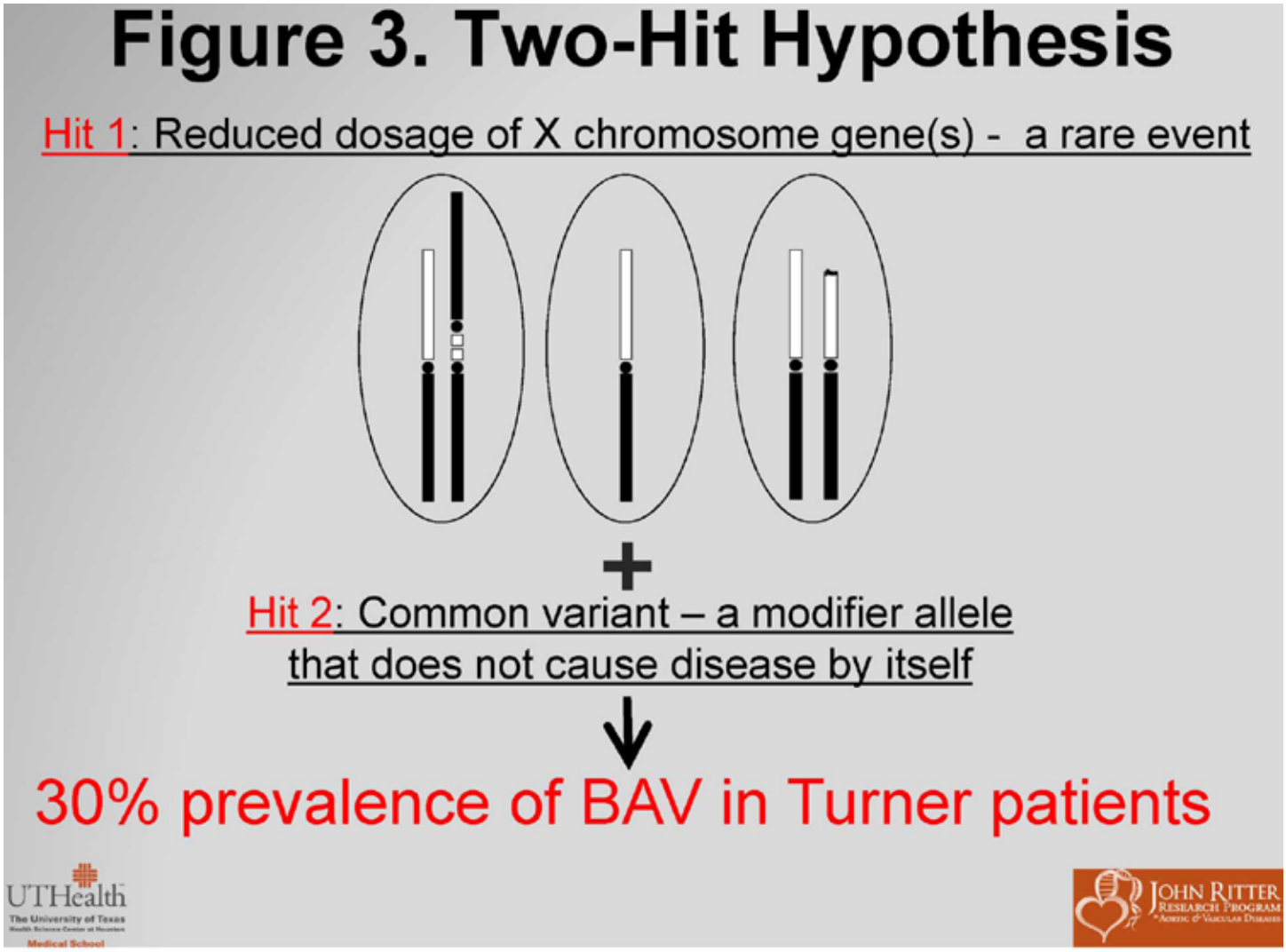
Why is BAV particularly important to Turner syndrome? According to recently compiled statistics, the impact of BAV on women with Turner syndrome is striking (see figure 2).



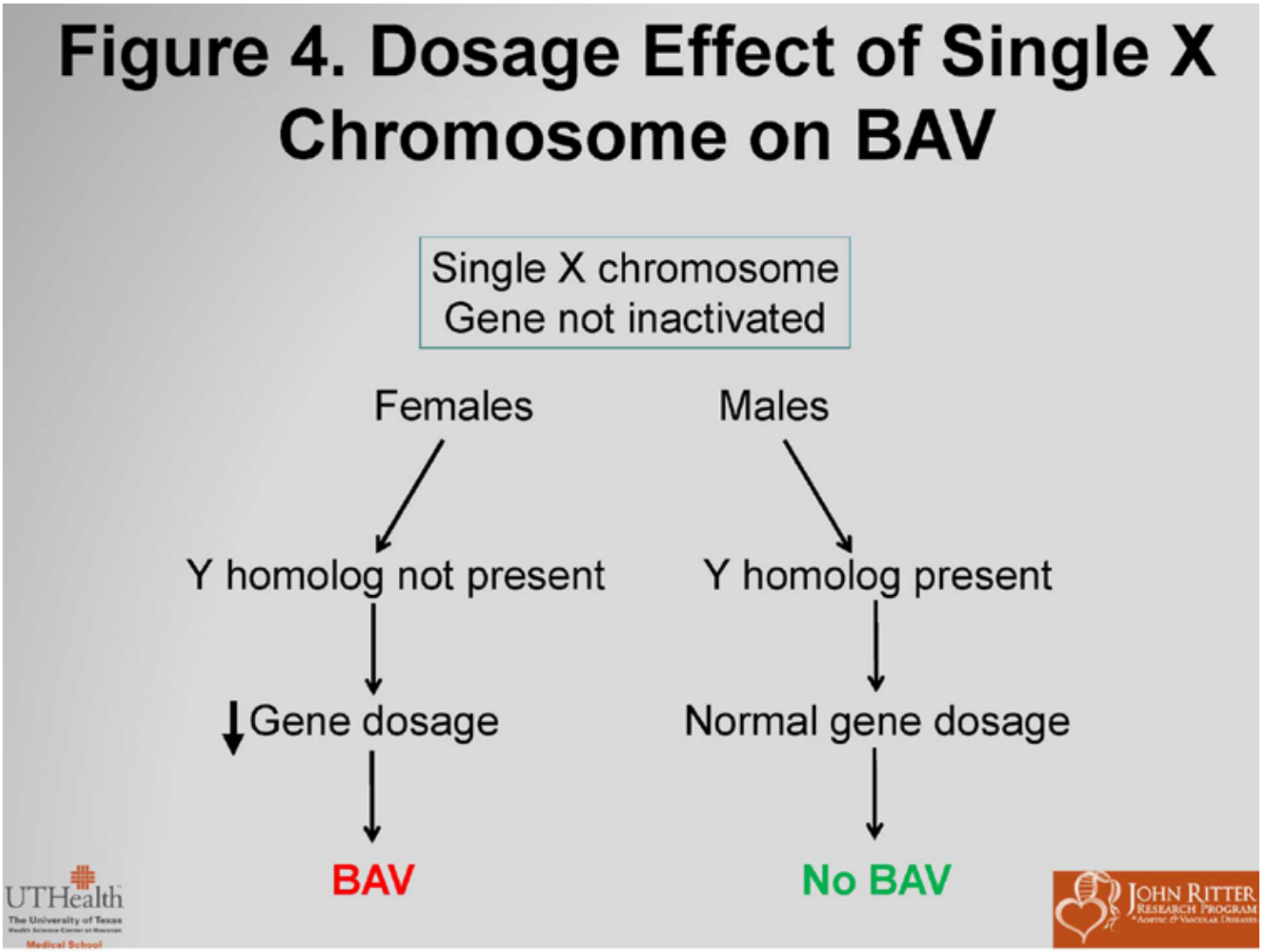
In sporadic cases, the prevalence of BAV is 1 percent. In Turner syndrome, the prevalence is at least 30 percent. BAV occurs in association with coarctation at more than double the rate in Turner syndrome. The prevalence of the rightleft- or "horizontal" subtype of BAV is enriched in Turner syndrome. Women with Turner syndrome also experience acute aortic dissections—which are strongly associated with BAV—at much younger ages than patients without Turner syndrome. Work by Carolyn Bondy and others has shown that aortic dilation and dissection are prevalent in Turner syndrome. Approximately one-third of women with TS exceeded the ninety-fifth percentile for their indexed aortic size, which is a measure of aortic size in relation to body size. In Carolyn Bondy's study, the presence of a BAV was the strongest determinant of aortic size. For dissections among TS women in that study, the presence of a BAV was the major risk factor. In comparison to the rate of acute aortic dissections in the general population, which was a much older study, dissections among Turner women were strikingly elevated. Turner syndrome by itself is a risk factor for dissection, and in combination with a BAV, the incidence of dissections is incredible. Aortic disease is a leading cause of death for women with TS.

Our group and several others have hypothesized that at least two genetic hits are required for susceptibility to aortic disease in Turner syndrome (see figure 3). The first hit is a reduction in the dosage of X chromosome genes due to the various structural variants of the X chromosome in women with Turner syndrome. A second hit could be a common variant or a modifier allele that

does not cause disease by itself, but may interact in an Xspecific or -genomewide- fashion with the loss of material in the X chromosome to account for the 30 percent prevalence of BAVs in women with Turner syndrome. We evaluated many potential models for the genetic contributions to BAV in TS, but this model is the most likely explanation based upon our observations. Another way to look at this problem is that women with Turner syndrome are sensitized to exceed a threshold for BAV development. TS women with BAVs have both sex chromosome factors and autosomal modifiers that greatly increase the prevalence of aortopathy in TS.



What is the dosage effect of a single X chromosome on the development of BAV in women with Turner syndrome? Let's imagine a candidate gene on the X chromosome for BAV. As David Page described, genes that do not undergo X inactivation are implicated in Turner phenotypes. If a single X chromosome is present, this candidate gene would be expressed at a lower level than in a woman without TS. In females, a Y homolog for this gene is not present, and this would lead to reduced gene dosage and precipitate BAV (see figure 4). In males with a Y homolog, which could partially compensate for the absence of one X chromosome, the total dosage of this candidate gene would be relatively normal and no BAV would develop. This could explain why Turner females are different than normal males in the population.



Since Carolyn Bondy and others linked cardiovascular defects in Turner syndrome to reduced dosage of Xp, the short arm of the X chromosome, we decided to investigate this issue more closely. As David Page mentioned, relatively few genes on the X chromosome have Y homologs and escape X inactivation. In fact, only six genes on the short arm of the X chromosome meet these criteria and are expressed in the heart. Haploinsufficiency of these genes may contribute to BAV formation in women with Turner syndrome. In addition, previous work led to the identification of a critical genetic region for lymphedema in Xp11.4. Lymphedema has been tightly associated with BAV and may also contribute to the congenital defects in TS. We believe that common autosomal variants may interact with these pathways to promote BAV.

One challenge in mapping the genes responsible for BAV in Turner women has been the relatively small samples available from single investigators, which resulted in limited power to detect lowerfrequency-variants that may play a role in BAV development. Adjudication of the BAV phenotype was also a significant challenge until recently, because there were no universally accepted standards for identification or characterization of BAVs. X chromosome genotypes merit special consideration in Turner women due to the frequency of structural variants. Moreover, these issues are compounded by mosaicism in a substantial proportion of cases. Because genetic results are usually based on peripheral blood samples, it is often impossible to determine the precise genetic contribution of any mosaic cell line to tissue-specific defects such as BAV.

To test our hypothesis that common autosomal variants are associated with BAV, we performed a genomewide association study. I will present preliminary data for 288 TS women. Sources of data and samples for the analysis were primarily from GenTAC and NICHD, with additional samples from collaborators in Mayo Clinic and in Naples. GenTAC- is a collaborative NIH-sponsored registry of many different types of patients with thoracic aortic disease, including women with Turner syndrome. Three batches were combined into a metaanalysis-, because they were analyzed at separate times.

After appropriate quality controls, we were left with 257 eligible cases: 103 were bicuspid valves and 154 were tricuspid aortic valves. The majority of BAV cases had coexisting coarctation, and the majority of all cases with coarctation had BAVs. The average age was thirty, including both pediatric and adult cases. Approximately 60 percent had 45,X karyotypes and 20 percent had isochromosomes. Ten cases had identifiable Y chromosome material.

Evaluation of population stratification is a critical component of GWA studies, because ethnicity differences by themselves can lead to associations that may not reflect true differences in genetic content with respect to the disease. We observed significant admixture with African ancestry, which is not unusual for a random North American sample. We were effectively able to adjust for stratification using principal component analysis to obtain a relatively flat Q-Q curve,[6] without significant inflation of the test statistics. The upward deflection at the right indicates SNPs with near genome-wide significance and is likely to reflect true associations.

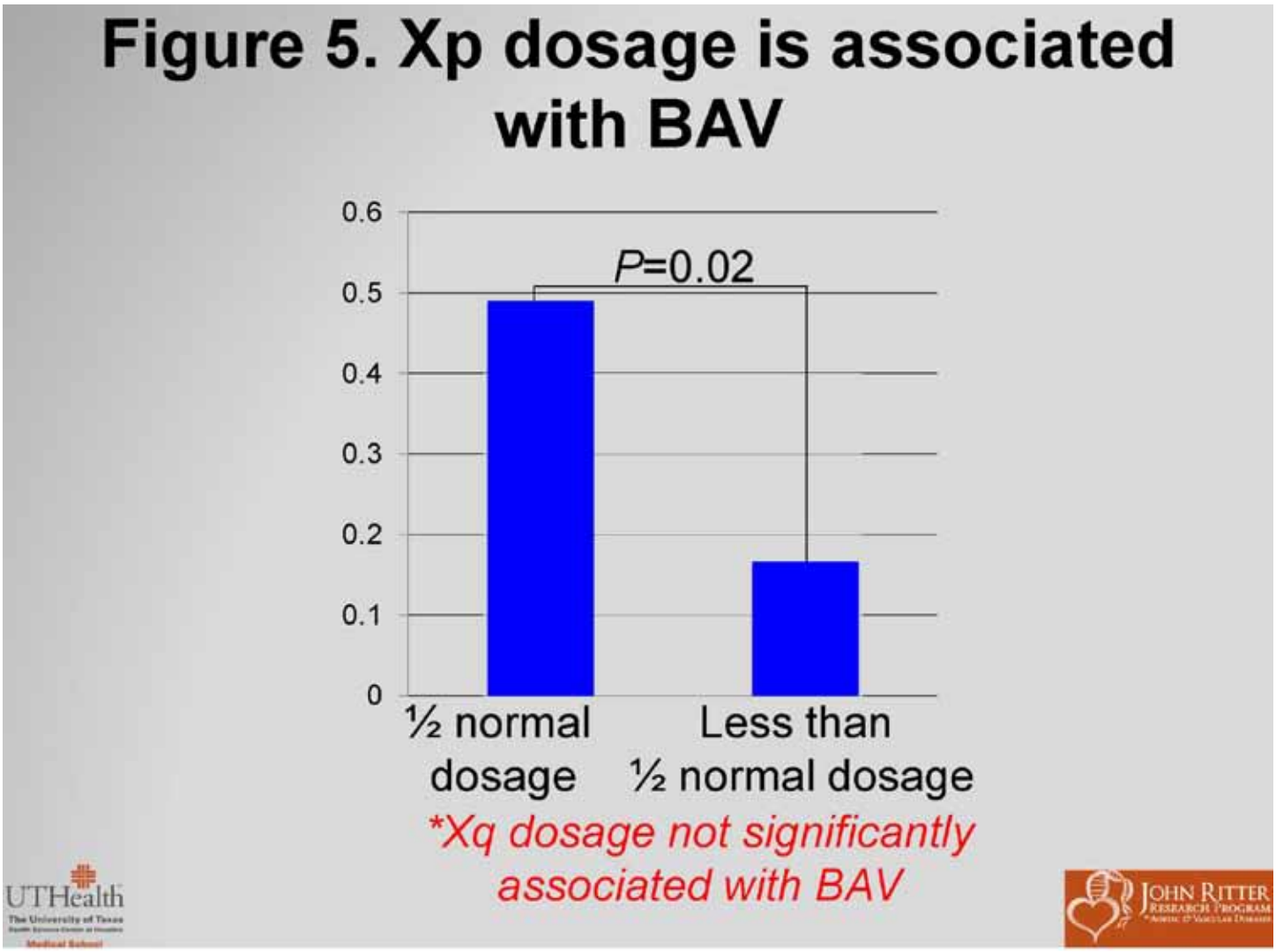
This Manhattan plot[7] summarizes our preliminary genomewide- association results, including only the autosomes. We observed a very suggestive peak on chromosome 18, with a minimum p-value around $1x10^{-7}$. P-values reflect the likelihood that a specific distribution of genotypes would occur by chance. Therefore, a p-value this low is very unlikely to occur at random and indicates a significant association between genetic content at this location in the genome and BAV. In addition to the chromosome 18 peak, we also identified similar peaks on chromosome 4 and chromosome 22. When you consider that our TS sample was much smaller than a typical genomewide-association study, you realize that a significant association such as this is very unusual, and it encouraged us to validate and replicate our findings.

In total, we identified seven autosomal hits with p-values less than $1x10^{-5}$. None of the new loci overlap previously described genetic association or linkage loci for BAV. We also identified one locus that is associated with coarctation and appears to overlap with the BAV locus on chromosome 22. This is consistent with our hypothesis that women with Turner syndrome are uniquely susceptible to BAV and associated aortic defects.

Association tests on the X chromosome were a particular challenge due to complex X chromosome structural variants and mosaicism with two or more cell lines in the majority of our sample. In order to collapse the numerous X chromosome variants for convenient analysis, I developed a method specifically for Turner women to quantitate the copy values of X chromosome SNPs, which also incorporates mosaicism into the analysis. The end result of these calculations is an "X chromosome dosage index," which is a ratio of the amount of DNA at each point on the X chromosome and the expected amount of DNA in an XX woman. We found that the presence of a BAV in women with Turner syndrome is strongly associated with the dosage index of Xp, but not Xq, the long arm of the X chromosome (see figure 5). Women with half the expected dosage of Xp genes, compared to those with a dosage of more than half of expected Xp gene content, were much more likely to have a BAV. When we performed association tests between the genotypes of SNPs in Xp and BAV, we found no significant results, although we were underpowered for this type of test because we analyzed the single alleles. These findings were not surprising and are consistent with prior studies.

[6]. Q-Q curve not shown as the data is still preliminary and not ready for publication.

[7] . Manhattan plot not shown as the data is still preliminary.



Using the SNP data, we were also able to very accurately determine the locations of the breakpoints of X chromosome structural variants. When we combined our results, we found that the breakpoints tended to cluster in one region of Xp11. The dosage of some candidate genes in this region is significantly correlated with the presence of BAV. Obviously, this is a very small subset of cases with particular breakpoints in Xp11, and any additional cases will help refine this breakpoint map. With investigators in the TRN network, we are beginning to replicate these promising variants in additional cases.

At the same time, we also explored the hypothesis that rare copy number variants (CNVs) contribute to the risk of BAV in women with Turner syndrome. Our group uses specialized software to deduce structural variants such as CNVs from genome-wide SNP or sequence data. This Manhattan plot[8] shows p-values for CNVs, rather than for single SNPs, ordered across the entire genome. We identified many rare CNVs in Turner women that are not present in 10,000 control genomes. CNVs marked by green dots were actually observed in more than one Turner woman, and are prioritized as recurrent CNVs. CNVs marked by red dots are prioritized because they are significantly associated with BAV in Turner syndrome. These rare CNVs are also quite large. To provide some perspective, many of these CNVs are at least one megabase, or one million base pairs, in length and involve ten to fifteen genes. This plethora of large and rare CNVs raises the possibility that there may be genome-wide instability in Turner syndrome. While this hypothesis had been developed decades ago in cytogenetic studies, we plan to investigate the burden of rare CNVs in TS women using SNP array data and other current high-resolution genomic tools.

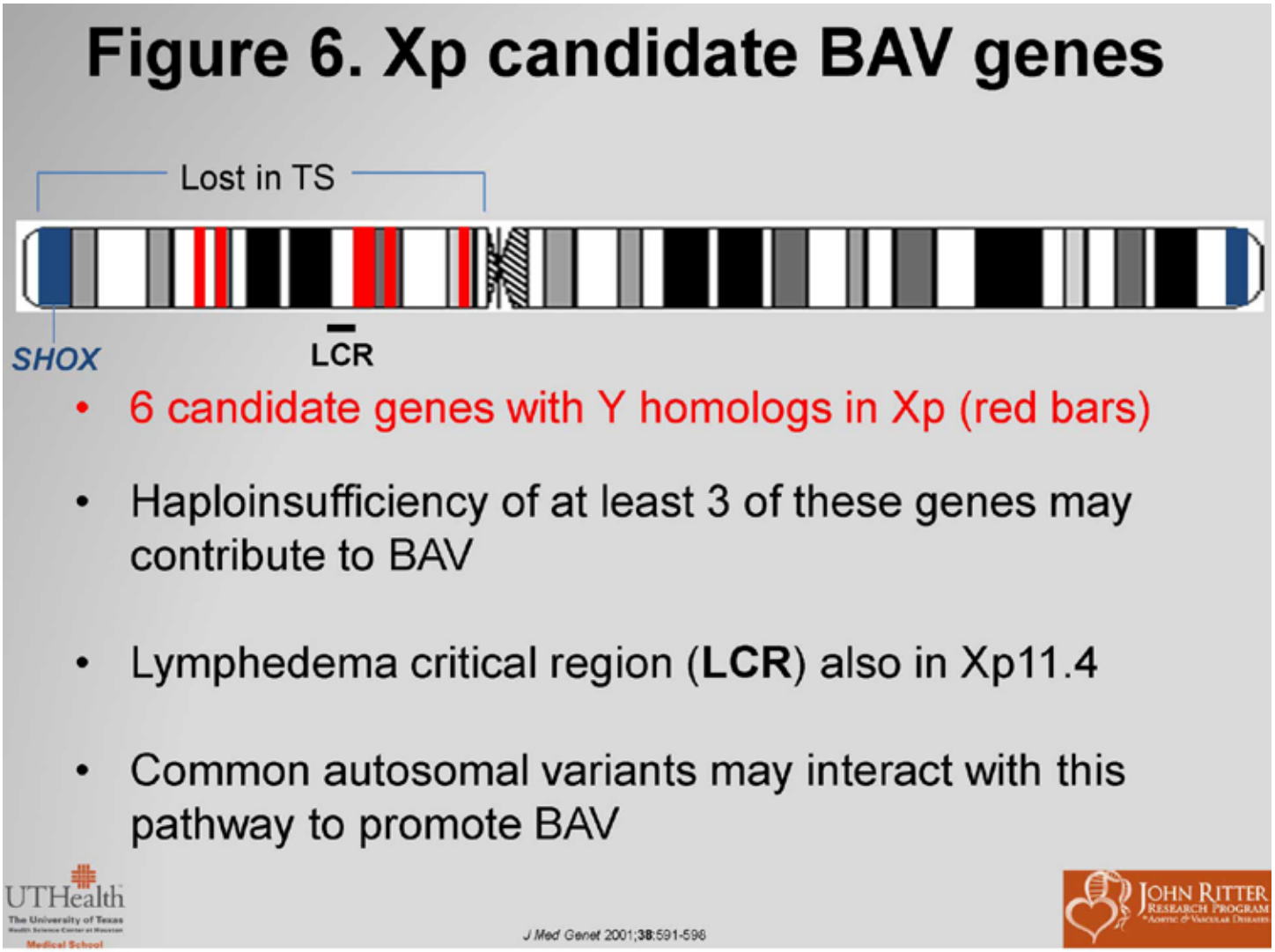
[8]. Manhattan plot not shown as the data is still preliminary.

What is the genetic architecture of BAV in Turner syndrome? We know that BAV is strongly associated with the dosage of Xp genes, and that common autosomal variants appear to interact with X chromosome dosage to increase the risk for BAV (see figure 6). We think that this may also explain the increased prevalence of BAV in males compared to females in the general population. More than threequarters of sporadically occurring BAV cases are male, and we hope to identify similar BAV susceptibility genes in a larger- cohort of sporadic BAV patients.

DR. HAWLEY: We have time for one very quick question.

DR. BONDY: What's the frequency of the allele that you pick out as associated with a higher risk in the general population?

DR. PRAKASH: The allele frequencies of our most significantly associated SNPs are all around .25 to .3, which is approximately the prevalence of BAV in TS women. We suggest that this frequency is appropriate, considering that we expect interactions between these common variants and X chromosome dosage.



Chapter 10

Insights into Congenital and Ischemic Heart Disease from Studies in Patients with Turner Syndrome

Carolyn Bondy, MD, and Melissa Crenshaw, MD

An area of common interest among experts on Turner syndrome and our colleagues is the question of the genetic causes of congenital heart defects and anomalies in Turner syndrome patients. There has been a great deal of progress in this area since the last National Institutes of Health (NIH) guidelines were published.

The NIH study was started in 2000. From the outset, the goal was not only to improve the diagnosis and care for girls and women with Turner syndrome, but also to relate Turner syndrome to six basic health problems in the general population. This has been central to the work done in the NIH study for the last ten to fifteen years. In this chapter we will focus on two gender related- issues in Turner syndrome that we thought were relevant to the general population, and for which the medical community could significantly expand its knowledge on.

The first cardiac issue that has relevance to the general population is congenital heart defects. We thought that since congenital heart defects were so widespread in Turner syndrome patients, that learning about this aspect of Turner syndrome would help us shed a light on what was going on with the development of these defects in the broader population.

From the very beginning, we were interested in the concept that parental origin of the single normal X chromosome might have some specific cardiovascular effects. The question was whether this could be expanded to an excess of coronary disease in normal men. Furthermore, could this be studied in patients with Turner syndrome to perhaps elucidate some new causes of coronary disease that are not related to previously known risk factors.

There are several pieces of evidence that suggest that congenital heart disease in Turner syndrome patients may be related to the occurrence of congenital heart disease in the general population. First, there is a sex bias in the frequency of left outflow tract abnormalities of the heart. The fact that there is a high prevalence of aortic coarctation and aortic valve and left heart hypoplasia in Turner syndrome patients implicates the involvement of a sex chromosome, as these defects are more common in males than females in all the populations across the world. That these problems really explode in prevalence in women with Turner syndrome, who lack a second sex chromosome, strongly suggests sex chromosomes are involved in some way in congenital heart disease, and in this particular type of congenital heart disease.

To know the prevalence and spectrum in Turner syndrome patients, it is important to start from the fetal development, because we lose 95 percent of our cases before birth or around the time of birth. So when we're looking at the seven-year--olds- and older patients in the NIH study, we are looking at the very rare population of survivors. The prevalence of heart disease in the embryonic and fetal development stage is very high. An important study conducted by a Japanese group (1) before the turn of the century looked at thirteen midgestation- Turner fetuses that had died. All of them had interruption or tubular hypoplasia of the aortic arch in a particular part of the arch. In addition, they all had aortic valve defects and degrees of left heart hypoplasia. There are other studies of fetuses with Turner syndrome in the mid-gestational- period that show similar findings in much smaller sample sizes.

Approximately 10 percent of newborns with Turner syndrome have left heart hypoplasia associated with aortic arch hypoplasia (2). While these newborns made it to birth, with left heart hypoplasia, a syndrome, and aortic arch compromise, many very rarely survive. Thus, a large part of our study population is not available for our study at the present time, but we are trying to incorporate more advanced imaging into our clinical evaluation. The girls and women that are evaluated are the ones who were able to survive through fetal development. Therefore, they may have milder, survivable congenital heart disease.

There is a signature profile in Turner syndrome that involves a left ventricular outflow tract. We imaged about 450 girls and women with Turner syndrome, using both MRI and cardiac echo imaging modalities. The most common thing that we saw were peculiarities of the transverse arch. Vincent Ho described these peculiarities as elongated aortic arches. In the NIH study, 50 percent of eighty-five patients had this elongated transverse arch. This would include people that had coarctation or pseudocoarctation-, aneurysms of the left subclavian artery, and aberrant right subclavian arteries. Of those with the abnormal aortic arch, 70 percent had bicuspid valve abnormalities, and those range from unicuspid or bicuspid to partial fusion.

Figure 1 depicts the spectrum of aortic arch abnormalities in Turner syndrome patients. At the left is a patient with a normal curvature of the aortic arch. This is a patient who is healthy and asymptomatic. The next image shows an elongated transverse arch in which the distance between the left carotid and the left subclavian is clearly flattened out and elongated. The next image is of another patient. She has an aberrant right subclavian artery. She also has a small kink in the aorta at the site where coarctation usually occurs. The kink goes into the interior of the aorta and can cause obstruction to flow. This woman has no obstruction. Her aortic diameters are relatively normal.

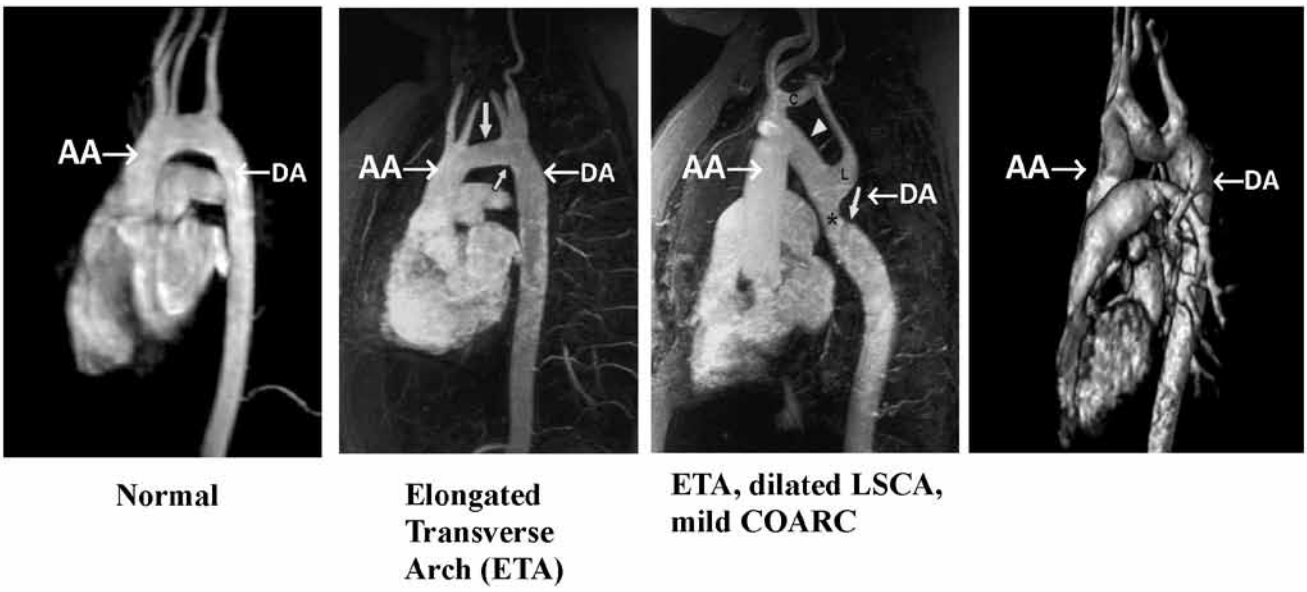


Figure 1. Spectrum of aortic arch abnormalities in Turner syndrome. AA is ascending aorta, and DA is descending aorta.

Some patients with Turner syndrome have even more complex configurations of the aortic arch. The final image in figure 1 shows a woman with a pronounced prolongation of the aortic arch between the carotid and left subclavian, an enlargement of the subclavian takeoff, and a form of coarctation. This individual does not have hemodynamic compromise.

Of importance, all these patients had bicuspid valves, and none of the issues discussed were known before they had an MRI. They also all had an echo. Most of the echoes were reported as normal; however, the images the MRIs produced showed that the architecture of the patients' transverse arches is clearly abnormal. These images provide a truer depiction of the phenotype, which is essential to understanding the genesis of these vascular changes and what genes may be involved in the cardiac features of Turner syndrome.

In Turner syndrome, the most extreme incarnation of this aortic arch disease is the formation of aortic arch aneurysms. Figure 2 shows the aorta of a twenty-three-year-old woman with Turner syndrome. Her cardiologist had monitored her with echocardiography

for some years and had felt her to be well. At the NIH, it was found that she had this six centimeter aneurysm at the site of the typical kink and at the end of the elongated portion of the arch. This aneurysm was fixed emergently at the NIH. She also had an ascending aortic aneurysm of 4 by 3.5 cm and aortic valve regurgitation. The aortic valve was also bicuspid. She returned later for an aortic root replacement.

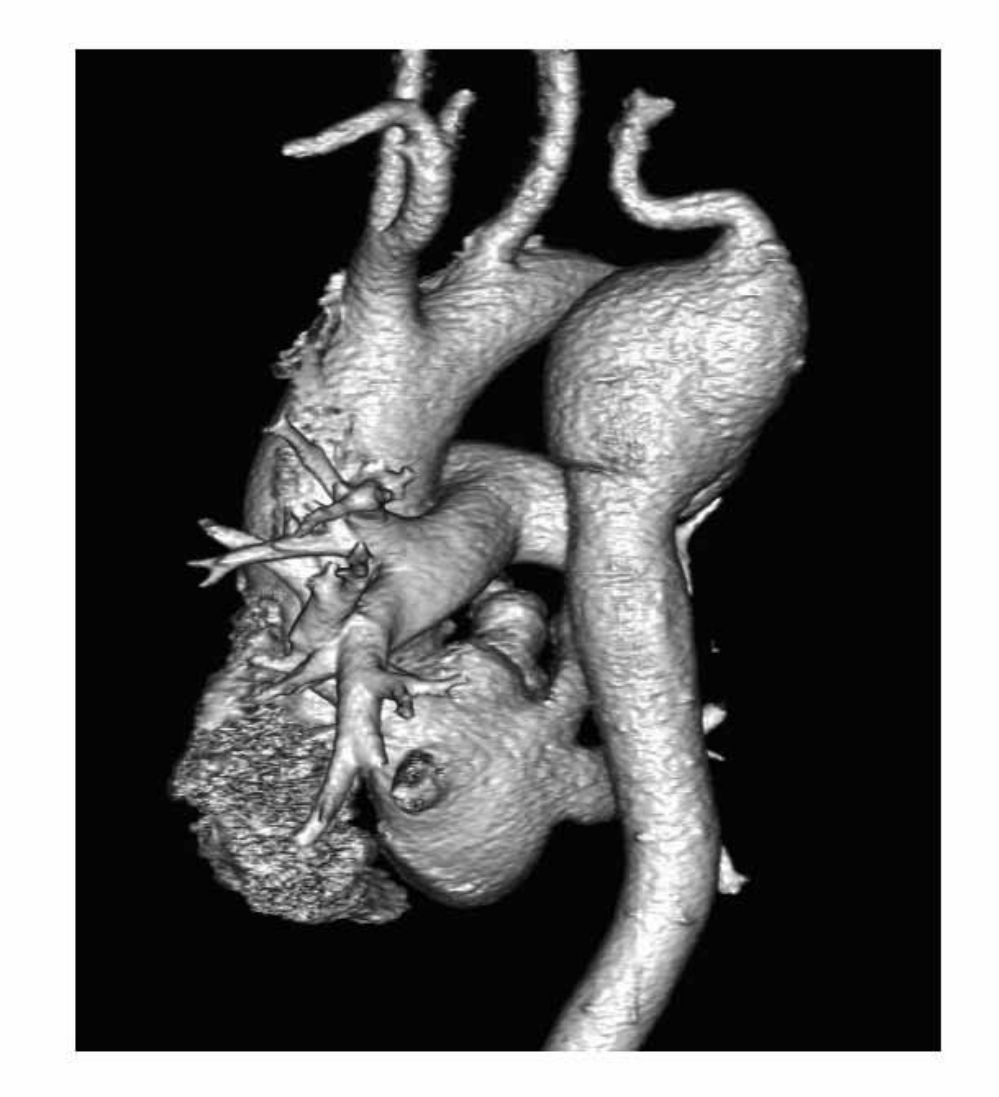


Figure 2. The aorta of a twenty-three-year-old woman with Turner syndrome with no cardiac symptoms, showing an aneurysm of the descending aorta at site of the "kink," and bicuspid aortic valve with ascending aorta aneurysm measuring 4 x 3.5 cm.

These cases clearly illustrate the value of using MRI scans. Medical issues that were not seen on an echo and that may be quite asymptomatic until they dissect or burst can be detected before they become a problem. MRI scans also help in identifying the phenotype and performing genetic analysis.

In regard to considering the embryonic origin of these vascular anomalies, the fourth aortic arch, which supplies the fourth branchial or pharyngeal arch, contributes to the region between the left carotid and the left subclavian (see figure 3). This is where we find the majority of Turner syndrome arch anomalies. We also find them at the origin of the right subclavian artery.

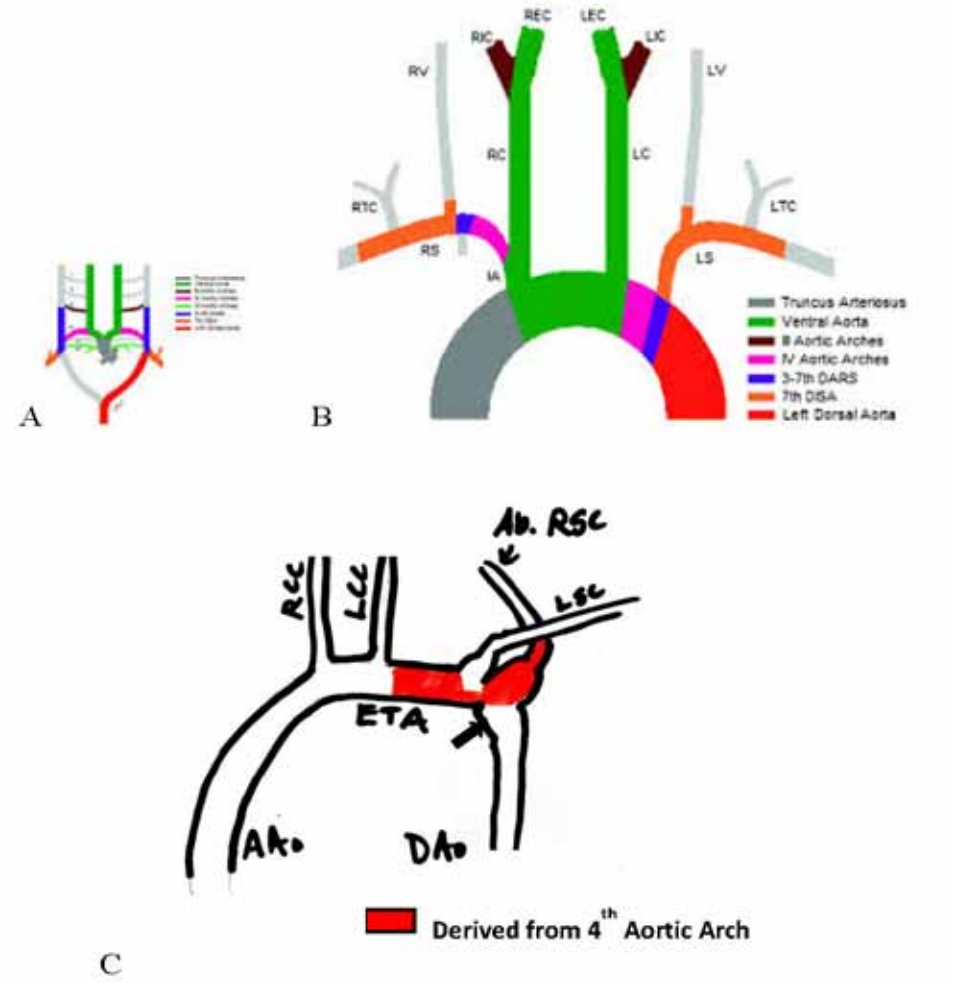


Figure 3. Embryonic origins of the aortic arch. (A) Pharyngeal arch arteries. Neural crest cells migrate to the arches and contribute to the differentiation of smooth muscle cells forming structures of the mature aortic arch. (B) Forming aortic arch. The structures in pink are derived from the 4th embryonic arch. (C) The structures in red are selectively affected in Turner syndrome.

There are several theories about how these anomalies occur. One possibility is an obstruction to the outflow tract during embryonic development, which then leads to defects in valve and left ventricle development. Alternatively, neural crest cells that remodel these fourth aortic arches are also involved in the endocardial cushions of the valve development.

By more precisely defining the phenotype, progress can be made toward establishing the genetic basis for these cardiac features. In a previous article (3), we showed that the valve disease relates to the deletion of the short arm of the X chromosome after p11.4 (see figure 4). It is felt that all the features of the Turner phenotype reside in this part of the X chromosome and its Y homologous genes.

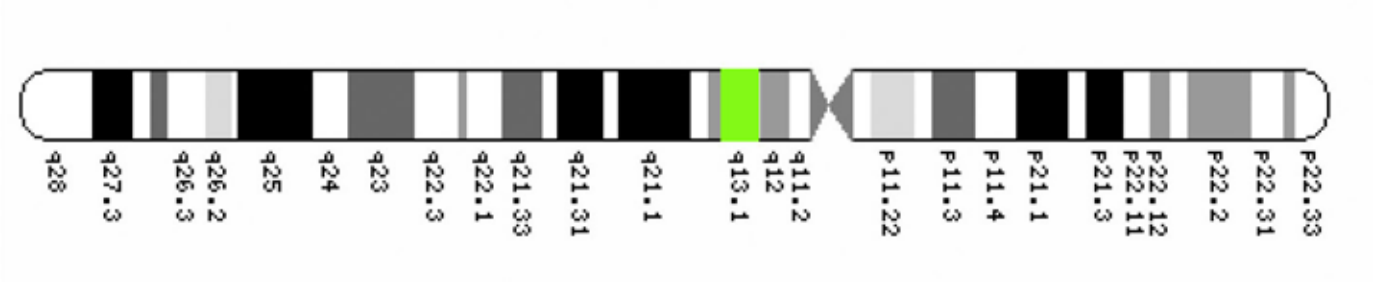


Figure 4. Deletion of the telomeric portion of Xp at p11.4; chromosome X 41,500,000 is sufficient to cause the signature congenital heart disease phenotype. The deleted region includes the pseudoautosomal region and several other genes that escape X inactivation and have Y chromosome homologues (3).

Here we propose that haploinsufficiency for XY homologous genes leads to the phenotypic aspects of short stature and lymphatic developmental problems (see figure 5).

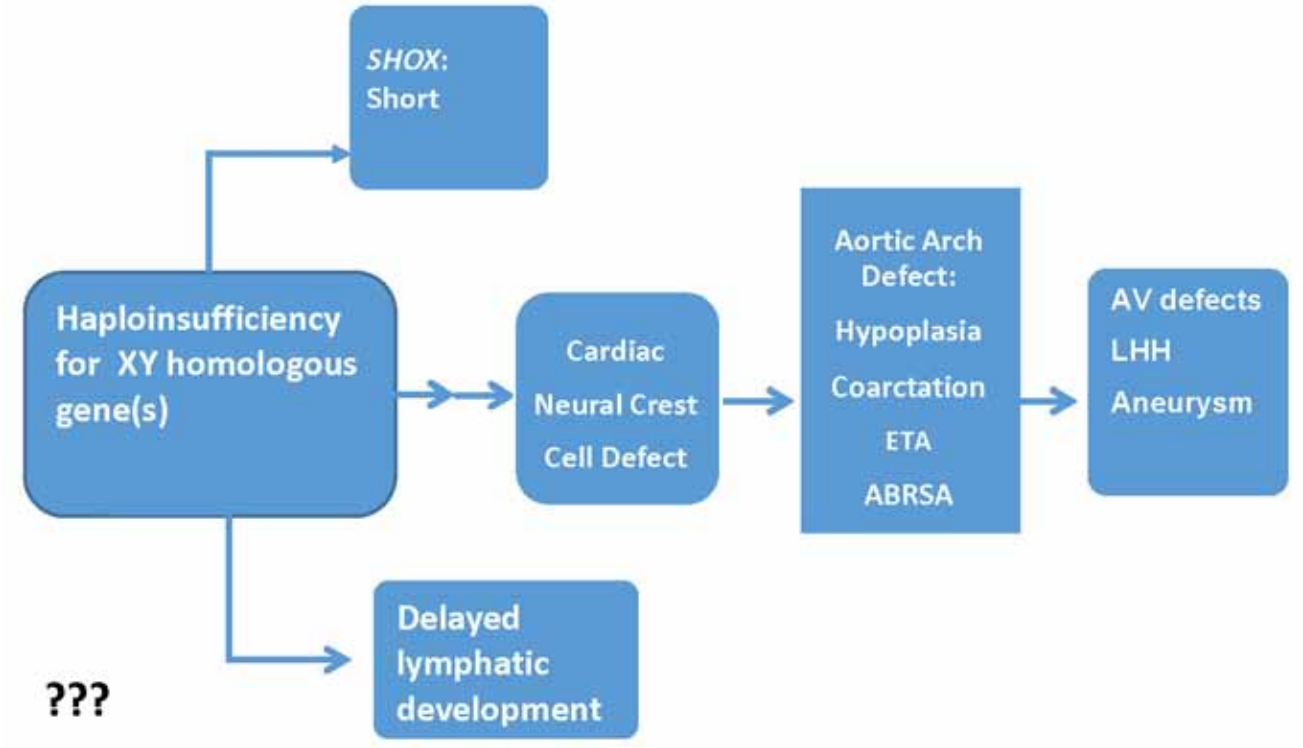


Figure 5. Proposed pathogenesis depicted from XY haploinsufficiency to development of short stature, heart anomalies, and delayed lymphatic development.

It can be further hypothesized that there may be defective migration or function of cardiac neural crest cells, which lead to aortic arch defects, which then lead to aortic valve and left heart hypoplasia and aneurysms. We suggest that the reason males are more prone to these types of left sided diseases is because there's a gene on the Y chromosome that's involved in some of these frequent recombination events necessary for Y chromosome integrity. The more frequent recombination, then, makes it more vulnerable to microdeletions or gene interruptions that would then lead to a higher prevalence of bicuspid valve and coarctation in men (see figure 6).

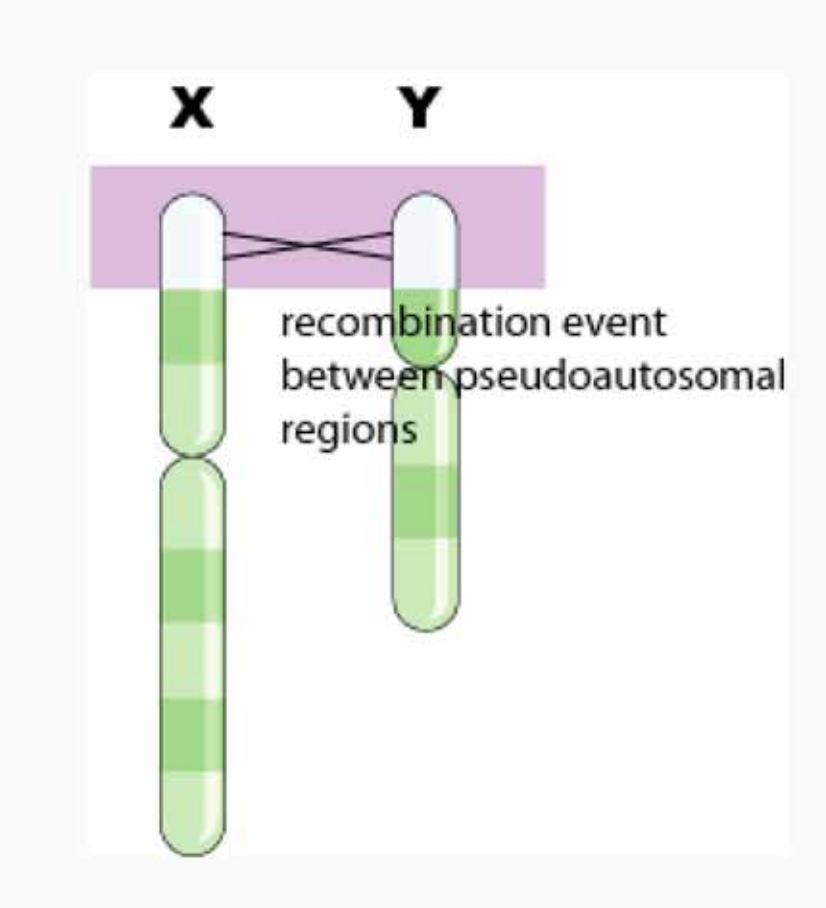


Figure 6. Diagram depicting recombination in the pseudoautosomal region of the X and Y chromosome hypothesized to lead to increased frequency of bicommissural aortic valve in males.

Another consideration is the prevalence of atherosclerosis among women with Turner syndrome. Despite recent attempts to publicize atherosclerosis in women, it is more common in men at a much earlier age, say after forty. This raises the question of whether this may be because men only have a maternally derived X chromosome. We hypothesized that if there's a gene that's selectively expressed from the maternal X, then men would have that gene in all their cells, where women would only have a 50 percent exposure (see figure 7). And if this gene caused some factor that led to coronary disease, then men would be at greater risk.

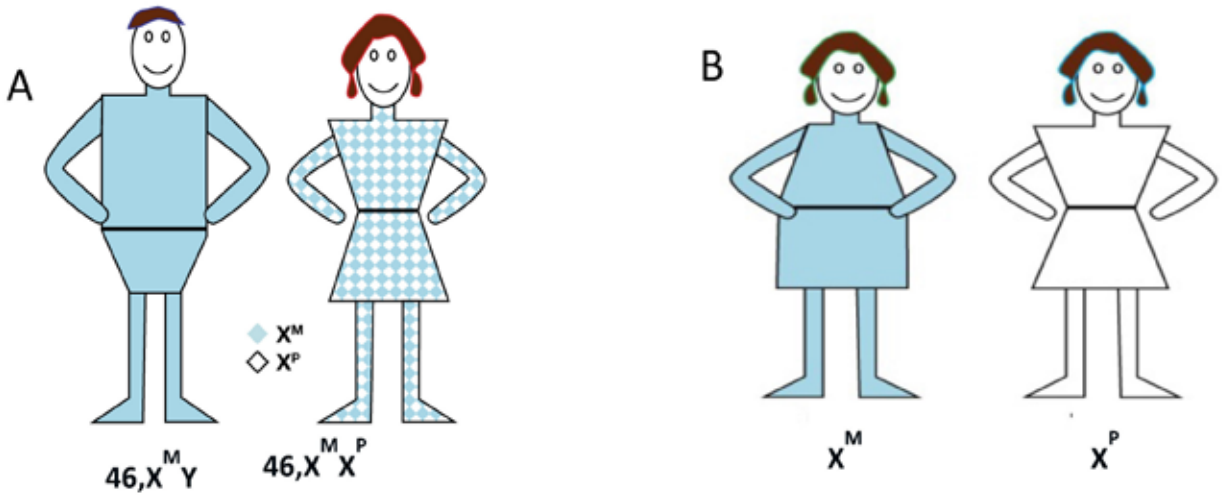


Figure 7. Selective expression of genes on the maternal X (Xm) predisposing to atherosclerosis. (A) Men have the gene in all of their cells, and women in 50 percent. (B) Expression in women with Turner syndrome with the maternal X or paternal X (Xp).

We wanted to study this without the factors related to many other aspects of sexual differences. So we looked at this question in women with Turner syndrome minus Xm or Xp. We looked at abdominal fat, visceral fat, and many other known markers or risk factors for coronary disease. We noted a different body habitus in the women with a single Xp compared to those with Xm (4). (See figure 8.)

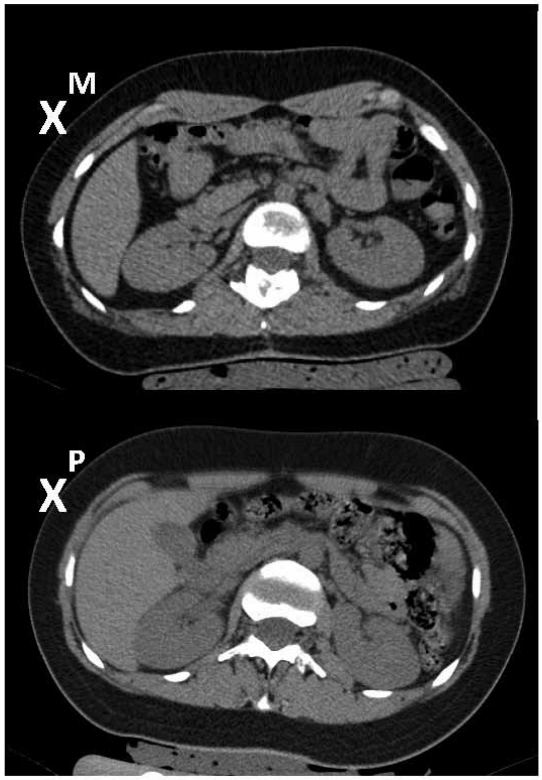
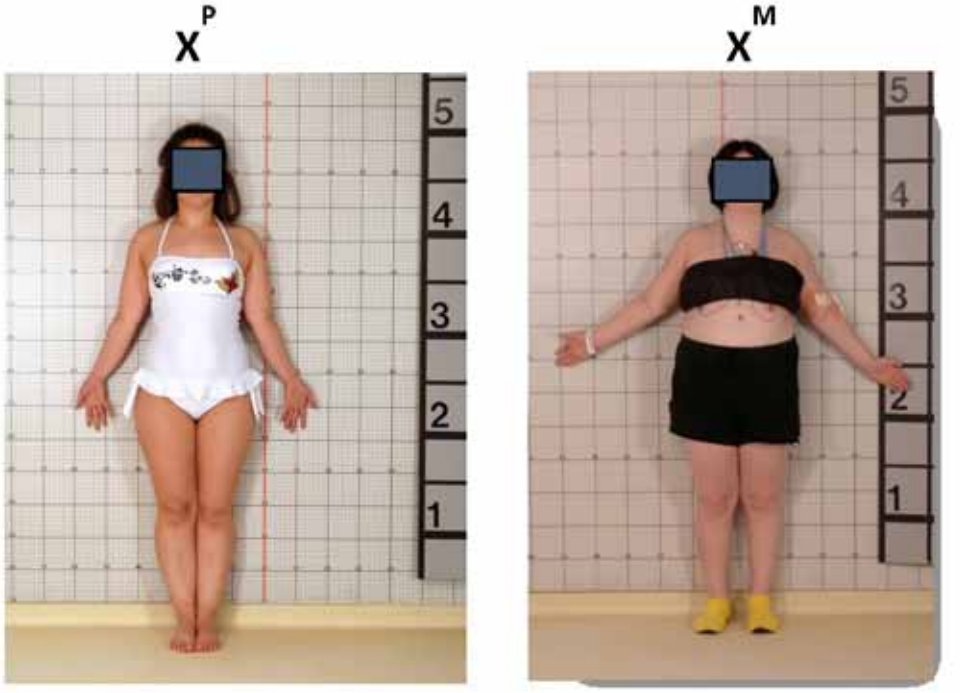


Figure 8. CT scans showing visceral and subcutaneous adipose tissue in women with Turner syndrome with the maternal X (Xm) or the paternal X (Xp).

We also found that the women's BMI and body fat were the same, but the selective location of the fat was different in the two groups. The Xp group had an increased amount of subcutaneous adipose tissue at the thighs or hips, whereas the Xm group had more visceral, ntra abdominal fat, similar to men (see figure 9). Our population was too young to determine whether this could be extrapolated to an association with coronary artery disease.



	X^P	X^M	P
Body Mass Index (kg/m^2)	26 (6)	27 (6)	0.15
Body Fat %	36 (8)	37 (8)	0.55
Visceral Abdominal Fat (mL)	14 (8)	25 (19)	<0.001
Triglycerides (mg/dL)	101 (42)	132 (60)	0.02
Low-density Lipoprotein Cholesterol (mg/dL)	103 (41)	137 (44)	0.005

Figure 9. Women with Turner syndrome who inherit the maternal X (Xm) have increased visceral fat and heart disease risk factors over those with the paternal X (Xp). Data from Van P and others (4) is shown as mean (standard deviation).

After two years, there were then enough participants between the ages of forty and fifty to assess coronary calcium. This was done using a fast, low radiation CT (see figure 10). This is a highly accepted method of quantifying coronary disease as a calcium score. We used this study to look at women with Turner syndrome that were monosomic for Xm and Xp (5). (See figure 11.)

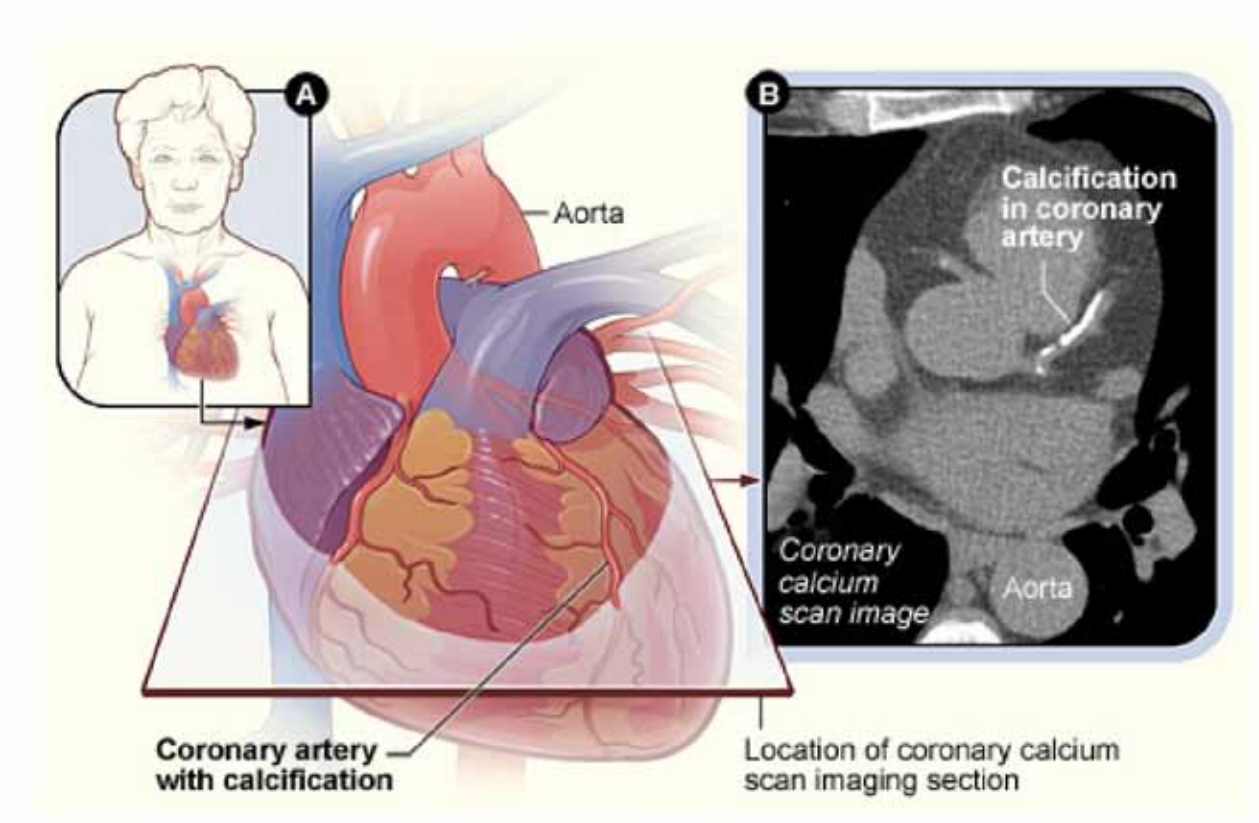


Figure 10. Fast, low radiation CT evaluation of coronary calcium content.

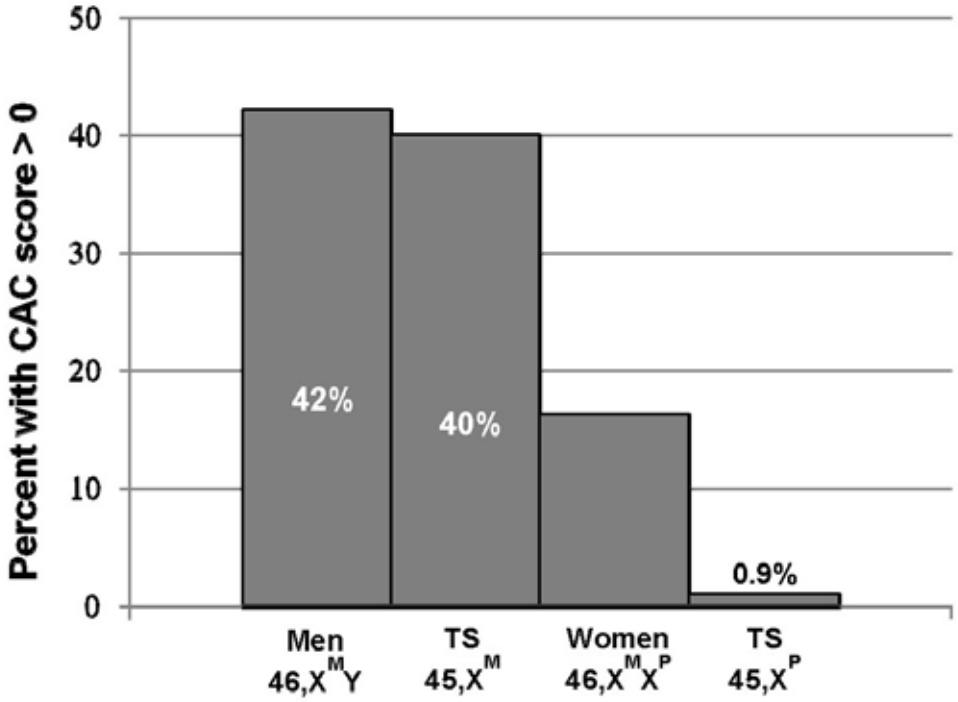


Figure 11. Quantitative comparison of coronary artery calcium (CAC) in the male population (100% Xm), Turner syndrome with Xm (100% Xm), female population (50% Xm/50% Xp), and Turner syndrome with Xp (100% Xp). Data from Abd-Elmoniem KZ and others (5).

The average age in both our Xm and Xp groups was forty-five; the range was from about thirty-five to sixty-five. Compared to the typical coronary score for men with no symptoms provided by the Framingham healthy heart project, which included about a thousand men aged forty to forty-five, we found that the women in our Turner syndrome Xm group had a coronary calcium score equal to those men who had no known coronary disease. A third group of women with normal karyotypes and no symptoms or risk factors volunteered for coronary calcium screening. They had a much lower proportion of coronary calcium than the Turner syndrome Xm group. And quite strikingly, our Turner syndrome Xp group had no calcium to speak of in their coronary. Thus we conclude that the monosomy for an X, maternally derived, chromosome does somehow relate to excessive atherosclerosis at an early age. Epidemiology studies have shown increased morbidity and mortality from coronary disease and cardiovascular disease in general. This suggests that the increase in coronary calcium may shorten a lifespan. A large proportion of older women in the NIH study had coronary artery disease; however, parental studies could not be performed for them.

This concern for atherosclerosis in Turner syndrome patients can be well illustrated by one patient who was not diagnosed until around the age of forty-five. Now sixty, she has short stature, no pubertal development, and many of the features of Turner syndrome. The NIH cardiology team led by Dr. Douglas Rosing evaluated her. They found a difference in blood pressure between her arms, which several people were able to replicate. Her left subclavian artery has a significant stenosis in it. It is an atherosclerotic stenosis creating a 95 percent blockage of her left subclavian (see figure 12). She also has an atherosclerotic dissection of her abdominal aorta. This is shown in this CT cross-section . The dissection interrupted the left renal artery.

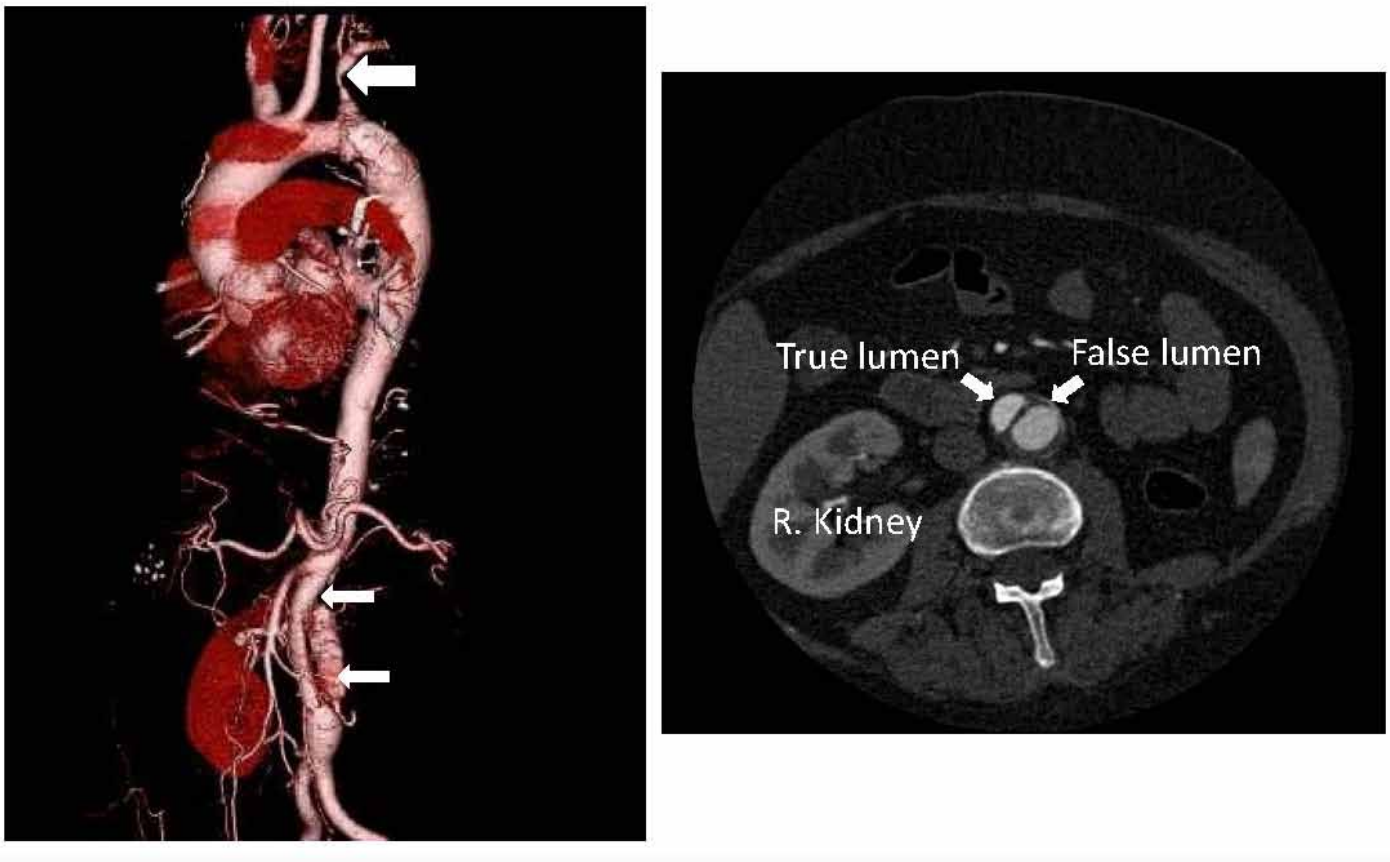


Figure 12. Aortic dissection in a sixty-year-old woman with Turner syndrome with reduced blood pressure in the left arm and back pain.

It is clear that atherosclerosis is a real challenge for Turner syndrome patients, just as congenital heart disease is. In this woman at age sixty, her condition was far more advanced than what we would see in women after many years of long-standing diabetes but similar to men between sixty and seventy.

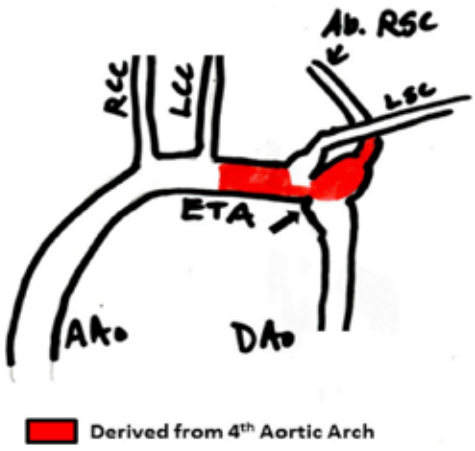
We can conclude that the pattern of segmental defects in the aorta most likely relates to problems with the development of the fourth aortic arch. This may be due to problems with neural crest cells that cause the remodeling of that arch. The nonlethal arch defects are associated with bicuspid aortic valve and are at risk for aneurysm formation. The cardiac phenotype links to the deletion of the terminal portion of the short arm of the X chromosome. There are at least thirty genes in this region that escape X inactivation and have Y homologs. Haploinsufficiency for one or more of these genes could cause the left outflow tract anomalies in Turner syndrome. A mutation or an interruption of genes on the Y chromosome could result in an excess of male linked left ventricular outflow tract problems.

Chapter 11

Genomic Approaches to Understanding Congenital Cardiac Anomalies in Turner Syndrome

Paul Kruszka, MD, and Karin Weiss, MD

Cardiac malformations in Turner syndrome are common, affecting roughly 50 percent of girls and women (1). Bicuspid aortic valve (BAV) and elongation of the transverse arch (see figure 1) are the most frequent anomalies; as yet, a putative gene, or genes, has not been implicated as a cause. In a large cohort of individuals with Turner syndrome, 34 percent were found to have bicuspid aortic valve (2). Dr. Carolyn Bondy and her group at the National Institutes of Health (NIH) showed that in a cohort with p-arm (short-arm) deletions, the most distal break associated with the BAV/COA trait was at cytologic band Xp11.4 and ChrX:41,500,000 (2).



Bondy C. 2015. Turner syndrome. In: Muenke, M., Kruszka, P., Sable, C., Belmont, J. (eds.): Congenital Cardiovascular Anomalies: Molecular Genetics, Principles of Diagnosis and Treatment. Karger Publishing, Basel, Switzerland.

Figure 1. Elongation of the transverse arch ETA is characterized by a flattened and elongated arch between the left common (LCC) and left subclavian (LSC) arteries. A kink in the lesser curvature of the descending aorta (DAo) is located at the common site of aortic coarctation. An aberrant origin of the right subclavian artery (Ab.RSC) is often seen with ETA and is accompanied by a bulbous swelling of the proximal left and right subclavian arteries. The areas colored in red in figure 1 are derived from the fourth pharyngeal arch arteries.

Aortic dissection is the most catastrophic cardiac anomaly in Turner syndrome. It has a hundredfold increase in young and middle-aged women compared to the general population (3). In addition to Turner syndrome, aortic dissection is associated with Marfan syndrome and isolated bicuspid aortic valve. In all three cases (TS, Marfan syndrome, and isolated BAV), histological evaluations show cystic medial necrosis is an intrinsic cause in the dissection of the aortic wall; however, the pathogenicity of aortic dissection in TS is not fully understood and more research is needed (3,4). Unfortunately, the one thing that these conditions do not have in common is that Marfan syndrome has an identified gene and molecular pathway. The molecular underpinnings of aortic dissection in Marfan are well understood. Dietz and others identified the first mutations in the FBN1 gene, which codes for the extracellular matrix protein fibrillin-1 (5). Over the last decade, it has been shown that fibrillin-1 is not only an important microfibrillar structural molecule but also a regulator of TGF-beta (transforming growth factor beta) signaling. In addition to the angiotensin II type 1 receptor blocking effect, the medication used for hypertension, called losartan, also has an inhibiting effect on TGF-beta signaling and is now being studied as treatment to prevent aortic dilatation and dissection in Marfan syndrome. Using Marfan syndrome research as a paradigm for the study of the pathogenesis of thoracic aortic aneurysms, we should be able to get an understanding of the molecular underpinnings of congenital heart disease in Turner syndrome, which should lead to new therapies.

There is a continuum of left outflow tract malformations in Turner syndrome ranging from bicuspid aortic valve to aortic dissection. In a registry of twenty individuals with Turner syndrome and aortic dissection, Carlson and others show that eighteen of the twenty individuals also have bicuspid aortic valve (6). Other studies have shown a statistically significant association between bicuspid aortic valve and aortic dissection.

Congenital heart disease in Turner syndrome occurs on a background of partial or full monosomy X. In 46,XX women, one X chromosome is inactivated and expresses a long noncoding RNA from the gene X-inactive specific transcript (Xist). Xist coats the inactivated chromosome, resulting in histone methylation and deacetylation. But the entire X chromosome is not inactivated. The largest difference in gene expression between 46,XX and 45,X is seen in the pseudoautosomal regions (PAR), where 46,XX women express both alleles of genes and 45,X women are haploinsufficient. The PARs are located telomerically on the short (PAR1) and long (PAR2) arms of the X chromosome and recombine with the respective loci of the Y chromosome during meiosis and mitosis. In addition to the PARs, there are genes that share similarity on the X and Y chromosomes but do not recombine; these genes are also not inactivated, which makes them candidate genes for the Turner syndrome phenotype (7).

Ross and others cataloged the homologous genes on the X and Y chromosomes: twenty-five of these genes are located on PAR1 and five genes are on PAR2 (7). Homology is maintained between the X and Y chromosomes in the PARs by obligatory recombination during male meiosis. The PARs are not subject to X inactivation, but other genes that have functional homologs on the X and Y chromosomes do not recombine. In addition to genes on the PARs, a study by Carrel and Willard showed that about 15 percent of X-linked genes consistently escape X inactivation (8).

A common and popular hypothesis is that the putative genes for congenital heart disease are haploinsufficient and located in the PARs. An early paper supporting this idea reported a fetus at eighteen weeks had aortic coarctation and was missing the PAR1 on the Y chromosome (9). In a similar haploinsufficiency scenario as the PARs, Tagariello and others reported that variations in TBL1Y located on the Y chromosome were associated in the genesis of non-syndromic coarctation of the aorta (10). TBL1Y has a functional homolog on the X chromosome (TBL1X), but TBL1Y and TBL1X do not recombine during meiosis in contrast to PAR genes.

Animal models are an invaluable tool in the study of congenital heart disease, especially the mouse model. One of the difficulties with Turner syndrome research is the absence of a suitable animal model. An XO mouse exists; however, these mice are fertile and they do not have cardiac malformations, so they

present a different phenotype than Turner syndrome (11). When you align the human X chromosome with the mouse X chromosome, the genes on the two chromosomes are conserved until reaching the very proximal area of the pseudoautosomal region (7); thus, the mouse X chromosome does not have an area that aligns to the human X chromosome in the PAR1 region. The failed alignment of the mouse and human proximal chromosomes, combined with the lack of cardiac phenotype in the XO mouse, suggests that PAR and haploinsufficiency are major causes of congenital heart disease and other Turner syndrome phenotypes. Some of these human genes on the PARs have orthologs located on the autosomes of the mouse and zebrafish. Future research should involve animal models that will simulate PAR haploinsufficiency with a knockout of these genes in the mouse and/or zebrafish. With the exception of SHOX, very little is known about the PAR genes, making future research in this area imperative.

There is much to learn about congenital heart disease in Turner syndrome. Hypotheses such as haploinsufficiency, cis and transacting factors, epigenetics, imprinting, and extrinsic nongenetic or environmental factors are well known. The reality is that we know very little about the causes of congenital heart disease in Turner syndrome. Fortunately, there are a number of new genetic tools that will impact research in Turner syndrome.

Many of the new genomic technologies will assist researchers in answering questions about congenital heart disease in individuals with Turner syndrome, and these new genomic technologies are more available, largely due to the decrease in their cost. Chromosomal microarray analysis uses thousands to millions of probes on a chip to hybridize patient DNA; these probes can also analyze for copy number variations (CNVs) and single nucleotide polymorphisms (SNPs). Prakash and others recently released a large study using single-nucleotide polymorphism array genotyping in a cohort of patients with Turner syndrome that supports the effectiveness of microarray genotyping to tackle clinical and research questions in Turner syndrome (12). Microarray technology is limited by the nature of the probes included in the platform and their sensitivity and specificity. Although chromosomal microarray is the most effective tool to evaluate for copy number variations, during the past several years, a number of computational algorithms have been developed to retrieve copy number from next generation sequencing, which will be discussed below.

For many years, DNA has been sequenced using Sanger sequencing technology, so a single stretch of DNA is sequenced at a time. Sanger sequencing, developed in 1977 by Dr. Frederick Sanger, was used to complete the Human Genome Project. Next generation sequencing has been around for almost ten years now, and has become much more widespread due to decreasing costs. This technology involves fragmenting DNA into many small pieces that are cloned and sequenced in parallel, generating millions to billions of reads (massive parallel sequencing). This technology in order of magnitude is faster than Sanger sequencing, but not as accurate.

Another relatively new tool that we have at our disposal is gene editing, especially in the animal model. Multiple techniques using engineered nucleases to act as molecular scissors to cut out and insert genes into precise locations are now available. These tools will give researchers new avenues to create more accurate animal models for Turner syndrome.

Although not as new as gene editing technology, gene expression studies should be employed in research in Turner syndrome. We know that in peripheral blood there are significant differences in gene expression between Turner syndrome and age-matched controls (Bondy, unpublished data). An important consideration in gene expression is that gene expression in cardiac development depends on both temporal and spatial parameters that may not be captured accurately in peripheral blood. But studying human embryonic gene expression is an unlikely scenario.

In conclusion, there is much work to do in cardiac research in Turner syndrome. Fortunately, the last decade has brought new genomic technologies that can now be applied to research in Turner syndrome. The research community looks forward to answers to the questions posed in this chapter.

Section 3

Crossroads of Health Policy and Health Research—Road Map to the Turner Resource Network

Chapter 12

The Pediatric Heart Network: A Model for Conducting Effective Research

Victoria Pemberton, RNC, MS, CCRC

The purpose of this chapter is to outline the steps for creating a research network in which to conduct clinical and observational research. By using the organizational structure of the Pediatric Heart Network (PHN) and other pertinent models as examples, the stakeholders here today will be able to select elements that might facilitate Turner syndrome research via a research network.

The Pediatric Heart Network (PHN) was established in 2001 and is funded by the National Heart, Lung, and Blood Institute (NHLBI) of the NIH. The PHN's mission is to improve health outcomes and quality of life in children, adolescents, and young adults with congenital heart disease, and in those who acquire heart disease during childhood. Before the PHN existed, multicenter pediatric cardiovascular research was exceedingly rare. Many children's hospitals across the country had clinical programs in congenital heart disease and were excellent at caring for patients with congenital or acquired heart disease, but little research had taken place and almost no collaborative research had been done. Up until that time, there were only about forty randomized clinical trials that had ever been conducted in this patient population. Most were single center studies that each enrolled just a few dozen patients. There was a compelling need for a network or collaborative in order to conduct multicenter clinical research. Like Turner syndrome, congenital heart disease and pediatric-acquired heart disease are sufficiently rare that single centers don't typically have an adequate number of patients to conduct trials and obtain meaningful results.

Currently (in 2014), the PHN has evolved into a collaborative of more than thirty clinical-research sites and one data-coordinating center. The red dots in figure 1 indicate the PHN core centers. These are clinical centers that NHLBI funds to support one to two nurse coordinators and a principal investigator (PI). The NHLBI also provides travel funds for these individuals to attend two in-person steering committee meetings each year.

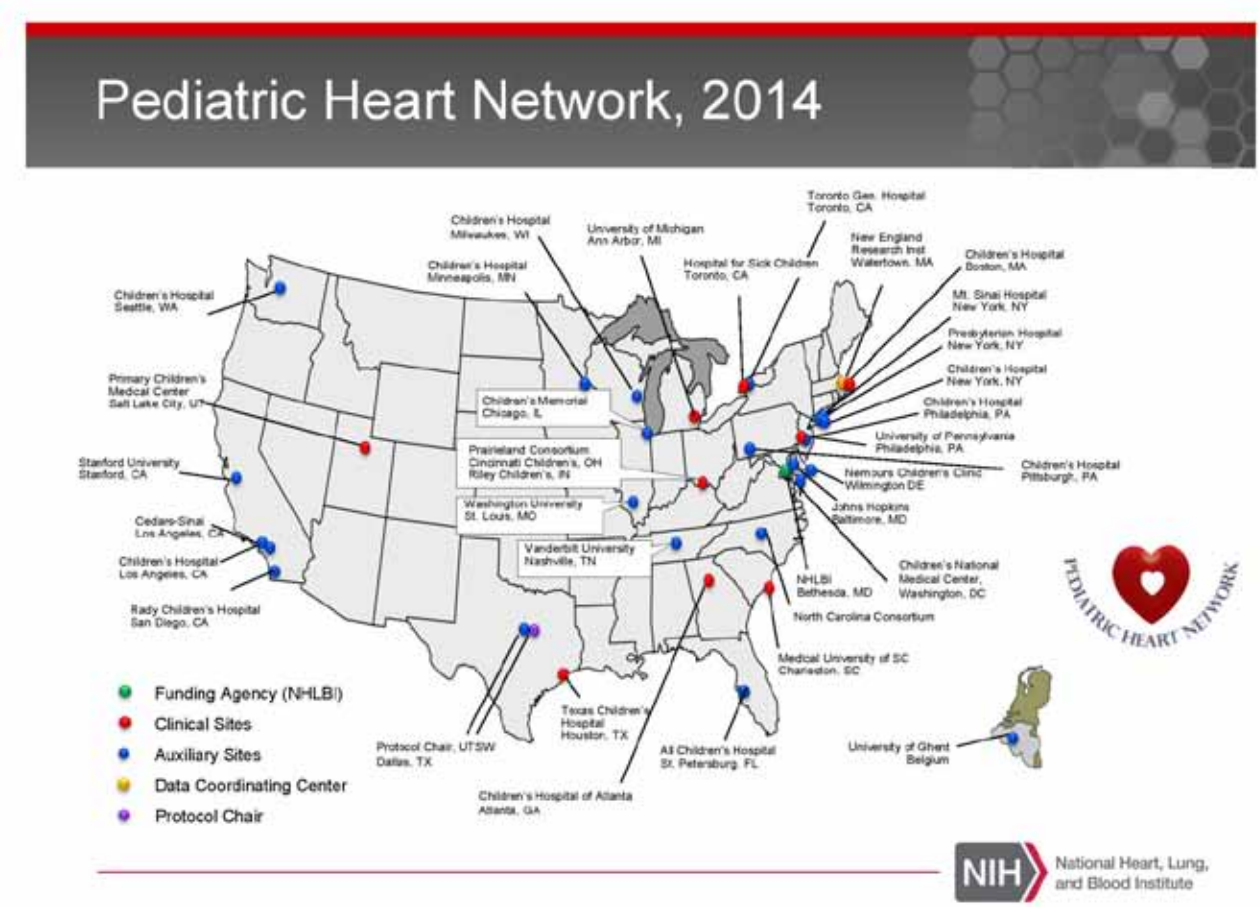


Figure 1: The Pediatric Heart Network clinical centers (2014).

The blue dots represent auxiliary sites. These sites do not receive grant funding to support personnel; instead, they are paid a higher rate than the core centers for each patient enrolled into a study. They contribute to PHN trials by bringing research ideas to the network, enhancing the scientific expertise of the network, and supplementing recruitment. Depending on the needs, up to twenty auxiliary centers may be invited to participate in any given project.

The yellow dot in figure 1 is the PHN data-coordinating center (DCC). The DCC is a critical element within a multicenter collaborative. Its main functions are to support clinical research through trial operations management; data acquisition, management, and analysis; quality assurance and quality control; and subject protection and regulatory guidance.

Figure 2 describes the organizational structure of the PHN. One important component is the independent review boards, which include a protocol review committee (PRC) and a data and safety monitoring board (DSMB). Each board makes recommendations to NHLBI about the scientific importance and feasibility of the proposed study protocols, the quality of the data, and the safety to research participants.

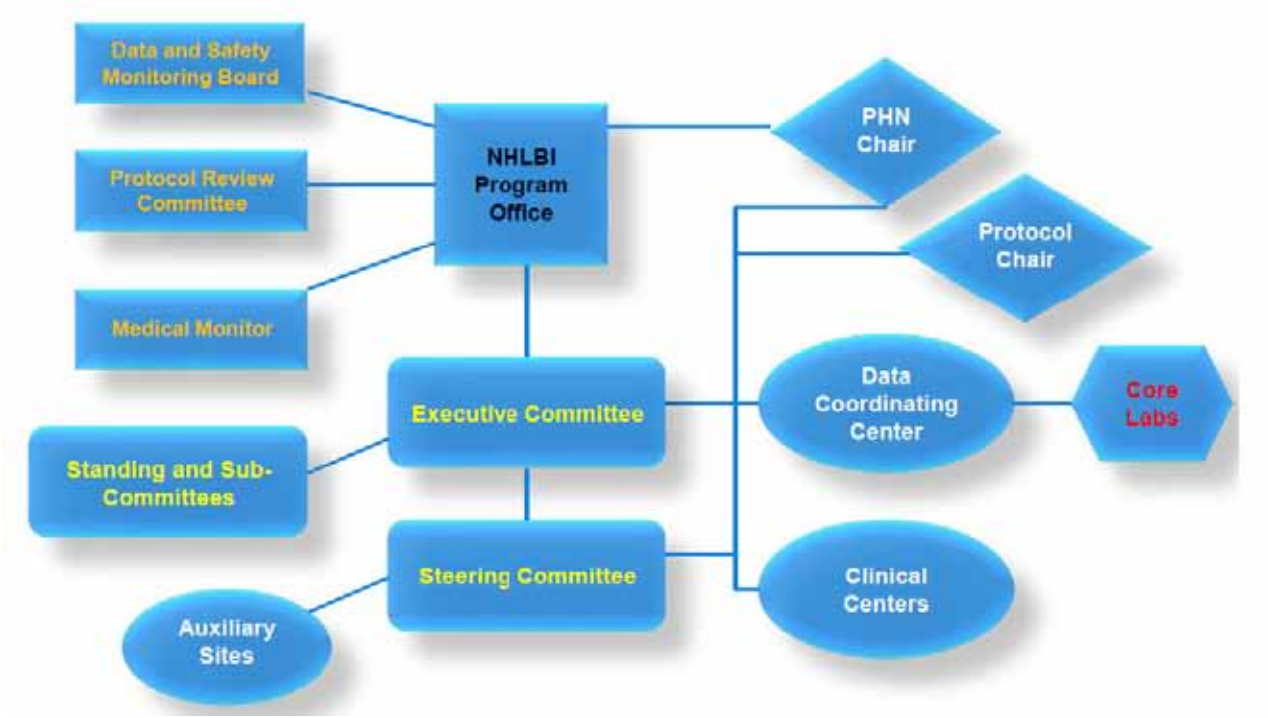


Figure 2: PHN infrastructure.

The other components of the PHN serve as the operational force of the network. The steering committee includes all PIs, nurse coordinators, and data managers from the core and auxiliary sites, as well as staff from the DCC, including the biostatisticians, project directors, and NHLBI program staff. During calls and the twice-a-year, in-person steering committee meetings, there can be in excess of seventy people in attendance. These meetings serve as an opportunity to incubate and discuss potential research concepts, receive updates on previously launched research studies, and hear presentations of study results that will be reported at scientific meetings or published.

The decision-making body of the PHN is a smaller group referred to as the executive committee. Each of the nine core sites and the DCC chooses one representative to serve on it; a few critical committee chairs as well as NHLBI program staff are also included. The responsibilities of the executive committee include setting the overall scientific agenda, determining which studies will be conducted, developing PHN policy and procedures, ensuring compliance with policies, determining which auxiliary sites will be added, reviewing study protocols before PRC and DSMB meetings, and resolving conflicts that affect the conduct of studies or dissemination of results.

This infrastructure serves to enhance operations and communication, and most importantly, to standardize procedures. It is critical in multicenter research to address the issue of variation in practice amongst centers. For example, when one of the PHN's first studies was launched, an echo protocol was developed. Over time, most of the PHN centers adopted that echo protocol as the protocol used in their echo labs for clinical care. This has resulted in a uniform way of procuring the images, and as a side benefit, has allowed us to use data from patients' "clinical" echoes for research purposes, which eliminates research costs and duplication of procedures.

One tremendous benefit of the network infrastructure is that it fosters working relationships, collective decision making, open lines of communication, and shared resources. When sites share expertise, educational materials, and lessons learned, the entire network benefits.

Leveraging the PHN Infrastructure

The PHN has been able to support a number of activities that have advanced the field of pediatric cardiology. We have worked with the US Food and Drug Administration (FDA) on biologics and device studies. The FDA Office of Orphan Products Development

has graciously provided funds to support a number of studies, such as testing drugs that have little or no pediatric data in congenital heart disease, pediatric acquired heart disease, and Marfan syndrome. And the PHN has worked closely with the Marfan Foundation and the Children's Heart Foundation on trials of special interest to these organizations.

In 2013, the PHN began a scholars program. Since then, it has been able to award grant scholarships to thirteen junior investigators. The funds cover the research project as well as travel to PHN steering committee meetings. The opportunity to be involved in these meetings often leads to increased engagement with other PHN activities and ancillary studies. Scholars can sharpen their skills in pediatric cardiology research by attending the annual PHN career day. Scholars receive appropriate mentorship and can network with PHN investigators from other centers who may have similar scientific interests. As a direct result of their scholars awards, three of our scholars have received additional grant funding from other sources, and four had abstracts accepted for a major scientific meeting this year, which resulted in two publications. Training the next generation of investigators is essential to every field, particularly in fields of rare diseases where opportunities may be limited.

Finally, the PHN is quite unique in that it provides funds for nursing research. The PHN nursing research committee has conducted a number of ancillary and pilot studies and published several articles over the last five years. The opportunity afforded by the PHN promotes scientific curiosity and improves retention among the PHN nurse coordinators.

Proposing, Prioritizing, and Conducting Studies in the PHN

During steering committee meetings or calls, individuals are invited to present new study concepts. Ideas can be presented by the PHN core, by the auxiliary sites, or by an investigator not involved in the network. After the presentation, there is a discussion where questions are raised and suggestions are made. Each site then discusses the study among all the investigators and nurses at their site. Here are several important questions that the sites are encouraged to ask: Is this study of scientific importance? Is it feasible? Do we have the resources and the equipoise at our site to conduct this trial? Is this study one that requires the infrastructure of the PHN?

Once the study is approved to move forward, it is then put in the queue. Figure 3 illustrates the studies and trials that the PHN has conducted since its beginning in 2001. An ongoing prioritization process occurs that considers the availability of resources and funds. For example, the PHN may move something up in priority

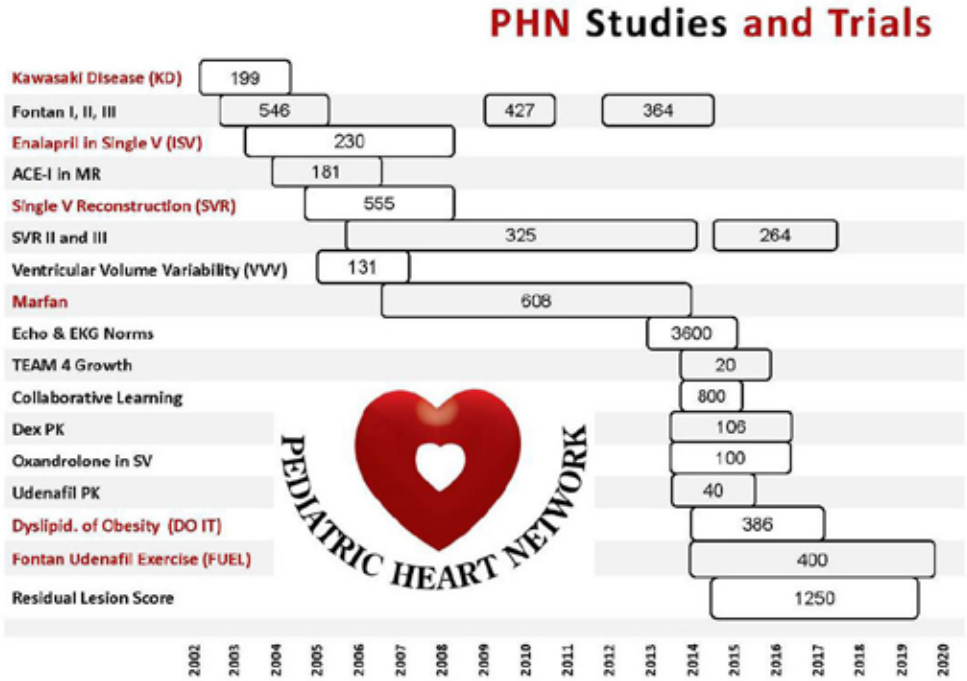


Figure 3: PHN studies (black) and PHN trials (red) with number of subjects enrolled.

because there is a unique opportunity or the timing is right to answer a compelling question. This was the case with the Marfan trial, where a discovery in the animal lab led to a human investigation.

In 2005, Dr. Hal Dietz from Johns Hopkins University approached NHLBI and PHN leadership about conducting a clinical trial in individuals with Marfan syndrome. Dr. Dietz had engineered a mouse model of Marfan syndrome that recapitulated the phenotype in humans, particularly the potentially fatal effects of the disease: aortic dilatation and dissection. Dr. Dietz found that a commonly used antihypertensive agent called losartan not only appeared to prevent aneurysms but also reduced the amount and rate of dilatation in the aortas of the mice. In addition to the mouse data, two children who had been treated in Dr. Dietz's clinic had compelling results with losartan. There was concern that, based on this paucity of data, widespread treatment with losartan might be adopted without appropriate testing. Therefore, the PHN prioritized this study and mobilized resources so that within fourteen months of the concept being presented, the first patient was enrolled.

This marked a new paradigm for the PHN in which stakeholders from the broader community partnered with the PHN to support this trial. For example, not only did we receive funding from the FDA Office of Orphan Products Development, but we were able to secure donations of the study drugs from Merck (losartan) and Teva Canada (atenolol). The PHN also developed a unique partnership with the Marfan Foundation, a patient advocacy group that provided financial, educational, and study participation support. They provided travel scholarships for patients who lived a considerable distance from a study site so the patients could attend study visits, and they also provided other types of financial support for patients without medical insurance to cover the required echocardiograms and tests. But most importantly, the Marfan Foundation helped their community understand the importance of participating in the study to answer the question about whether losartan might be more effective than atenolol, the medication that was being used. They repeatedly emphasized in their newsletters, on their website, and in their patient materials that adopting treatments without evidence could have negative consequences. They also motivated their members to participate in the study. In four years, 608 patients with Marfan syndrome volunteered to be randomly assigned to one of the two drugs. This is a testament to the determination of this very strong community of individuals and their advocates.

While considered the gold standard, randomized clinical trials can take a long time, a lot of resources, and careful planning. The Marfan trial, which took approximately seven years from when the first patient enrolled to the last follow-up visit, commandeered the majority of the PHN's resources, which left little for other projects during this time. Undertaking a randomized clinical trial, such as the Marfan trial, as a first project for a new network requires serious consideration.

But observational studies, registries, or quality improvement projects may be optimal for a newly formed network. The PHN's collaborative learning study (see figure 3) is an example of such a project. Data from one of the PHN core sites suggested that early extubation of infants after cardiac surgery led to improved outcomes and decreased length of stay. Through a series of roundrobin visits, doctors, nurses, and anesthesiologists at a particular site would join Georgia Tech engineers and visit another site to observe their procedures and steps for the early extubation of these infants. A clinical practice guideline (CPG) was then developed by all the sites around early extubation of babies after cardiac surgery. Half of the PHN sites are "active sites," meaning they are implementing the CPG at their sites, and the other half are control sites. Data from patients undergoing specific types of heart repair is being collected to ascertain whether the CPG was implemented effectively at active sites and to assess what the extubation success rates and outcomes are.

Other Considerations and Lessons Learned

After participants, data is the next most important element on which to focus. Having databases or electronic data capture systems will allow a network to collect data from multiple sites with the appropriate edit and audit controls needed. There are some systems that can be purchased off the shelf and others that are "home grown" for particular networks. And some are free of charge to use, like REDCap. Whichever type is selected, it is vital that networks have staff with expertise in managing them, and auditing and analyzing the data.

Regarding publications and authorship, the PHN established a policy in the early years, which has been updated periodically. It permits each site that participates in a given study to nominate one

individual for authorship on a paper, abstract, or presentation. The nurse coordinators are also allowed one nomination, and all authors must adhere to strict guidelines for authorship. For networks and other types of multicenter collaborations, it is important to have a publication policy in place before papers are developed to help diffuse conflicts and questions that might arise otherwise.

The Pediatric Heart Network has learned a lot about our disease, including survival rates, surgical sequelae, neurocognitive outcomes, and patient perceptions of quality of life. More importantly, we have learned a great deal about the "science of research," or how to conduct research more efficiently. We have learned that patients are always harder to find and qualify than we originally thought. We have tried to address this by reviewing the medical records of potential patients and using the actual inclusion/exclusion criteria proposed for the study. We believed that we could define the actual number of patients that were eligible or alter our criteria more appropriately. We now know that it often takes more sites, more time, and more money than originally planned. We have trained a number of productive auxiliary sites to act as additional recruiting centers when we need them, we have become more conservative in our time estimates, and we have improved our budget processes in an effort to finish our projects on time and within budget.

One of the biggest lessons we learned is that our clinical colleagues are not always as fascinated with our research as we are. And they may not have equipoise for the study question or expertise in clinical trial conduct. This can become challenging when studies are conducted in hospital and clinic settings where a wide range of clinical care staff may be overseeing the patient's treatment. Taking the time to educate, communicate, and partner with clinical colleagues during a study will go a long way toward helping them to become invested in the study too.

We have been fortunate to have many partners. Partnerships with patients and their families, foundations, granting agencies, patient advocacy groups, and industry can provide benefits to a study—beyond what the PHN could provide alone. In collaboration with our partners, we have sought ways to "give back" to each group through acknowledgements and participation in joint endeavors where our goals overlap.

The last lesson I'm going to share we learned early in the PHN. The families we were approaching for our studies had very little knowledge of what being in a clinical research study meant. They were uncomfortable with research language and long, complicated consent forms. It can be frightening when a parent hears or reads that they will be "blinded" to the study treatment, and they have little concept of what randomization means.

We began looking for resources to help our families understand more about clinical research and found very little information that was readily available. So NHLBI, in conjunction with other institutes at the NIH and private partners, created a website called Children and Clinical Studies (1). This website highlights a series of videos featuring researchers, parents, and children sharing their clinical research experiences. Parents discuss their struggle to decide whether to enroll their child in a research study or not. Children talk about what happens during their research visits and how they might have missed school or other activities because they were in a study. So, for those networks that will enroll pediatric subjects into some or all of their studies, this website can be used to convey information to parents about children and research.

Alternative Network Models

While the PHN is structured like many other NIH-funded networks, there are other ways to model a network. An example is PECARN (Pediatric Emergency Care Applied Research Network). This network is funded by the Health Resources and Services Administration (HRSA), the federal government agency that improves access to health care services for the medically vulnerable or uninsured. HRSA funds a certain number of clinical sites to execute or conduct research in pediatric emergency care. This funding is limited; it only pays for salary support for a PI and study coordinator. So this model requires that the PIs seek alternate funds from NIH or other grant-making agencies or associations to pay for the costs of the trials themselves, which is different from NIH models where the funds to support the actual trials are built into the network.

Another model for conducting multicenter research is based on the use of electronic medical records (EMR) as the source for data. Large health networks (like Kaiser) are interrogating their data networks to generate

questions and answers. It is an approach that minimizes costs by minimizing data collection from patients and employing data collectors. The Society for Thoracic Surgeons (STS) national database is an example of this. Each participating site pays a membership fee to be in the STS. They enter their data into the database from EMRs and other sources. Once there, sites can review their own data or they can compare their outcomes, or numbers of patients receiving a certain treatment or drug, with the other sites that participate. The national database offers a platform for conducting clinical research out of which more than one hundred publications have emerged.

In summary, as the Turner Syndrome Society considers its particular network, it may benefit from models that have been tried and that have proven successful. Or it may choose to select successful elements from a number of different models to create a hybrid approach unique to the needs of the researchers and the Turner syndrome patient community.

Chapter 13

How to Secure a National Turner Syndrome Program in an Era of Declining Federal Resources

David Cole, PhD, and Cheri Svagerko, MOT, OTR/L, BA

A critical part to implementing the programs that nonprofit organizations develop is getting the money to do the work. In this chapter, we will address how the Turner Resource Network (TRN) can secure funding in an era of declining federal resources. In an effort to propose suggestions for acquiring funds, we will present the challenges of building a resource base. We will also discuss assumptions for consideration, the fundraising status of the primary stakeholder groups, and the perspective on national philanthropic trends. All this will lead up to six final suggestions on how to engage major stakeholders of the TRN to ensure future success.

Establishing a network for the Turner syndrome (TS) community to utilize for research in this era of declining federal resources presents many obstacles, especially in our ongoing negative political climate. We thought a lot about this problem. What do we do when the NIH, especially in inflationadjusted terms, is not providing what it used to provide? So much of taking the idea of a TRN further is resource dependent. How, exactly, is the governing body going to get the money? It begins with addressing the fundamental challenge: securing a funding base. To do this, there are three primary elements that require our attention.

The first element is that the resource base needs to be sufficient to meet longterm research goals. Perhaps this is an obvious point, but one cannot underestimate the amount of funds required to make the TRN productive and to advance it beyond just the building out of the infrastructure. Other authors in this book have made it very clear what is involved in building infrastructure for various network models, and various presenters during the symposium made it very clear how much money it really takes to do just that piece of it. And that doesn't even begin to address how every principal investigator will require assistance to sustain them, so that they are not just forced to rely on investigatorinitiated grants. The investigators will need to seek out grants, but in designing this network, the goal should be to have flexible funds available to top off the investigatorinitiated grants when they fall short. Being able to do this will be vital to the network's long-term success.

This brings us to the second element of the challenge: securing fungible, unrestricted, and sustainable money. The potential research agenda proposed by David Page revealed a high-level plan that is ambitious and risky. We know many funders out there—both at the federal level and in the public foundation environment—are often risk averse. To be able to raise fungible, unrestricted money to feed projects is really important and really expensive. You will need money that is sufficient to meet the longterm goals and money that is sustainable. Unfortunately, funders such as the federal government, various public foundations, and corporations are more fickle than they used to be. The requirements for measuring impact, application acceptance, and repeat funding are higher and harder too. All these things present a challenge, because we want to fish for money in waters where we think we can go back multiple times.

A third element of the challenge to build a resource base concerns getting the best possible return on the dollars and the human resources that have been invested. The organizations that are now doing the principal fundraising for the advocacy, education, and research of Turner syndrome are the Turner Syndrome Society of the United States (TSSUS), the Turner Syndrome

Foundation (TSF), and the Turner Syndrome Global Alliance (TSGA). Each is leanly staffed but still does a lot despite this. An important aspect of moving forward in the weeks and months and years to come is to staff up in such a way that the investment you put into staffing gives you the absolute largest amount of money back. Figuring out the return on investment question is not easy. That is why it is a major challenge to overcome.

In discussing this and putting together a few recommendations at the end, we decided that there are a few assumptions that you should have. First, the outlook for federal funding is grim. Second, monies raised for the TRN should be as unrestricted as possible, which will require looking for money from sources that allow funds to be collected and distributed according to current needs, thus maximizing their effectiveness. Third, the funds will need to be collected in some sort of a center. Whether that's the TSSUS, TSF, and TSGA all working together as a kind of central hub, or whether it's some other body to be determined, someone needs to be able to take in the monies that are flexible and then that someone needs to be able to send the monies back out to be distributed in ways that maximize their effectiveness.

If you assume that the roughly five thousand girls and women who are already members or affiliates of the three primary organizations (TSSUS, TSF, and TSGA) are already being tapped at their maximum giving capacity for respective annual funds, then it's shortsighted to rely on their increased giving potential. It's an ideal world if everyone's giving capacity has been completely tapped out. This leads us to another assumption: each respective annual fund needs to be as productive and efficient as possible. That is, how to ask and ask often of these five thousand or so folks to make sure the maximum amount of support is being realized. If that's not the case right now, a critical action item would be how to address the annual fund piece for each organization. But for the moment, let us assume that that piece is going well.

And then, finally, let's presume that the money required to make this successful is many times what is already being raised annually, probably millions of dollars more than what's being raised. With that in mind, let us take a look at where the primary organizations are right now.

Information taken from 2013 and 2014 IRS 990 forms for TSSUS and TSF reveals that collectively the organizations are generating about $750,000 a year in fundraising and program service revenue, which is quite good considering how leanly staffed these two organizations really are. (TSGA is newly formed and no financial information was available for inclusion.) Additionally, TSSUS and TSF come close to spending what they bring in, and that's a good thing. They collectively spend about $700,000 a year to fund events, walks, conferences, educational programs, and educational tools. From looking at each website and talking to key individuals, it appears that most of what is being done is really focused on building awareness, advocacy, and education for patients and clinicians. This is the kind of work that absolutely needs to be done and should continue, but there are costs and benefits to using time this way.

The benefit is obviously that some money is raised and put toward that very good work just described, thus advancing the missions of awareness, advocacy, and education of Turner syndrome. Each organization is creating wonderful resources for people who want to know more. And that's terrific. But the cost is that some money is being left on the table because personnel is being used to pursue these kinds of activities. Undoubtedly the organizations are consciously pursuing these activities because education, awareness, and advocacy have to come first. While these programs, walks, conferences, local fundraisers, or whatever else they may be, are great because they raise awareness and understanding in a community, they are not the most efficient way to raise money. And they are certainly not the best way to raise big money. That is what is needed: major gifts. Going forward, it is major gifts in the six and seven figure range that will most likely be needed. The model of each organization is good, and it serves each organization's needs right now. But if the desire is to build a TRN model as presented earlier, then the current models with the current level of staffing will not be sufficient.

So, what's going on in the national scene in terms of philanthropy? What's out there in the environment that may give us some clues about where some targets are or about where some pitfalls to avoid are?

Remember, each organization is leanly staffed, so the authority figure, as determined by the chosen TRN model, is going to have to find the shortest route to success and avoid some of the pitfalls common in the current environment. The philanthropic trends began to shift in 2008 or 2009, and since then, corporate grant making has been on the decline. Corporations still write a lot of checks, but not the way they used to. In

fact, corporate grant making has not even approached what it was in 2006, and that's after you take into account adjustments for inflation. Nine years and the environment is still not back to that level. Corporations have cut back, and they've made it harder to get the money, because now, in many cases, grant-seekers do not just talk to the corporate grant maker whose job it is to listen and write the checks, they have to talk to the marketing people, too. That's a lot of conversations and a lot of elbow grease and time invested.

This crucial information needs to be taken into account when thinking about how to use precious human resources to go after big money. It may even be best to not send them after the big money. A better strategy would be to leave the acquisition of corporate grants to principal investigators (PI) who are already out in the network and who may already have established relationships with pharmaceutical companies, biotech companies, or other allied industries.

Additionally, public-facing foundations—those large, highly visible philanthropies that invite proposals and advertise how to pursue their money—are being besieged by a record number of applications. As a result, these foundations are giving out less money to more organizations to make sure that everybody stays afloat; they are not concentrating their bets in a strategic way.

Corporate and foundation grant makers are allocating less money to requests for indirect cost recovery, which forces attention back to unrestricted money. Everyone wants the unrestricted money. The TRN will need this unrestricted money. Building a network with the steps involved and the investments that must be made will be hard money to raise.

Years ago, a corporate foundation may have given 15 to 20 percent for indirect cost recovery. Many of these foundations have reduced that percentage considerably, and there are others—Microsoft, for example—that give virtually nothing for indirect cost recovery and pride themselves on it. The idea is that they provide money for investigations, for direct project expenses. Charities then have to figure out how to get the money to keep the lights on, pay people, and so forth.

Applications themselves have become increasingly burdensome and time consuming. But first you've got to find them, which can be an issue. Foundations have made the application process harder, with more hurdles that have to be overcome, in an effort to stem the tide of applications they are besieged with. Should you get their money, you then have to meet stewardship requirements, which increasingly require documents that show measureable impact, regular communication that shows specific data when the foundation wants to see it, and site visits at the foundation's will. These stewardship requirements will require time and money that hasn't been allocated for these requirements. So they've made the burden on the front end and on the back end heavier than it used to be at a time when the overall number of dollars they're going to give out has decreased. This environment isn't helpful to funding the TRN, given how the primary TS organizations operate.

The best recommendation we have for today's environment is to access the enormous dollars that are being professionally managed that fly "under the radar." Up until two years ago, the largest single philanthropy in this country was the United Way, which is the heir to the community chest tradition. United Way is an enormous enterprise. It gives away billions of dollars. But it's no longer the biggest charitable giver.

The organization that gives out the largest amount of philanthropic money by any one organization in the country is Fidelity Investments. Collectively, these donoradvised funds represent the single largest pool of money available for philanthropic purposes. The folks who manage donoradvised funds, like Charles Schwab and private banks, are taking over the process of helping people with their philanthropy by aggregating those funds, providing recommendations for the funds, and handling the tax implications. And the banks and financial services firms that these folks work at are growing by leaps and bounds. The business at Fidelity Investments grew over 20 percent in 2013 and it already looks pretty mature. So that's where the money is going. This is the current philanthropic environment, and the governing body of the TRN must make an effort to engage in it.

So how is that accomplished? As we've already discussed, the current outlook for trying to raise money from corporations and public-facing foundations is grim. But the needs of the TRN are great. The money the TRN requires needs to be fungible and accessible, and the means by which it is attained should not wear out the staff but should maximize their time and efficiency. Acquiring the needed money is not going to be an easy task; however, we have six suggestions.

The first suggestion is to focus on incremental fundraising expenditures. In other words, spend the money you're not spending now on a major gifts program.

The second suggestion is to reach out to the five thousand or so affiliates of the TSSUS, TSGA, and TSF—and rather than just ask them to host an event for money or to support your advocacy and education programs—ask them to introduce you to anyone in their network who is a philanthropic advisor at a major bank, a private bank, or a multifaceted financial services firm; who is an estate and trust manager; or who is a professional that manages under the radar private foundations. These people manage a team of folks who in turn manage the affairs of thousands of private foundations, private foundations that often represent the interest of wealthy individuals. You'll never hear about any of these foundations. They're hidden, with good reason: they don't want to be bothered.

So the way to find this money is to have the folks at the TSSUS, TSGA, and TSF network their way through their affiliate lists looking for connections into these organizations. Once the connection is made, they ask to present a proposal describing the work the TRN is doing. Managers often rarely know what to do with the money they manage and are looking for places to put it. These managers tend to have more lenient application and reporting expectations too, unlike big public foundations where the giving parameters have been well established and the professional program managers have high expectations.

Our third suggestion is to allow the PIs who are working on the periphery of the network at the various research sites to seek out partners at the NIH, pharmaceutical and biotech companies, and other major foundations to fund their specific project needs. Let them do that work, but they'll need to report back in.

The fourth suggestion we have plays off the previous one. In addition to allowing the PIs to seek out partners, someone in the center of the TRN network needs to develop a centralized moves management stewardship function to support the partners. So if a PI gets a grant from Biogen or the NIH, someone in the center of the TRN needs to know about it, and that same someone needs to be on top of the letters, communications, tax information, and whatever else is necessary to steward that relationship long term so the PI isn't burdened with that responsibility. The PI's job is to provide the necessary content for strong application, but the follow up, if you will, should be handled at the center, which will require the necessary staff.

Another suggestion, our fifth, is to raise the profile of the major gifts philanthropy on the TSSUS, TSF, and TSGA websites. If you don't ask, then you don't get. And right now, what is being asked for on the websites is not much. Each organization needs to make it clear that there's a program with set goals in need of big money.

And then, finally, the sixth suggestion we have is that these three extraordinary organizations—the TSSUS, TSGA, and TSF—need to find some way to work together to maximize their human resources and to reallocate some resources toward this major gift-sourcing function, and they need to do it in a costeffective way. Then, together, the three organizations need to agree to take some of the precious funds saved in cash and cash equivalents, which is approximately $335,000, to hire a couple of development people for a couple of years to assess the function's productivity. These development people will also need to look at the three organizations to see where redundancies exist, specifically in administrative functions and programming functions. These people can also help to find one or two people who can dedicate themselves to major gift fundraising that any TRN model will require.

In conclusion, major funding is fundamental to the plan to establish a successful Turner Resource Network. Relying on traditional funding from federal resources will not support the network's long-term goals, or operational goals. Securing fungible monies from "hidden" sources will likely prove profitable, but will require that a center or authoritative body have comprehensive oversight of every funding initiative. At the core of the success of the TRN will be the cooperation and collaboration of the major stakeholder organizations in the TS community, specifically the Turner Syndrome Society of the United States, the Turner Syndrome Foundation, and the Turner Syndrome Global Alliance. Partnering with one another to advance the TRN and increase productive efficiencies will prove beneficial, as will communicating with their supporters to identify key contacts within the financial environment and creating websites that relay the cooperative goal for TRN success, which requires significant donations.

The tasks and obstacles ahead are many, but in keeping with the vision, passion, and steadfastness of the Turner syndrome stakeholders, each task can be accomplished and each obstacle can be overcome in order to successfully secure the funding needed for the TRN.

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