5-2011

Prevalence of Premature Ovarian Failure in Women with Tuberous Sclerosis

Emily Gabitzsch

PREVALENCE OF PREMATURE OVARIAN FAILURE IN WOMEN WITH TUBEROUS SCLEROSIS COMPLEX

by

Emily K. Gabitzsch, BS

APPROVED:

_____
Michael J. Gambello, MD, PhD
Supervisory Professor

_____
Hope Northrup, MD

_____
Shala Nader-Eftekhari

_____
Mary Kay Koenig, MD

_____
Syed Hashmi, MD, PhD, MPH

_____
Marianna Raia, MS, CGC

APPROVED:

_____
Dean, The University of Texas
Graduate School of Biomedical Sciences at Houston

Prevalence of Premature Ovarian Failure in Women with Tuberous Sclerosis

A THESIS

Presented to the Faculty of

The University of Texas

Health Science Center at Houston

and

The University of Texas

M.D. Anderson Cancer Center

Graduate School of Biomedical Sciences

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Emily Gabitzsch, BS

Houston, TX

May 2011

Acknowledgements

To individuals with Tuberous Sclerosis and women participating in the questionnaire. Without your support in and optimism toward research, this type of work would not be possible.

Dr. Michael J. Gambello, my thesis advisor and mentor for this project. It has been a complete honor to work with you on this project, and I appreciate your authentic enthusiasm and support for my endeavors. I never actually expected to impress you, but you have always had full confidence in every portion of this project. Thank you for guiding me through each step with substance and style. I will miss discussing the project with you!

Drs. Hope Northrup and Mary Kay Koenig, committee members. My formal committee meetings were extremely successful as a result of your attentive and thoughtful input. Thank you for not only being leaders in this field of research and medicine, but in providing excellent suggestions and encouragement for the completion of my thesis project.

Dr. Shala Nader-Eftekhari, committee member. We wouldn't have any data, interpretation, or any questionnaire without your professional expertise and careful consideration of reproductive issues. Thank you for introducing us all to additional issues we hadn't considered.

Dr. Syed Hashmi, committee member and biostatistician. I will always remember the six hours one Friday you devoted to helping me code my data. You have shown enormous dedication and involvement in the education of genetic counseling students, and I appreciate your patient and thorough introduction into statistical tools I hadn't thought I could accomplish on my own.

Marianna Raia, committee member. As a graduate of this program, you always knew exactly how I was (or was not) getting through my thesis and second year. I feel fortunate to have benefitted from your supervision that's extended from clinic to this research project.

The University of Texas Genetic Counseling Program faculty and staff. I can't believe I have actually accomplished what I have and learned so much in what seems like lightning speed. You have all consistently showed more confidence in my abilities than I, and I will always be grateful for the opportunity to train and work with you all.

To my classmates, the only group of 12 girls I know that share the same, nerdy interests as myself. We will only have each other to recount our experiences here and show appropriate emotion to the discussion of cases. Thanks for putting up with my behaviors and disorganization.

To my parents, Kurt and Elizabeth Gabitzsch, and brother, Justin Gabitzsch. I feel extra support and encouragement because of your love. Thank you for setting a block of time in your day to allow me to vent about my stress and difficulties, and suggest helpful ways to manage my time and accomplish this thesis project.

Prevalence of Premature Ovarian Failure in Women with Tuberous Sclerosis

Publication No. _____

Emily K. Gabitzsch, BS

Supervisory Professor: Michael J. Gambello, MD, PhD

Tuberous Sclerosis Complex (TSC) is an autosomal dominant tumor suppressor disorder characterized by hamartomas, or benign growths, in various organ systems. Inactivating mutations in either the *TSC1* or the *TSC2* gene cause most cases of TSC. Recently, the use of ovarian specific conditional knock-out mouse models has demonstrated a crucial role of the TSC genes in ovarian function. Mice with complete deletion of *Tsc1* or *Tsc2* showed accelerated ovarian follicle activation and subsequent premature follicular depletion, consistent with the human condition premature ovarian failure (POF). POF is defined in women as the cessation of menses before the age of 40 and elevated levels of follicle stimulating hormone (FSH). The prevalence of POF is estimated to be 1%, affecting a substantial number of women in the general population. Nonetheless, the etiology of most cases of POF remains unknown. Based on the mouse model results, we hypothesized that the human TSC1 and TSC2 genes are likely to be crucial for ovarian development and function. Moreover, since women with TSC already have one inactivated TSC gene, we further hypothesized that they may show a higher prevalence of POF. To test this hypothesis, we surveyed 1000 women with TSC belonging to the Tuberous Sclerosis Alliance, a national support organization. 182 questionnaires were analyzed for information on menstrual and reproductive function, as well as TSC. This self-reported data revealed 8 women (4.4%) with possible POF, as determined by menstrual history report and additional supportive data. This prevalence is much higher than 1% in the general population. Data from all women suggested other reproductive pathology associated with TSC such as a high rate of miscarriage (41.2%) and menstrual irregularity of any kind (31.2%). These results establish a previously unappreciated effect of TSC on women's reproductive health. Moreover, these data suggest that perturbations in the cellular pathways regulated by the TSC genes may play an important role in reproductive function.

Table of Contents

List of Figures

Page Number

List of Tables

Page Number

Background

Tuberous sclerosis complex (TSC) is an autosomal dominant disease characterized by the formation of hamartomas, or benign growths, in various organs of the body. TSC is estimated to affect 1/6000 – 1/10000 individuals. The disorder is caused by an inherited or de novo mutation in either the *TSC1* or *TSC2* gene. Approximately 30% of cases are inherited from an affected parent, and 70% represent de novo germline mutations. While TSC can affect any organ system, major manifestations often occur in the brain, skin and kidney. Approximately 50% of individuals will develop seizures and many have neuropsychiatric illness and/or behavioral problems. TSC is diagnosed clinically, but molecular testing of *TSC1* and *TSC2* is available for confirmation, accurate recurrence risks, and information for families.

History

The French neurologist Désiré-Magloire Bourneville is credited with naming tuberous sclerosis, or, as he stated in 1880, “tuberous sclerosis of the cerebral convulsions [Bourneville].” Bourneville described several findings in a deceased young woman with a history of some degree of mental retardation, facial angiofibromas, and epilepsy since infancy. Several sclerotic lesions in her brain were noted to be dense and “potato-like,” and Bourneville concluded they were the likely cause of her seizures. He also noted masses in both of her kidneys but believed they were independent of the brain pathology. The eponym Bourneville disease was used well into the 20th century. The term Tuberous Sclerosis Complex is attributed to Sylvan E. Moolten, who in 1942 proposed the name to reflect the heterogeneity of the disorder. He also established “hamartoma” as the term for the pathologic tumors [Moolten].

The genetic etiology of TSC would not be uncovered until the early 1990s, but other important genetic concepts were realized as early as 1910. Kirpicznik documented the presence of TSC in three successive generations of one family [Kirpicznik]. Years later, Gunther and Primrose confirmed the hereditary nature of TSC, and commented that a high mutation rate must be present to account for the number of affected individuals with no family history [Gunther]. Lastly, the term “forme frusta” TSC (from the French word for fluster, or defaced) was used by Schuster to refer to those individuals who met some, but not all of the existing diagnostic criteria. This early recognition of variable expression foreshadowed a difficulty in the recognition and diagnosis of TSC that still exists today; fewer than 40% of affected individuals meet the classic “triad” of seizures, mental retardation, and facial angiofibromas [Kwaiatkowski; Curatolo, 2003].

Early studies of TSC involved patients in mental institutions, and indeed the presence of mental impairment was an initial part of TSC diagnostic classification [Chao]. However, a study of individuals with TSC performed in the late 1960s showed over one third of individuals were of average intelligence, and over 60% had some learning disabilities [Lagos]. This marked phenotypic variability, contributed to multiple revisions of diagnostic criteria [Gomez; Roach, 1998; Roach, 2004]. The current diagnostic criteria for TSC are separated into major and minor criteria. Individuals with two or more minor features or with one major feature alone are considered "Possible TSC." Those with one major feature and one minor feature are "Probable TSC." Finally, the presence of two major features or of one major feature plus two minor features is deemed "Definite TSC." These features are listed in the table below [Roach, 2004].

Major Features	**Minor Features**
Facial angiofibromas or forehead plaque	Multiple randomly distributed dental enamel pits
Nontraumatic ungual or periungual fibromas	Hamartomatous rectal polyps
Three of more hypomelanotic macules	Bone cysts
Connective tissue nevus	Cerebral white matter radial migration lines
Multiple retinal nodular hamartomas	Gingival fibromas
Cortical tuber	Nonrenal hamartoma
Subependymal nodule	Retinal achromic patch
Subependymal giant cell astrocytoma	
Cardiac rhabdomyoma, single or multiple	
Lymphangioleiomyomatosis	
Renal angiomyolipoma	

Manifestations

TSC has the potential to affect any organ system in the body. Descriptions of the most common findings are presented below.

Dermatologic Lesions

Virtually all individuals with TSC have skin lesions that are often clues for the diagnostician. Hypopigmented macules, the most common skin findings, are present in nearly all individuals. These lesions usually have a sharp border, are elliptical, and show improved

visibility under a Wood's light, which is useful for examining individuals with fair skin [Fitzpatrick; Leung]. Importantly, in the absence of prenatal findings, hypopigmented macules are often the first signs of TSC, as they are present at birth. The next most common dermatological lesions are facial angiofibromas, present in approximately 47-90% of affected individuals. These typically do not appear until late childhood and early adolescence [Curatolo]. The appearance of angiofibromas is often psychologically disturbing to patients. Another skin finding, hypopigmented macule (previously "shagreen patch)" is present in 20% - 80% of individuals. It is typically located in the lumbar region or the nape of the neck and often appears with satellite lesions. The skin is usually leathery, and can be dimpled like an orange peel [Webb].

Fibrous facial plaques are slow growing, raised, flesh-colored plaques seen in about 19% of individuals [Jozwiak]. Finally, ungual fibromas occur in 17- 87% of individuals with TSC. These are tumors of the fingernails and toenails and are found more commonly in the latter [Osborne]. The fleshy lesions can occur underneath and/or around the nails.

Neurologic manifestations

The neurologic manifestations of TSC often cause the classic features of intellectual impairment, epilepsy, and behavioral problems. These complications are the most common causes of morbidity and mortality, severely affecting individuals' quality of life. About half of individuals with TSC have normal intellect. Between ten and fifteen percent do not have seizures [O'Callaghan; Sparagana & Roach].

Intracranial lesions include cortical tubers (in 70% of individuals), subependymal nodules (in 90% of individuals), and subependymal giant cell astrocytoma (in 6 -14% of individuals). Tubers are typically usually present at birth and can be numerous [Leung]. Subependymal nodules are lesions lining the lateral ventricular system of the cerebral hemispheres. The lesions are usually multiple and small, and rarely cause clinical problems [Leung; Roach, 2004].

Subependymal giant cell astrocytomas (SEGAs) are benign tumors located in the lateral ventricles near foramen of Monro. It is hypothesized that they develop from sub-ependymal nodules, and indeed, the histology of both lesions are indistinguishable. However, they can be visualized if they sustain significant growth. There is a risk for SEGAs to lead to an obstruction of CSF outflow and become symptomatic. Prior to improvements in imaging and guidelines for routine screening, patients often presented with specific symptoms of acute hydrocephalus

such as headache, emesis, and altered visual and/or status. Current recommendations are screening every two years in individuals over the age of 21 [Campen; Leung; Sparagana].

Infantile spasms, partial motor and generalized tonic clonic seizures are present in 80-90% of individuals [Sparagana; Kandt, 2003]. Seizures are typically managed with epileptic medications. For some, surgical resection allows a favorable long-term outcome [Romanelli].

Consistent with other features of TSC, there is a spectrum of severity in intellectual dysfunction and behavioral disturbances. Approximately 50% individuals have intellectual disability [Kandt, 2003]. Most, but not all mentally retarded children with TSC have seizures. Conversely, many patients with TSC have seizures but not mental retardation [Leung; Sparagana; O'Callaghan]. In general, an earlier onset of seizures, particularly infantile spasms, confers a greater risk of intellectual disability [Prather]. Of note, earlier treatment is associated with a better prognosis for learning development and seizure termination. One small study found a seizure free status in 50%, normal or borderline mental development in 30%, and no mental retardation in ten children with an early diagnosis treated with vigabatrin followed long-term [Bombaradiere].

There are often specific learning difficulties in children with TSC. It is estimated that 25 - 50% of children with TSC have a diagnosis within autism spectrum disorder [Northrup]. Additionally, frequent behavioral disorders include attention deficit disorder, aggressive and/or self-injurious behavior, hyperactivity, and sleep problems [Curatolo; Prather]. These may occur in combination or as isolated problems [Leung; Roach].

Renal manifestations

The renal manifestations of TSC are the second most common cause of early death [O'Hagan]. Angiomyolipoma (AML), the most common renal finding, is present in approximately 70% of individuals. AMLs are often bilateral and progressively increase in size and number. As the name implies, these growths are comprised of blood vessels, smooth muscle cells, and adipose tissue [Henske]. Some degree of gender predilection exists in that females are affected 3 to 4 times more often than males [Leung].

The second most common renal lesions are cysts, affecting approximately 50% of individuals [Dixon]. Similar to AMLs, these may increase in size, and are usually numerous. Cysts over 4 cm in diameter are often symptomatic and visible on CT scan [Roach, 2004; Leung]. Individuals with a contiguous gene deletion involving *TSC2* and *PKD1*, the gene responsible for autosomal dominant polycystic kidney disease (ADPKD), will show symptoms

of ADPKD such as hypertension, flank pain, and pyelonephritis, and are at increased risk for end stage renal disease [Franz].

Lastly, renal cell carcinoma occurs in less than 2-3% of individuals, at an average age of 28 [Kwiatkowski, 2005; Patel]. The growth rate of carcinoma is much slower in TSC [Leung].

Ophthalmologic Abnormalities

Up to 75% of individuals with TSC have ocular findings. The most common finding is retinal hamartoma, present in 40-50% of individuals. One-third of these patients will have bilateral lesions [Leung].Lesions are best identified with pupil dilation and ophthalmoscopy. The majority of lesions are asymptomatic, though they could lead to visual impairment if large enough [Leung].

Cardiac Lesions

The most common cardiac manifestation in TSC is cardiac rhabdomyoma, present in 66% of newborns. The maximum growth period occurs at 22-26 weeks gestation and thus is often visualized in the third trimester of pregnancy. After infancy, these lesions are usually asymptomatic and gradually disappear over time. Rarely, they result in outflow obstruction (requiring surgery), valvular dysfunction, or arrhythmia [Leung].

Pulmonary Disease

In TSC, overall pulmonary dysfunction is infrequent. However, the most common finding, lymphangioleiomyomatosis (LAM), may develop in up to 39% of women with TSC, typically at ages 20-40 [Moss; Roach, 2004]. LAM is thus a well-established gender-specific manifestation of TSC and mutations in TSC have been found in individuals with sporadic LAM [Carsillo]. Common symptoms of pulmonary disease include cough, dyspnea, hemoptysis, and pneumothorax [Roach, 2004]. Disease can progress to the need for oxygen therapy and/or lung transplantation.

Less commonly affected systems

A lesser known vascular manifestation of TSC involves aneurysm in the descending aorta, primarily in the abdominal aorta [Cao]. Though rare, they occur more commonly in children under the age of 5 years (the youngest reported at 4.5 months) and impart a risk for premature death. Molecular correlation has identified at least one patient with a *TSC2* mutation; subsequent work in Tsc2 haploinsufficient mouse models demonstrated proliferation of smooth

muscle cells and reduced expression of contractile proteins [Cao]. The naturally occurring "Eker" rat harbors a *Tsc2* mutation and can develop pituitary adenomas, uterine leiomyomas, splenic hemangiomas, lesions in the brain resembling SENs, cortical tubers, and renal tumors [Wilson].

Also unappreciated is the extent of gender predilection in specific manifestations. AMLs are more common in women with TSC in general, but also in women with TSC-associated LAM compared to those with sporadic LAM. Estrogen and progesterone are thought to be important in the development of these lesions. Additional effects of sex hormones or reproductive manifestations in TSC are poorly understood. **Perhaps further examination of other possible organ manifestations will lead to the identification of newly appreciated aspects of TSC.**

Management

The severity of TSC is quite variable, and prognosis is based on each individual. As there is no cure for TSC, management and surveillance are dependent on the symptoms present and function to improve the patient's quality of life. Routine evaluations include EEG to manage seizures, cranial CT/MRI, renal ultrasonography and/or CT/MRI, echocardiography when cardiac symptoms are present, behavioral evaluations, and chest CT if pulmonary symptoms are present [Leung]. Recommended surveillance includes:

Individual	Surveillance	Frequency
Children/adolescents	Cranial CT / MRI	1-3 years
Individuals with no previously identified renal lesions	Renal ultrasound	1-3 years
Individuals with AML <3.5-4.0 cm	Renal ultrasound	Semiannually
Large / numerous renal tumors	Renal CT/MRI	As needed
Seizure management	EEG	As needed
If cardiac symptoms exist	Echocardiography	As needed
If pulmonary symptoms exist	Chest CT	As needed
Children entering school / in response to concerns	Neurodevelopmental evaluations	As needed
As visual loss is uncommon, routine ophthalmologic evaluations are unnecessary for the majority individuals with retinal lesions		
Routine dermatological evaluations are unnecessary for most individuals.		
Source: GeneClinics		

Inheritance

TSC is an autosomal dominant condition. Affected individuals have a 50% risk with each pregnancy to have an affected child. Approximately one third of children diagnosed with TSC have an affected parent while a majority (approximately two thirds) occur as a result of a *de novo* mutation [Sancak; Rose]. TSC-causing mutations are fully penetrant, though TSC shows marked variable expressivity within families and among individuals. Thus it is recommended that the parents of a child with no family history of TSC be thoroughly evaluated. Some individuals often have a very mild phenotype and/or could have eluded recognition from a health care provider. The recurrence risk for parents with an affected child but no apparent family history is complicated by somatic mosaicism [Verhoef].

Molecular Genetics

The *TSC1* gene is located on the long arm of chromosome 9 (9q34) [van Slegtenhorst]. It encodes the protein hamartin. Almost all *TSC1* mutations are truncating. Over half are small deletions and insertions, and 40% are nonsense mutations. Splice site mutations have also been described; large deletions and missense mutations are rare [LOVD, TSC1]. Recently, deletions in the promoter of *TSC1* were identified by MLPA [Ouweland]. The *TSC2* gene is located on the short arm of chromosome 16 (16p13.3) and encodes for the protein tuberin [European Consortium]. The majority of *TSC2* mutations (64%) are small deletions and insertions, splice site mutations, and nonsense mutations. Approximately 28% are missense mutations, and the remaining 8% are due to large deletions or rearrangements; 3.7% involve the PKD1 gene [LOVD, TSC2].

Approximately 20% of mutations identified occur in *TSC1* and 60% in *TSC2* [Crino]. The remaining 20% of individuals have no identifiable mutations. There is a reportedly high rate of somatic mosaicism in TSC, estimated between 10% and 25% [Verhoef]. However, a recent study used deep sequencing analysis to identify mutations in affected individuals with possible low-level mosaicism and concluded the rate of somatic mosaicism may be overestimated and actually as low as 6% [Qin].

DNA testing is available clinically, but is not necessary for a diagnosis of TSC. Studies have demonstrated an overall mutation detection rate as high as 85% in patients with a clinical diagnosis [Sancak; Kwiatowski, 2010]. The majority of mutations in *TSC1* can be detected through sequence analysis. In general, the overall detection rate of *TSC1* mutations is 30% and 15% in familial and simplex cases, respectively. Sequence analysis of *TSC2* has a detection rate of approximately 50% in familial cases and 60-70% in simplex cases.

Deletion/duplication analysis comprises an additional 0.5% of familial cases, and 5% of simplex cases [Northrup]. The detection of large deletions requires additional techniques, such as Southern analysis or array comparative genomic hybridization (CGH).

Several studies have shown genotype and phenotype correlations [Au; Sancak]. *TSC2* mutations are known to result in a more severe phenotype than *TSC1* mutations and are more common in individuals with *de novo* mutations. Familial *TSC2* mutations and individuals with *de novo TSC1* mutations have a milder phenotype. Lastly, individuals for which no mutation was identified also had milder phenotypes. In spite of these genotype-phenotype correlations, mutational analysis should not currently be used for prognostic prediction [Jansen].

Pathogenicity

Hamartin and tuberin function as tumor suppressors. The pathogenesis of TSC involves both the "two hit hypothesis" as well as haploinsufficiency. The former involves a loss of heterozygosity (LOH). Complete loss of either *TSC1* or *TSC2* leads to a cellular loss of tumor suppression and unregulated cell cycle progression, resulting in uncontrolled growth and the development of hamartomas [Castro; Jozwiak]. A somatic "second-hit" mutation has been best described in renal lesions, and LOH has also been observed in advanced stages of murine renal lesion progression [Osborne Wilson]. The TSC genes have also been implicated in individuals with sporadic lesions. In an analysis of AMLs taken from women with sporadic LAM, TSC2 LOH was detected in six of seven AMLs [Niida].

Haploinsufficiency may be another explanation for the developmental of lesions. Observations of renal tubules in mouse lesions have indicated a loss of heterozygosity (LOH) in only about 20% of lesions, suggesting that haploinsufficiency may be sufficient to lead to their development [Wilson]. As LOH is seen less frequently in cortical lesions, some have proposed haploinsufficiency to explain the formation of some cortical lesions.

Hamartin and Tuberin form a biochemical complex that acts as a major regulator of cap-dependent translation in response to growth factors, energy status, oxygen tension and mitogenic signals [Rosner]. This translational inhibition occurs at the level of the mTORC1 kinase, a key enzyme that controls the activation of multiple proteins of the translational apparatus. The TSC complex inhibits mTORC1 through the GTP-ase activating domain of tuberin on the RHEB protein, preventing mTORC1 mediated translation and cellular growth (Figure 1) [Tee; Castro; Wang].

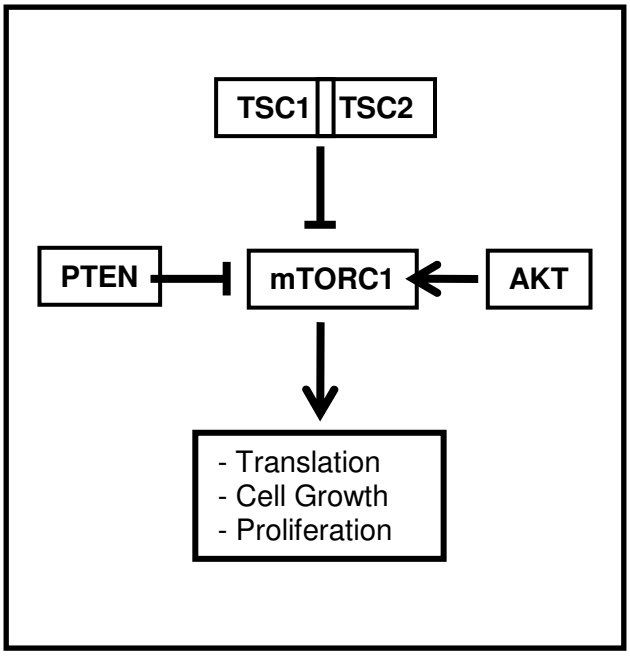


Figure 1. TSC and mTORC1. The TSC1/2 complex forms a heterodimer and inhibits mTORC1 (bar), down-regulating cellular translation, cell growth, and proliferation. Separately, mTORC1 is also inhibited by the action of PTEN. In contrast, AKT enhances mTORC1 activity (arrow).

Separately, the TSC complex directly binds a second enzyme, mTORc2. This results in the phosphorylation and activation of Akt [Tee; Castro]. As Akt is an oncogene implicated in cancer formation, promoting such activity is a seemingly curious role for tumor suppressor genes [Vivanco]. It is plausible that mutations in the TSC genes could accelerate tumor growth through a loss of negative regulation on mTORc1, while a simultaneous loss of mTORc2 activity may attenuate Akt and could prevent hamartomas from becoming malignant [Huang]. Of note, mTORC1 is rapamycin-sensitive and responds to nutrients and energy inputs, and mTORC2 is rapamycin-insensitive [Dann; Huang]. The potential for effective symptom alleviation in TSC patients with rapamycin treatment is being explored [Koenig].

Mouse Models

Any organ system could potentially be affected by TSC. The use of conditional mouse models has been important for the study of the roles of the TSC genes in TSC pathogenesis and normal organ function [reviewed in Kwiatkowski, 2010]. Adkihari, et al., have examined the role of *Tsc1* and *Tsc2* in the mouse ovary, an organ not thought to be affected in humans

with TSC. They created mice devoid of either *Tsc1* or *Tsc2* exclusively in the ovaries [Adkihari, 2009, 2010]. These ovarian –specific knockout mice demonstrated premature activation of primordial follicles and subsequent depletion of the ovarian reserve in young adulthood, leading to infertility. In essence, these animals experienced the equivalent of the disorder termed in humans as premature ovarian failure (POF).

These Tsc1 and *Tsc2* ovarian specific knockout mice also showed sub-fertility; each produced an average of 2 litters, significantly less than control mice with normal ovarian TSC expression. The mice became infertile in young adulthood. Of note, despite accelerated activation of primordial follicles, the size of the litters was unaffected.

The increase in follicular activation from loss of TSC function was associated with increased mTORC1 activity, as demonstrated by increased phosphorylation of two downstream substrates, p70 S6 kinase 1 (S6K1) and ribosomal protein S6 (rpS6). Furthermore, treatment with the mTORC1 inhibitor, rapamycin, reversed the over-activation of primordial follicles in knockout mice. Another ovarian specific knockout mouse model further supports a crucial role for mTORC1 in ovarian biology. In mice lacking the expression of *Pten*, a suppressor of mTORC1 upstream of the TSC complex (Fig 1), the entire primordial follicle pool was activated and depleted in early adulthood, features of murine POF consistent with the *Tsc1* and *Tsc1* ovarian specific knockouts. These results indicate elevated mTORC1 activity is the driving force of follicular activation, and that proper suppression from the TSC heterodimer or other suppressors of mTORC1 activity is necessary to regulate appropriate activation, maintaining dormancy of the follicular pool and preserving reproductive function. These animal studies suggest that abnormal mTORC1 regulation might contribute to human POF, a condition which remains largely idiopathic.

Premature Ovarian Failure (POF)

The studies discussed above indicate a crucial function of the TSC genes, and mTORC1 regulation, in murine ovarian function, but the extent to which human ovaries may be affected from mutations in *TSC1* or *TSC2* is unknown. The authors concluded the knockout mice underwent the phenotypic equivalent of POF. In humans, POF is defined as the cessation of menses in a woman under the age of 40 and displays a clear disruption in the normal human reproductive lifespan [Rebar, 2008]. Although widely used in the literature, the term POF may be inaccurate, as it indicates a finality of menstrual functioning and fertility that is not consistently observed. Other terms such as premature ovarian insufficiency, or POI, have

been suggested, to account for a decline in ovarian reserve, and irregularities in menstrual functioning concomitant with rising levels of FSH and reproductive symptoms such as hot flashes [Rebar]. For the purposes of this reading, the term POF will be used throughout as a term to refer to declining ovarian reserve, with the caveat that POI may indeed be more representative of the menstrual issues subsequently discussed, particularly in the presence of self-reported data and limited facts obtained in this study.

Ovarian function

The progression of a woman's reproductive function begins prenatally, when millions of follicles develop and are arrested at meiosis I in the developing gonads of the fetus [Altchek]. After reaching a maximal number of about 7 million at approximately 28 weeks, a decline of follicles begins. At birth, 1-2 million follicles remain, declining further to approximately 200-300 thousand at menarche, less than 10% of the original supply. After menarche, a highly regulated, cyclic process of ovulation is observed. At the age of 40, most women have about 8 thousand follicles remaining [Altchek]. This process continues until menopause, occurring at an average age of 51 years.

Beyond the well described feedback mechanisms involving the hypothalamus, pituitary gland, and ovaries, the oocyte itself governs a significant portion of ovulation in response to highly controlled regulation of activation and development. However, as evidenced by the previously mentioned mouse knockout studies, the mechanisms that govern the growth and recruitment of follicles are poorly understood. The maintenance of dormancy in the ovaries is required for the preservation of the follicular reserve; similarly, appropriate activation ensures proper ovarian function, allows for pregnancy, optimal hormonal regulation, and ultimately, ovarian senescence at menopause.

In each menstrual cycle, only one or two eggs are released during ovulation for the approximately 40 years of reproductive lifespan. The majority of the remaining oocytes do not reach a mature state, or are "recycled." Thus, one can actually consider atresia the norm, and ovulation a rarity. The rate of follicular atresia appears to increase with age. However, it is not known whether increasing numbers of follicles undergo atresia prior to activation and growth, accelerating the decline in follicular reserve, or if increasing numbers of follicles enter the growth phase and thus undergo degradation in the absence of ovulation [Hansen]. Previously the rate of follicular decline was thought to increase dramatically after age 38, when approximately 25,000 follicles remain [Faddy]. This parallels the decline of fertility, which peaks in the 20s and drops off substantially after age 35-38 [Menken]. However, recent

evidence suggests the rate of decline is actually increasing constantly throughout life. Therefore both a woman's initial endowment of follicles and the increasing rate of atresia leading to follicular depletion are responsible for a progression toward menopause [Hansen].

While menopause is a clear, well-defined end to a woman's reproductive lifespan, it is preceded by a dynamic process of a decline in ovarian function, occurring over several years. These stages may involve sub-fertility, which often escapes detection because of adequate and appropriate hormonal secretion [Hansen]. It is estimated that fertility begins to decline approximately 10 years prior to the age of menopause, with an average of 41 marking the end of fecundity [Murray]. Later, perimenopause occurs; this is a stage characterized by an irregularity in menstruation cyclicity and increased gonadotropin levels. Additionally, the number of follicles falls below a certain threshold (1000-1100) [Faddy]. Fertility is compromised as many remaining oocytes contain chromosomal aberrations and make a viable conception difficult. Eventually, there are no remaining follicles to allow for ovulation, and menopause occurs.

Clinical Presentation and Diagnosis

The most common scenarios for POF evaluation include sub-fertility and menopausal symptoms. Difficulty in conceiving often brings women to the attention of health care providers, in the setting of an infertility specialist, obstetrician-gynecologist, or reproductive endocrinologist. Others present with the onset of menopausal symptoms, including hot flashes, dyspareunia, night sweats, and vaginal dryness [Altchek]. In particular, hot flashes are a characteristic symptom of POF and are distinguished by their brevity, with an average duration of 3.5 minutes. The hypothalamus senses a perceived discrepancy in set and core temperature and quickly triggers homeostatic heat loss through increased heart rate, perspiration, and vasodilation. In the general population, 85% of women overall will experience hot flashes, 30% within three years of menopause [Kronenberg]. Additional indications include oligomenorrhea, absence or regression of sexual secondary characteristics, ceasing birth control pill without resolution of menstrual cyclicity, or after giving birth [Nippita].

Once POF is suspected, the clinician often pursues diagnostic testing such as a progesterone challenge to distinguish between anovulatory states and hypothalamic suppression [Rebar; Nelson, 2005]. Measurement of serum gonadotropin levels may also be indicated. FSH and LH are elevated in POF, but must be measured on separate occasions at least one month apart, as fluctuations in levels from intermittent ovarian activity can mask

underlying disruptions [Shelling]. In addition, estrogen levels are typically low, approximately 50 pg/ml in women with an absence of functional follicles [Rebar]. Naturally, other reasons for a lack of menstruation, such as pregnancy, thyroid abnormalities, polycystic ovarian syndrome, and elevated prolactin must be excluded [Shelling; Rebar]. Lastly, a thorough history is imperative in determining an etiology for POF, which is considered idiopathic for the majority of women [Goswami, 2005]. A value of FSH over 40 IU/ml with concomitant amenorrhea for 3-6 months (variable among physicians) is consistent with a diagnosis of POF [deVos; Shelling].

Epidemiology

POF affects about 1% of the US population [Rebar]. The estimated prevalence increases by a factor of about 10 with each decade of life, ranging from 0.1% at age 30 to 1% at age 40 [Rebar]. Some ethnic disparities exist: there appears to be a higher prevalence (1.4%) in African-American and Hispanic women; the prevalence in Caucasians is about 1%; the lowest figures, 0.1and 0.5%, are in Japanese and Chinese women, respectively [Nippita].

Etiology

In hypothesizing possible mechanisms of early ovarian decline, one appreciates the complexity of the menstrual cycle. The time period for a primordial follicle to progress through activation from dormancy, further follicular development, and selection for ovulation, atresia, or rescue varies dramatically from days to decades [Altchek]. Additionally, there are many stages of folliculogenesis or ovulation from which a depletion of significant magnitude may hasten the progression to menstrual cessation. First, as early development of primordial follicles to primary and pre-antral follicles occurs with each cycle, a smaller initial number of primordial follicles to begin with would deplete the supply sooner. Atresia occurs concurrently at each of these stages, resulting in a decreasing number of developing follicles maturing toward ovulation. Thus, any increase in the rate of atresia will parallel the rate of decline in the follicular supply. Thirdly, follicles must mature from the antral stage toward ovulation. Errors or alterations in signaling, secreted factors, gonadotropin receptors, or numerous supporting granulosa and thecal cells could all lead to ovarian dysfunction and early ovarian failure [Laml, Persani]. Lastly, follicular damage or destruction from autoimmune factors or toxic effects from excess metabolites could preclude successful follicle maturation [Nelson, 2009].

Studies of POF have been hindered by the inaccessibility of the ovary and by the occurrence of follicle depletion before symptom onset. A **direct cause of POF remains unknown in about 90% of cases** [Nelson, 2009]. Broadly speaking, established etiologies can be categorized into genetic conditions, autoimmune disease, exposures, postsurgical complications, and other causes, and will be discussed below. [Strauss; Gravholt; Hundscheid; Goswami; Laml].

Genetic

It is of no surprise that mutations in genes encoding proteins for the functions mentioned above would be found in women with reproductive dysfunction. In fact, many mutations have been found in small samples of women with isolated POF. Variants in gonadotropin receptors, *GDF9*, *NOBOX*, *NR5A1*, *Inhibin*, *FSH*, *POLG*, *AIRE*, and *FIGLA* have been reported at low frequencies [Laml; Persani].

Though comprising a small proportion of POF, the additive effect of genetic causes accounts for approximately 5% of POF etiology [Shelling; Persani]. By far the largest part of this is due to errors in the X chromosome.

Each female has two X chromosomes. Through a modification known as X-inactivation, one X chromosome is effectively silenced in each cell. Studies have shown that while differentiation of ovaries requires only one X chromosome, genes on the second X chromosome function in ovarian maintenance [Laml]. Some regions of the X chromosome accordingly "escape" inactivation and may be crucial for ovarian function, as evidenced by POF and amenorrhea in women with breakpoints or deletions on the X chromosome including Xp11.2-p22.1, Xq26-28, Xq13-22 [Laml; Conway]. It follows that global aberrations of the X chromosome would be implicated in ovarian dysfunction, and indeed are present in 40-50% of girls with primary amenorrhea and a portion of women with reproductive dysfunction. This includes structural rearrangements such as translocations, mosaicism of sex chromosome dosage, and aneuploidy such as Turner syndrome or triploidy.

Specific mutations in genes on the X chromosome can also lead to POF, the most well-known being Fragile X syndrome. This is an X-linked mental retardation syndrome and the most common cause of mental retardation in boys. Abnormal expansions of a CGG repeat in the promoter regions of the FMR1 gene lead to abnormalities in gene methylation and gene silencing. The inactivating expansion occurs when an allele containing between 55 and 200 CGG repeats is inherited from the mother [Chonchalya]. These intermediate sized alleles are termed "premutation," and carrier females have an absence of cognitive disease but an

increased risk to pass on an expanded allele and have an affected boy [Corrigan]. The frequency of POF in premutation carriers is estimated at 11.5% in those with a family history of POF, and 3.2% in those with sporadic POF [Rohr].

The mechanism behind the increased frequency of POF in premutation carriers is unknown, but is hypothesized as similar to that of Fragile X-related tremor/ataxia syndrome, a neurological disorder that may present in premutation carriers. Expanded CGG repeats in the 5' untranslated region of the gene shift the transcription start site upstream. A 5-fold elevation of FMR1 mRNA may result in a toxic gain of function from a buildup of abnormal gene products. It may lead to excess binding of proteins to the expanded repeat region, further altering the translational efficiency; protein levels (FMRP) are known to decrease with increasing repeat size [Hagerman]. Some have proposed that a cumulative effect of toxic RNA may lead to increases in follicular atresia, leading to POF. Alternatively the FMRP-regulated translational suppression on a subset of mRNA could lead to haploinsufficiency of proteins needed in oocyte development [Rohr].

A diagnosis of POF, especially with a significant family history, warrants exploration of the possibility of Fragile X. A 2006 statement from ACOG recommends that in a woman with ovarian failure or elevated FSH level of unknown cause before 40 years "fragile X carrier screening should be considered to determine whether she has a premutation" [ACOG].

More recently, chromosomal microarray has been utilized to identify copy number variations in affected individuals. A recent study identified 44 losses and gains potentially causative for POF in 74 patients. Many of the candidate genes are involved in meiosis, DNA repair, and folliculogenesis or male fertility in homologs of model organisms [Ledig].

Autoimmune

Autoimmune disease contributes another significant portion of causes and may be present in 10-20% of women with POF, often correlated with adrenal disease [Shelling]. These include Addison's disease, hypoparathyroidism, diabetes mellitus, pernicious anemia, vitiligo, myasthenia gravis, autoimmune polyglandular syndrome types I and II, Crohn's disease, Sjogren's syndrome, and systemic lupus erythematosus [Nippita].

Exposures

Radiation and chemotherapy for the treatment of malignant disease is one of the leading causes of POF [Nippita; Rebar]. Chemotherapy depletes the number of oocytes, and affects the structure and function of oocytes and granulosa cells. The risk to develop POF from

radiation therapy is primarily from treatment within the pelvic area, but disruptions in the hypothalamic-pituitary-ovarian axis must be considered. Of note, an additional factor of importance involves the age at which radiation therapy occurs; prepubertal ovaries are fairly resistant to radiation. Secondly, there is a dose-dependent effect, and the greatest risk for ovarian failure occurs at doses of radiation 9 grays or higher [Nippita; Rebar]. Infection is another possible cause of ovarian depletion. A study in 1998 demonstrated that 3.5% of women with POF had varicella, shigella, or malaria exposure. In addition, some women may develop oophoritis during an exposure to mumps [Goswami]. Other exposures leading to POF may include surgeries, particularly in the pelvic region, that carry the potential to disrupt vascular supply to the ovaries.

Other

Several reported associated causes of POF include cigarette smoking, epilepsy, endocrine disruptors, and other environmental exposures, though these are equivocal and poorly studied [Goswami; Conway]. Exposures to industrial 2-bromopropane, a cleaning solvent, was associated with primary ovarian insufficiency in 16 Korean women [Nelson, 2000].

Management

The management of POF involves a combination of medical and psychological assistance. A negative, often traumatic psychological impact has been consistently reported in the literature, including social anxiety, depression, and lowered self-esteem [Rebar; Nippita; Shelling; Schimdt; Liao; Groff; deVos]. Many women experience frustration from an inability to recognize clinical reasons for their symptoms; one study found it took up to 5 years for a diagnosis in a quarter of affected women [Albuzaidi]. Secondly, the term “failure” alone can bring feelings of inadequacy and guilt [Nelson, 2005]. Indeed the rates of depression in women with a diagnosis of POF seem to be higher than the general population. A recent study in 174 women with sporadic POF found the onset of depressive systems seemed to occur prior to the actual diagnosis of POF but after the onset of symptoms [Schmidt].

It has been suggested that the term POF is inadequate to reflect the spectrum of ovarian dysfunction, uninformative and difficult for patients, and to some degree, inaccurate [deVos; Shelling; Rebar]. For example, in three studies, 50%–84% of women with POF had new follicle growth and 16%–49% ovulated [Welt]. The term “primary ovarian insufficiency” is also used by clinicians and was in fact the original term coined in 1942 by the endocrinologist Albright Fuller [Rebar]. It is a vague term that allows for the heterogeneity and possible irreversibility of

disease, and does not imply finality [deVos; Shelling; Rebar]. Additional terms include "hypergonadotropic amenorrhea," "hypergonadotropic hypogonadism," and "primary hypogonadism." These terms may be less recognizable to patients, but also account for etiologies outside of a defect in the ovaries [Shelling; Rebar].

A third psychological factor involves women who are seeking pregnancy at the time of diagnosis. As increased numbers of women delay childbirth, the age of which childbearing is most desired may be concurrent with the onset of POF [Shelling]. Estimates of the percentage of women with POF who may still become pregnant range from 5-10% [Shelling]. However, there are no known reliable predictors to identify these women, nor are there specific identified measures to increase the chances of conception [deVos]. In fact, ongoing assessments of patients have found that women with no oocytes observed by imaging have become pregnant, while women with residual ovarian function have not [Dr. Michael J. Heard, personal communication]. There is recent evidence that conception may occur with the suppression of gonadotropin-releasing hormone (GnRH) analogues and subsequent ovulation induction, but no published trials exist [deVos]. The lack of prognostic information makes it difficult for clinicians to offer definitive information to women, and frustrating for patients who face the possibility that they may not conceive.

The low chance of success in using a patient's own oocytes for conception usually prompts discussion of other pregnancy options for those women who desire children. Adoption is certainly a choice for many families. More commonly, the use of donor eggs is utilized and studies show higher success rates than those observed in traditional *in vitro* fertilization [Rebar]. Appropriate counseling on familial risks of POF is essential, as many women choose to have a family member, such as a sister, donate oocytes. Approximately 20-30% of women have a family history of POF [Woad].

Additional management for patients involves mitigating the risks to the patient from a lack of estrogen, which is important for bone health and reducing the risk of adverse cardiac events. In studies of women with natural early menopause, the risk of mortality from cardiovascular events was significantly higher than in women with late menopause, suggesting that longer exposure to endogenous estrogen may be protective against cardiovascular disease [van der Schouw]. A study of women with primary ovarian insufficiency demonstrated a change in the lipid profile of patients, including elevated triglycerides and lower HDL cholesterol concentrations [Knauff]. Evidence from longitudinal studies such as the Women's Health Initiative (WHI) have assessed risks for the development of breast cancer, heart attacks, and strokes in menopausal women who had a prior hysterectomy undergoing

hormone replacement therapy (HRT) [LaCroix]. There are marked differences in women who have experienced natural menopause and patients with POF; the former are typically seeking symptom alleviation, while the latter utilize HRT to counteract the deficit of estrogen [Shelling]. Recent releases from the WHI have indicated that the risks for breast cancer appear lower in those women who received estrogen only after menopause, and younger estrogen users had a lower rate of heart attacks [LaCroix]. Estrogen is also important in protection against osteoporosis. In the absence of exogenous estrogen supplementation, routine bone density scans, medication, and lifestyle changes are useful in preventing osteoporosis and a reduction in bone mineral density [Woad; Shelling]. Finally, as autoimmune disorders are considered an etiology of POF, approximately 20% of women with spontaneous POF will develop autoimmune hypothyroidism [Kim]. Therefore, it is prudent to include measurements of TSH and free T4 during a workup for POF, and to check for the presence of serum thyroid peroxidase autoantibodies [Nelson, 2009].

Despite the mechanisms of POF identified through numerous studies among various medical disciplines, diagnosis includes a direct cause in only a small portion of women. This fact necessitates further attempts to understand disruptions to ovarian function, and the eventual establishment of risk factors to identify women for which further attention is implicated.

Goals of Project

As the majority of women are given a diagnosis of idiopathic POF, many are left with little guidance on appropriate management and information for family members [Altchek]. Therefore, more information and research is necessary to pinpoint specific mechanisms of POF in humans and identify possible genetic components to account for the relatively high observed familial associations. Until recently there was no evidence to suggest the TSC genes were crucial for follicular recruitment, survival, maturation, or demise. Recent evidence from of *Tsc1* and *Tsc2* conditional knockout mouse models suggests otherwise. Identifying a possible association between ovarian dysfunction and TSC-causing mutations is thus an appropriate endeavor. The simplest way to assess the possibility of a connection between the TSC genes and POF is to sample a selection of women with Tuberous Sclerosis Complex, since the majority harbor a mutation in either TSC gene. Determining if an association between the two conditions exists will shed more light on the function of the TSC genes and their possible pathogenic role in the ovaries, and about mechanisms behind the pathogenesis of POF in women. There is currently no evidence to suggest women with TSC have a higher incidence of POF compared to the general population, or that the *TSC1* and *TSC2* genes play a similar role

in human ovaries as was demonstrated in the mice [Adkihari]. **The aim of this project is establish a prevalence of POF and associated reproductive dysfunction in a sample of women with TSC**. We hypothesize that no significant association exists between women affected with TSC and the likelihood of developing POF. Thorough self-reports of menstrual function, reproductive history and symptoms, as well as clinical manifestations of TSC will be collected from individuals. Expert medical guidance will be useful in determining which women may have an unlikely, possible, or probable diagnosis of POF, or features pointing to perhaps an occult and as of yet unrecognized problem. In the absence of a defined control population, current scientific literature will be used to compare these women to what is reported about POF in the general population. This information will allow for more interpretation of the results of the conditional knockout mice studies, and further insight into possible roles of these genes in human ovarian function.

Materials and Methods

Participants

Participants were woman with tuberous sclerosis complex over the age of 18 who are members of the national Tuberous Sclerosis Alliance (TSA) support group. This study, HSC-GSBS-10-0329, was granted exempt status by the University of Texas Health Science Center Committee for the Protection of Human Subjects. A total of 1000 questionnaires in return addressed envelopes were sent to the TSA. These were sent to randomly selected women from a database maintained by the TSA. Identifying information was only available to members of the TSA and questionnaires were returned in blank, preaddressed envelopes. Approximately one-fourth of the women to whom the questionnaire was sent were "dependent," meaning they are entered in the TSA database as individuals who live under the care of another individual and thus the questionnaire was completed by a caretaker or family member. We received a total of 200 questionnaires; five were blank, twelve were completed with respect to an individual who was under the age of 18, and one was sent to an affected male, giving a final sample size of 182. These individuals were excluded from analysis. An email address was created in the event that women may have questions; one response was received regarding the appropriate female in the household to complete the questionnaire. A telephone number was also provided, and two women did make contact with questions and concerns about the questionnaire; no information from email or telephone correspondence was kept.

Questionnaire Design

The questionnaire was developed to encompass three areas: demographic information, reproductive health and history, and information about the individual's history of TSC. In the absence of access to validated questionnaires assessing POF and without clinical information from respondents, the questionnaire was designed through extensive literature review and clinical expertise. Please see Appendix A for a complete questionnaire. A description of the questions included in each section follows:

Demographics: Independent/proxy respondent, current age, height and weight, ethnicity, employment status, marital status, household status, educational level, and annual household income.

Reproductive Health: Age of menarche, age and number of pregnancies, live births, and premature births, number of and ages at miscarriages, and number of terminations; a detailed menstrual history from ages 16-40, indicating menstrual cycle regularity and the use

of contraception; perimenopausal symptoms, blood tests indicating thyroid disease, elevated testosterone levels, or a menopausal state; history and age of radiation therapy, chemotherapy, hysterectomy, and/or oophorectomy; diagnosis (age, and by whom) of any of the following: POF, polycystic ovarian syndrome (PCOS), Addison's disease, Autoimmune disease, Anorexia, Infertility, Turner syndrome, Galactosemia, Amenorrhea; a family history (relation to individual, and age of diagnosis) of Fragile X syndrome, POF, and unspecified autoimmune disorders; current medication, dose, and frequency.

TSC: Age of diagnosis, family history (relation to individual, and age of diagnosis, and genetic testing (if pursued, and results, if known); history of a SEGA (type of treatment received, if applicable); history of seizures (age of onset, frequency, and if surgery was performed for seizure control); current and previous seizure medication (number of years taken, and an indication of medication effectiveness on a 1-3 scale); diagnosis (current/previous diagnosis and age of diagnosis) of any of the following: learning disability, attention deficit hyperactivity disorder (ADHD), autism, bipolar disorder, depression, dyslexia, intellectual disability, obsessive compulsive disorder (OCD), other); clinical manifestations of TSC (age of diagnosis, indication of current vs. prior manifestation, where applicable): facial angiofibromas, hypopigmented macules, ungual tumors, renal cysts, angiomyolipoma, renal cancer, pulmonary cysts, lymphangioleiomyomatosis, shortness of breath, rhabdomyomas, and irregular heartbeat. Lastly, an opportunity for women to write in additional comments or concerns was provided.

Menstrual Classification

Menstrual regularity was defined as 10 or more periods per year. Menstrual irregularity was defined as 9 or less periods per year, or 0. Contraception was indicated by use of oral contraceptive pills or an intrauterine contraceptive device. Time frames for age ranges of menstrual history were divided into the following year increments: 16-20, 21-25, 26-30, 31-35, 36, 37, 38, 39, and 40. Women reported their history in regards to the majority (more than half) of the time in each age range. Time frames in which women reported using contraceptives were considered uninformative. For women reporting hysterectomy prior to age 40, only time frames prior to the age of their hysterectomy were considered informative. Seven women reported oophorectomy without hysterectomy; these were considered to be bilateral as the women were no longer menstruating. The information gathered from the responses to the menstrual history was classified into the following five categories:

Group	Classification	Number of informative time frames	Menstrual regularity
0	All Regular	At least one	Regular in all time frames
1	All Irregular	At least one	Irregular in all time frames
2	Regular to Irregular	At least two	Consistently regular in early time frame(s) changing to consistently irregular in later time frame(s)
3	Alternating Regularity	At least two	Menses alternate between regularity and irregularity, case not classified in Group 2
9	Unknown	None	N/A

TSC Classification

Women reported specific manifestations in several organ systems and these were compared to data in the current TSC literature to assess for reliability of data. The following classifications were made:

Category	Manifestations
Dermatological	Facial Angiofibromas
	Hypopigmented Macules
	Ungual Tumors
Renal	Renal cysts
	AML
	Renal Cancer
Pulmonary	Pulmonary Cysts
	LAM
	Shortness of Breath with Exertion
Cardiac	Rhabdomyomas
	Irregular Heartbeat
Seizure	History of Seizures

Statistical Analysis

Responses were entered into a password protected Microsoft Access database. Descriptive analysis was performed for all variables (frequencies, means, median, range, standard deviation, as applicable). All analysis was performed in Stata v.11 (College Station, TX). Fisher's exact t-test analysis was used with a significance p-value of <0.05.

Results

Participants and Demographics

A total of 200 questionnaires were received for a response rate of 200/1000 (20%). Five surveys lacked sufficient information, twelve surveys were completed with respect to individuals under the age of 18, and one survey was sent to the household of an affected male, resulting in a final sample size of 182. Participants were asked to indicate if the person completing the questionnaire was affected with TSC (independent participant), or if they were completing the questionnaire with respect to an affected individual (dependent participant). A total of 42 women were dependent or this information was missing, and the remaining 140 were labeled independent. Demographic information for both groups is provided in Table 1. The average age of independent participants was 44 years, and 35 years for dependent women. The overwhelming majority of the study population (90.1%) indicated their ethnicity as Caucasian. Differences in marital status, employment, educational level, and income between independent and dependent participants are also provided. All women were presumed to have a clinical diagnosis of TSC based upon their membership in the TSA. In spite of this involvement, the questionnaire was constructed to further characterize TSC disease of the participants. This data follows the reproductive data.

Table 1. Demographic information for Independent and Dependent Women with TSC* N=182

Age		
Median:	44 years	35 years
Range:	18 – 77 years	18 – 68 years

Independent / Dependent Status n (% of total sample)	
Independent	140 (76.9)
Dependent/ Unknown	42 (23.1)

	Dependent Women	Independent Women
	n (% of 42)	n (% of 140)
Ethnicity		
Caucasian	34 (81.0)	130 (92.9)
African-American	3 (7.1)	3 (2.1)
Hispanic	0.0	1 (0.7)
Asian	2 (4.8)	1 (0.7)
Other	3 (7.1)	5 (3.6)
Marital Status		
Single	37 (26.4)	37 (88.1)
Married	82 (58.6)	4 (9.5)
Divorced	16 (11.4)	1 (2.4)
Widowed	5 (3.6)	0.0
Household Status		
Alone	22 (15.70)	5 (11.9)
With family	81 (57.9)	25 (59.5)
With S/O	34 (24.3)	4 (9.5)
Assisted living	0 (0)	7 (16.7)
Other	3 (2.1)	1 (2.4)

	Dependent Women	Independent Women
	n (% of 42)	n (% of 140)
Employment Status		
Unemployed	29 (20.1)	20 (48.8)
Part-time	14 (10.0)	6 (14.6)
Full-time	64 (45.7)	4 (9.8)
Student	6 (4.3)	2 (4.9)
Disabled	10 (7.1)	6 (14.6)
Retired	10 (7.1)	1 (2.4)
Other	7 (5.0)	2 (4.9)
Annual Household Income		
< $10,000	8 (6.6)	13 (37.0)
$10,000 – 24,000	12 (9.8)	8 (22.9)
$25,000 – 49,000	25 (20.5)	3 (8.6)
$50,000 -74,999	20 (16.4)	3 (8.6)
$75,000-99,999	21 (17.2)	2 (5.7)
> $100,000	16 (13.1)	1 (2.9)
Decline / blank	38 (27.1)	12 (28.6)
Highest Education Level		
Under 12th grade	3 (2.1)	18 (43.9)
Completed 12th grade	30 (21.40)	12 (29.3)
Some college	43 (30.7)	6 (14.6)
Bachelor's degree	31 (22.1)	4 (9.8)
Master's degree	33 (23.60	1 (2.4)

* "Dependent" includes women for which this information was missing

Reproductive History

All information obtained in this study was self-reported and thus reproductive information is unconfirmed by medical chart review. Data are presented in Tables 2 through 10 and separated with respect to independent and dependent women. The study question of interest, the prevalence of premature ovarian failure in women with TSC, is presented afterward.

The reported average age of menarche was 12.5 years and did not differ between dependent and independent women (Figure 2). This age at menarche is consistent with that of the general population and suggests initial menstrual function is intact in this population of women with TSC. Though some degree of pubertal irregularity is not uncommon, the majority of cycles normalize by age 16-20. Thus, irregularity reported during the time period of 16-20 years was still considered irregular even if followed by consistently regular cycles.

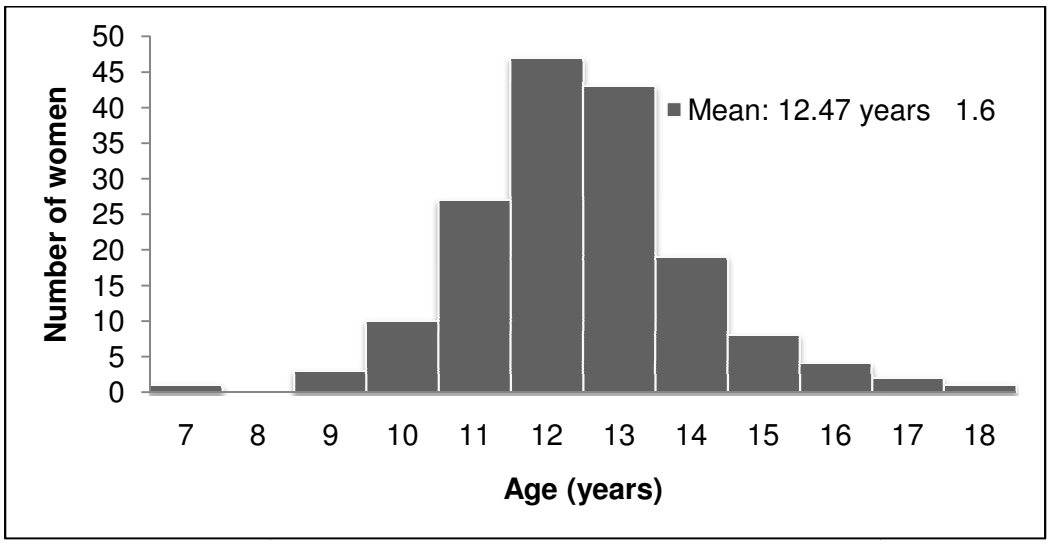


Figure 2: Age of menarche of entire sample of Women with TSC

History of menstrual irregularity was determined by self-reported cycle regularity, and use of oral contraceptives, for five year intervals from 16-35, and one year intervals from 36-40. Women were asked to report if cycles were regular or irregular for the majority of the time in each category. Regular was defined as “10 or more periods in a year,” and irregular was defined as “less than 9 periods a year, or 0.” Oligomenorrhea is consistently reported in the literature as fewer than 9 periods in one year. Women with some irregularity were then divided into those with only irregular cycles, women progressing from consistently regular to consistently irregular periods, women progressing from consistently irregular to consistently regular periods, and women whose periods alternated between regular and irregular cycles. Of 182 women, 36 had a history that was uninformative because of unclear responses and

oral contraceptive use. Nonetheless, 57 women (31.3%) displayed some irregularity in menstrual cyclicity between the ages of 18 to 40 (Table 2).

Table 2. Menstrual History

	Independent Women N=140	Dependent Women N=41	All women N=181
Menstrual category	**n (%)**	**n(%)**	**n (%)**
Always regular	71 (50.7)	17 (41.5)	88 (48.6)
Always irregular	16 (11.4)	8 (19.5)	24 (13.3)
Regular to irregular	15 (10.7)	3 (7.3)	18 (9.9)
Irregular to regular	5 (3.6)	2 (4.8)	7 (3.9)
Alternating regularity	7 (5.0)	1 (2.4)	8 (4.4)
Uninformative	26 (18.6)	10 (24.4)	36 (19.8)

Pregnancy

Pregnancy data included a history of pregnancy, maternal ages at delivery of all live and/or premature births, the number of miscarriages and maternal ages at occurrence, as well as the number of voluntary terminations. 92 (50.1%) women indicated that they had become pregnant. (Table 3) Of these, there were 10 women who had no live births, while a total of 8 women had premature births. The average number of pregnancies for women was 1.71, with an average age of 25.3 years for the first pregnancy. Lastly, 3 of 18 total women who reported a voluntary termination were dependent, and 15 were independent.

Table 3. Pregnancy

	Independent Women N=140	Dependent Women N=42	All Women N=182
Women pregnant at any time	n (%)	n (%)	n (%)
	85 (60.7)	7 (16.7)	92 (50.1)
Average number of live births	1.7 ±1.5	1.3 ± 1.0	1.7 ±1.5
Average age at first birth (years)	25.6 ± 5.5	21.8 ± 2.5	25.3 ± 5.4
Women who had preterm birth	7	1	8
Women who had voluntary termination	3	15	18

Miscarriage

Of 92 women who reported ever being pregnant, 38 (41.3%) reported having a miscarriage at one point in their life (Table 4). This rate of miscarriage is significantly higher than expected when compared to national estimates from literature and US epidemiological data from national surveys, which ranges from 15-25% for aged 25-39 years. Thirty-five of these women were independent, and the remaining two did not indicate a dependent or independent status. The ages at which the first miscarriage occurred varied from 19 to 40 with a mean of 28.5 years. The average number of miscarriages women reported was 1.6, ranging from one to six. Menstrual history for these women is also provided in Table 4. Of women who had a miscarriage, 34.2% had a history of some type of menstrual irregularity, while 50% reported a history of consistently regular cycles. Six (17.1%) women with a history of miscarriage never experienced a live birth, and 29 (82.9%) women with a history of miscarriage did have at least one live birth.

Table 4. Miscarriage History for Women with TSC Reporting Pregnancy
N=92

Women who had at least one miscarriage n (%)	38 (41.3)
Average age of miscarriage (years)	28.5 ± 5.9
Average number of miscarriages	1.6 ± 1.1
Menstrual category	**Women with history of miscarriage n (% of women reporting pregnancy)**
Always regular	19 (50)
Always irregular	4 (10.5)
Regular to Irregular	3 (7.9)
Alternating regularity	6 (15.8)
Uninformative	6 (15.8)

Reproductive symptoms, tests, and surgeries

The questionnaire was designed to screen for symptoms related to menopause, POF, and other reproductive disorders, as well as a history of blood tests and surgery related to

reproductive issues. We asked women who experienced reproductive symptoms to specify if they occurred currently or in the past. We also asked them to specify a duration (in years) for which these symptoms lasted (Table 5). We also inquired about blood tests indicating a menopausal state, thyroid disease, or elevated testosterone levels (Table 6).

A surgical history of hysterectomy was reported in 38 women below the age of 40 (Table 7). Eight women did not indicate an age at which this was performed, and were not considered further as we had no means of evaluating their cycles. Additionally 18 women reported oophorectomy at an average age of 42 years. Radiation therapy at unspecified locations was reported in six women. One woman indicated use of radiation for SEGA, but the rest did not indicate a diagnosis of cancer or other indications. The average age of radiation therapy was 31.7, reported by three women. One woman reported chemotherapy for an unspecified indication, performed at 45 years.

Table 5. Reproductive Symptoms in Women with TSC Less Than 40 years

	Independent Women		Dependent Women		All Women	
Symptom	**Current or previous symptom n (%)**	**Average duration (years)**	**Current or previous symptom n (%)**	**Average duration (years)**	**Current or previous symptom n (%)**	**Average duration (years)**
Hot Flashes	7 (14.9) N=47	5.8 ± 4.7	2 (7.7) N=26	2.3 ± 2.5	9 (12.3) N=73	4.9 ± 4.4
Night Sweats	10 (21.3) N=47	1.2 ± 0.5	2 (7.7) N=26	2.3 ± 2.5	12 (16.4) N=73	1.5 ± 1.2
Body Hair	8 (17.4) N=46	12.3 ± 6.3	3 (11.1) N=27	15.0 ± 7.1	11 (15.1) N=73	13.2 ± 6.0
High Blood Pressure	12 (26.0) N=46	4.5 ± 3.0	7 (25.9) N=27	12. ± 8.9	19 (26.0) N=73	7.7 ± 6.8
Milky breast discharge, excluding breastfeeding	7 (15.2) N=46	1.1 ± 1.3	1 (3.7) N=27	--	8 (11.0) N=73	1.1 ± 1.3
Weight gain of 25 pounds, excluding pregnancy	17 (35.4) N=48	N/A	9 (33.3) N=27	N/A	26 (34.7) N=75	N/A

Table 6. Reproductive Blood Tests in Women with TSC less than the age of 40			
	Independent Women	**Dependent Women**	**All Women**
Blood Test Indicating:	**N (%)**	**N (%)**	**N (%)**
Menopausal State	0 (0.0) N=50	1 (3.7) N27	1 (1.3)
Thyroid Disease	4 (8.0) N=50	4 (14.8) N=27	8 (10.4)
Elevated Testosterone Levels	3 (6.0) N=50	1 (3.7) N=27	4 (5.2)

Table 7. Surgical History of Women with TSC
Women Reporting Surgery Performed at Less Than the Age of 40

	Independent Women			**Dependent Women**		
Surgery	**n**	**% of Independent Women N=140**	**Average Age (years)**	**n**	**% of Dependent Women N=42**	**Average Age (years)**
Hysterectomy	31	(22.1)	35.6 ± 9.7	7	(16.7)	26.6 ± 10.9
Oophorectomy	14	(10)	41.6 ± 7.4	4	(9.5)	32 ± 5.3
Radiation therapy	0	--	--	3	(7.1)	31.7 ± 10.0
Chemotherapy	1	(0.71)	45	0	--	--

Other Reproductive Disorders

The presence of other reproductive disorders is presented in Table 8. Eleven women reported a diagnosis of PCOS, at an average age of 32.4 years. Features associated with PCOS can include menstrual irregularities, elevated BMI, and symptoms such as acne and body hair. Additionally, the use of valproic acid is thought to be a risk factor for PCOS, particularly in women with an elevated BMI. Twenty-one women reported using valproic acid in the past, for an average of 5.33 years. Seventeen women reported current use of valproic acid, for an average of 14.8 years. Of the 17 women currently using this medication, one did report a diagnosis of PCOS. Other common disorders among the women were infertility and autoimmune disease, present in 13 women each. Of note, 64 independent and 13 dependent women in the entire sample indicated a family history of autoimmune disease. The menstrual history classifications for these three diagnoses are presented in Table 9.

Table 8. Reported Reproductive Diagnoses in Women with TSC (not otherwise confirmed)

	Independent Women		Dependent Women	
Diagnosis	**n (%)**	**Average Age Diagnosed**	**n (%)**	**Average Age Diagnosed**
POF	4 (2.9) N=135	39.3 ± 4.9*	0 N=37	N/A
PCOS	11 (8.3) N=133	32.4 ± 14.7	0 N=38	N/A
Amenorrhea	10 (7.5) N=134	30.6 ±11.5	2 (5.3) N=38	Unknown
Infertility	13 (9.6) N=135	30.9 ± 6.0	0 N=38	N/A
Anorexia	5 (3.7) N=135	20.3 ± 1.5	1 (2.6) n-38	13
Addison disease	1 (0.8) N=134	41	0 N=38	N/A
Autoimmune Disease	13 (9.7) N=134	36.6 ± 15.0	0 N=134	N/A

* One woman indicated an age at diagnosis of 45; this age was excluded

Table 9. Menstrual classifications of common reproductive diagnoses

Menstrual Classification	**PCOS N=11**	**Infertility N=13**	**Autoimmune N=13**
	n (%)		
Always Regular	4 (36.7)	5 (38.5)	8 (61.5)
Always Irregular	2 (18.2)	1 (7.7)	2 (15.4)
Regular to Irregular	0	4 (30.8)	0
Alternating Regularity	2 (18.2)	2 (15.4)	2 (15.4)
Uninformative	3 (27.3)	1 (7.7)	1 (7.7)

Medication

Women were asked to list the current medications they were taking, including frequency and dosage. The classes of medications are listed in Table 10; we did not have information about specific indications for each woman's prescriptions. Data on seizure medications are presented subsequently. On average 80.8% of women took medications. Outside of multivitamins and supplements, the most common medications for independent women were analgesics, followed by medications for high blood pressure, and psychiatric illness. For dependent women, the most common were those for high blood pressure, gastric reflux, and psychiatric illness.

Table 10. Current Non-Antiseizure Medications

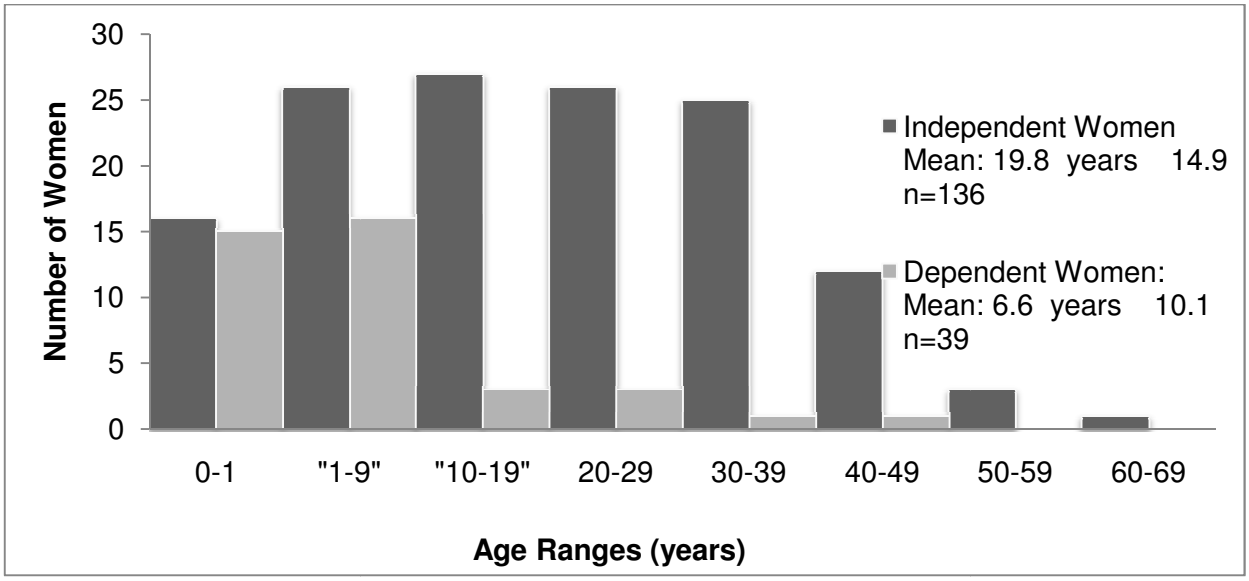
	Independent Women			**Dependent Women**	
Number of women reporting current medication	111 (79.3%) N=140			36 (85.7%) N=42	
Average number of medications used	4.7 ± 4.0			3.8 ± 2.66	
Type of Medicine	**Number of Women**	**% of 111**		**Number of Women**	**% of 36**
Analgesic	40	(28.6)		2	(5.6)
Anti-histamine	2	(1.4)		6	(16.7)
Anti-microbial	10	(7.1)		3	(8.3)
Asthma-related	21	(15)		6	(14.3)
Bisphosphonate	8	(5.7)		0	(0)
Contraceptives/HRT	29	(20.7)		5	(13.9)
High Blood Pressure	39	(27.9)		15	(41.7)
Multivitamin	39	(27.9)		10	(27.8)
Other	38	(27.1)		20	(55.6)
PPI/GERD	14	(10)		12	(33.3)
Psychotropic	52	(37.1)		18	(50)
Statin	29	(20.7)		2	(5.6)
Steroids	18	(12.9)		4	(11.1)
Supplement	51	(36.4)		15	(35.7)
Thyroid hormone	23	(16.4)		7	(19.4)

TSC History

Age at Diagnosis

The reported age of diagnosis of TSC varied widely. Some women were diagnosed prenatally, whereas one person was diagnosed as late as 62 years of age. The median age of diagnosis was 15 years. Frequencies and stratification by independent and dependent status are provided in Figure 3. Because the age of diagnosis was later than expected, we analyzed this variable in comparison to others. In general the more severely affected individuals received a diagnosis at a younger age. This is further discussed in a later section.

Figure 3. Ages of TSC Diagnosis



Family History

Of all the respondents, 73 (40.1%) indicated having another family member with TSC, ranging from 34 women listing one affected family member to one respondent listing six affected family members. The frequency of specific degrees of relation to the respondent is presented in Table 11. By this analysis, our sample appears to contain 76.9% of individuals with no family history of TSC. This is higher than the expected 66 – 70% of individuals expected to have a *de novo* mutation, but could also reflect less awareness or reporting of affected family members.

Table 11. Family History Involvement in Women With TSC		
	Independent Women	**Dependent Women**
	n (%)	
Women with no family history (*de novo*)	103 (73.6) N=140	37 (88.1) N=42
Women reporting family history	66 (47.1) N=140	7 (17.5) N=42
Average number of affected relatives	2.3 ± 1.7	1.75 ± 2.2
Affected Relative	Number of Women	
Mother	15	1
Father	8	1
Sister	16	1
Brother	14	1
Daughter	29	2
Son	25	0
Grandmother	3	1
Grandfather	4	1
Aunt	5	1
Uncle	0	1
Cousin – Female	7	1
Cousin – Male	3	0
Niece	8	0
Other	6	0

Genetic Testing

Table 12. Genetic Testing Results in Women With TSC Receiving Testing		
Mutation testing results N=93	**n**	**(%)**
TSC1	**9**	**(9.7)**
TSC2	**17**	**(18.3)**
No mutation identified	**28**	**(30.1)**
Other	**4**	**(4.3)**
Result Unknown	**35**	**(37.6)**

Ninety-three (51.1%) women indicated they did have genetic testing, while 69 (37.9%) said they did not; 13 (7.1%) did not know. Of those who did, 93 reported results presented in Table 12. Although over half of the women reported receiving genetic testing, over 37% of these did not know the results of their test, providing less information on the frequency of mutations in either TSC1 or TSC2.

Seizure History

Seizure history included age of onset and frequency of seizures, as well as past and current medications used for seizure control and whether surgery was ever performed (Tables 13-16). Overall, 114 (62.6%) women indicated having a history of seizures, with an average onset of 7.0 ± 10.8 years. The majority (59.1%) of women reported a frequency of seizures as less than once a year. A lower frequency than would be expected in TSC reflects milder disease in independent women. Thus the data is separated to show seizure history for dependent and independent women, the former of which have a higher prevalence of seizures. Fifteen women (13.2% of those with a seizure history) indicated having surgery for their seizures.

Table 13. Seizure History in Women with TSC

Seizure History	Independent women	Dependent women	All women
Number reporting seizures n (%)	76 (54.3%) N=140	38 (90.5%) N=42	114 (62.6%) N=182
Mean age of onset (years)	9.3 ± 11.8	2.5 ± 6.4	7.0 ± 10.8
Range in age of onset (years)	0 – 45	0 – 34	0 – 45
Frequency n (%)	Independent Women N=69	Dependent Women N=36	All Women N=105
At least once a day	5 (7.3)	6 (16.7)	11 (10.5)
At least once a week, but not daily	6 (8.7)	9 (25)	15 (14.3)
At least once a month, but not weekly	4 (5.8)	4 (11.10	8 (7.6)
At least once every six months, but not every month	8 (11.6)	1 (2.8)	9 (8.6)
Less than once a year	46 (66.7)	16 (44.4)	62 (59.1)
Number reporting surgery for seizures	10 (13.2)	5 (13.2)	15 (13.2)

In addition to asking about what current and previous seizure medications women used, we asked them to include the dosage, frequency, and an estimate of effectiveness on a scale of 1 to 3. Women used the scale to note how effective they believed the medication(s) to be; 1 “did not work well,” 2 “neutral,” “3 worked well.” Medication data is presented in Tables 14 -16b. The most common seizure medication in use by independent women was Keppra, at an average dose of 1000 mg an average frequency of twice per day, taken for an average length of 4 years, and rated an average effectiveness of 3. For dependent women, the most frequently used current seizure medication was Depakote, with an average dosage of 250 mg two times per day, an average length of 24 years, and average effectiveness of 3.

Table 14. Medication History in Women with Seizure History

	Independent Women N=69	**Dependent Women N=38**
Reporting current medications n (%)	47 (68.1)	36 (94.7)
Average number of medications	1.5 ± 0.9	1.7 ± 1.0
Reporting previous medications n (%)	62 (89.9)	29 (76.3)
Average number of medications	2.0 ± 1.6	3.1 ± 2.6

Table 15. Current Seizure Medications Reported by Women With TSC

	Name	Number of women	Median dosage (mg)	Median frequency	Median years taken	Median Effectiveness
Independent women N=47	Depakote	5	250	2/day	24	3
	Diastat	1	-	-	-	-
	Dilantin	2	300	1.5/day	27.5	2.5
	Felbatol	2	-	3/day	9	2.5
	Gabapentin	1	-	-	-	-
	Keppra	14	1000	2/day	4	3
	Klonopin	2	0.75	2.5/day	6.5	-
	Lamictal	11	150	2/day	5	3
	Lorazepam	1	-	-	-	-
	Neurontin	2	-	-	5.5	2.5
	Phenobarbital	6	60	1/day	36	2.5
	Tegretol	11	300	2/day	18	3
	Topamax	6	150	2/day	6	3
	Trileptal	3	600	2/day	8	-
	Valium	1	-	-	-	-
	Vimpat	1	-	-	-	-
	VNS implant	1	-	-	-	-
	Zonegran	2	-	-	-	2.5
Dependent women N=36	Banzel	2	-	-	-	-
	Clobazam	1	-	-	-	-
	Depakote	12	250	2/day	24	3
	Diastat	2	-	-	-	-
	Dilantin	2	300	1.5/day	27.5	2.5
	Felbatol	2	-	3/day	9	2.5
	Keppra	4	1000	2/day	4	3
	Klonopin	3	0.75	2.5/day	6.5	-
	Lamictal	4	150	2/day	5	3
	Mysoline	1	-	-	-	-
	Phenobarbital	4	60	1/day	36	2.5
	Sabril	3	-	-	-	-
	Tegretol	10	300	2/day	18	3
	Topamax	3	150	2/day	6	3
	Trileptal	6	600	2/day	8	-
	Vimpat	2	-	-	-	-
	Zonegran	2	-	-	-	2.5

Table 16a. Previous Seizure Medications Reported by Dependent Women With TSC

	Name	Number of women	Number years taken	Average Effectiveness
Dependent Women N= 29	ACTH	2	1	2
	Ativan	1	-	0
	Clobazam	1	2	3
	Clonazepam	1	7	-
	Klonopin	3	0.12	1
	Depakote	10	1.75	1.5
	Dilantin	12	4.5	3
	Felbatol	3	9	3
	Gabitril	2	1.5	1
	Keppra	4	0.12	1
	Lamictal	7	1	1
	Lyrica	1	-	-
	Mellaril	1	-	-
	Mysoline	2	-	1
	Neurontin	1	0.5	1
	Phenobarbital	8	4	1.5
	Sabril	3	6	3
	Tegretol	13	8	1
	Topamax	3	1.1	1
	Tranxene	1	-	1
	Trileptal	2	-	1
	Unknown	3	-	-
	Valium	2	5	0.5
	Zarontin	1	3	2
	Zonegran	3	4	1

Table 176b. Previous Seizure Medications Reported by Independent Women With TSC				
	Name	**Number of women**	**Number years taken**	**Average Effectiveness**
Independent Women N= 62	ACTH	2	0.5	2
	Carbatrol	3	9	1.5
	Depakote	12	5	2
	Diamox	1	1	1
	Dilantin	33	6	2
	Felbamate	1	1	1
	Keppra	3	2.5	1
	Klonopin	1	5	1
	Lamictal	4	1	1
	Mogadon	1	--	3
	Mysoline	2	1	1
	Neberil	1	--	1
	Neurontin	8	1	1
	Phenobarbital	24	8	3
	Tegretol	21	5	2
	Topamax	3	2	1
	Trileptal	2	0.25	1
	Unknown	2	--	--
	Vigabatrin	1	3	3

Clinical Manifestations

The last two sections of the TSC portion of the questionnaire focused on the presence of intellectual impairments, and specific clinical manifestations listed by affected organ. For each, women were asked to report a current /prior existence of diagnosis/manifestation, and the age at which they were diagnosed, where applicable.

By far, the most commonly reported neuropsychiatric diagnosis was depression, present in 72 (39.6%) women (Table 17). The finding is considered in light of rates of depression in women, in individuals with genetic disease, and in individuals with other psychiatric disturbances and is discussed later. We further analyzed this by combining depression with other diagnoses (anxiety, OCD, bipolar disorder) into a sub-category of “mood disorders.” We found a prior or current diagnosis of these mood disorders in 65 (47.8%) independent women, and 22 (52.4%) dependent women, for total of 87 women (47.8%) in the sample. Further analysis between women with a history of a “mood disorder” and those women without is provided in Table 18.

Other common diagnoses were learning disabilities, intellectual disability , and ADHD. Less common were OCD and Autism, and dyslexia. As with seizures, it is expected that dependent women would be more severely affected by cognitive impairment; thus, the data for neuropsychiatric manifestations of TSC is shown separately for dependent and independent women.

Table 187. Diagnosis of Neuropsychiatric Disorder in Women with TSC

	n (%)					
	Independent women			Dependent women		
Disorder / Diagnosis	Current / previous n (%)	Age Diagnosed (years)		Current / previous n (%)	Age Diagnosed (years)	
		Mean	Range		Mean	Range
Learning Disability	35 (25.9) N=135	7.9 ± 5.3	2 – 20	27 (64.3) N=42	5 ± 4.9	0 – 20
ADHD	9 (6.7) N=134	12.9 ± 7.5	5 – 25	8 (19.0) N=42	4.4 ± 3.5	1 – 10
Autism	3 (2.2) N=134	3 ± 1.4	2 – 4	9 (21.4) N=42	4 ± 5.4	1 – 16
MR	4 (3.0) N=133	3.3 ± 1.5	2 – 5	25 (59.5) N=42	3.2 ± 2.4	1 – 10
Dyslexia	8 (5.9) N=135	12.7 ± 4.6	10 – 18	2 (4.8) N=42	15 ± 7.1	10 – 20
OCD	8 (6.0) N=134	21.4 ± 9.5	14 – 38	6 (14.3) N=42	13 ± 4.2	10 – 16
Depression	59 (42.8) N=138	27 ± 10.6	13 – 46	13 (31.0) N=42	23.5 ± 9.6	10 – 35
Bipolar Disorder	5 (3.7) N=134	33.2 ± 10.9	18 – 47	4 (9.5) N=42	23 ± 11.3	15 – 31
Other	16 (28.6) N=56	26.4 ± 12	11 – 45	9 (21.4) N=42	10.9 ± 8.5	1 – 25

Table 19. Women with Mood Disorders: Statistically Significant Findings

	n (%)		(p<0.005)
Finding	Mood Disorder	No Mood Disorder	p value
Shortness of Breath with Exertion	38 (43.7) n=95	23 (24.1) n=87	0.022
Night Sweats	9 (28,1) n=32	3 (7.1) n=42	0.038
Hot Flashes	8 (25,0) n=32	1 (2.4) n=41	0.009

To assess the severity of brain manifestations of TSC, we asked women to report a history of SEGA, and any associated treatment. Twenty-two (12.5%) women indicated being diagnosed with SEGA, while 44 (25.0%) reported they did not know. Of those who did, ten had treatment, all of which was reported to be surgery; one woman indicated both surgery and radiation.

Clinical manifestations of TSC are presented in Table 19 and are separated into Dermatological, Renal, Pulmonary, and Cardiac categories. Specific manifestations are shown in Tables 20 – 23 for each organ system.

Table 20. Overall Organ System Involvement in Women With TSC

	n (%)	
Organ System	**Independent women**	**Dependent women**
Dermatological	133 (95.0) N=140	42 (100.0) N=42
Renal	80 (57.1) N=140	30 (71.4) N=42
Pulmonary	67 (47.9) N=140	18 (42.9) N=42
Cardiac	38 (27.1) N=140	16 (38.1) N=42

Table 21. Dermatological Involvement in Women With TSC

	n (%)	
Manifestation	**Independent women**	**Dependent women**
Angiofibromas	115 (82.1) N=140	39 (92.9) N=42
Hypopigmented Macules	124 (88.6) N=140	37 (88.1) N=42
Finger / Toe Nail Tumors	84 (60.0) N=140	25 (59.5) N=42

Table 22. Renal Involvement in Women With TSC		
	n (%)	
Manifestation	**Independent women**	**Dependent women**
Renal Cysts	63 (45.0) N=140	27 (64.3) N=42
Angiomyolipoma	63 (48.1) N=140	22 (52.4) N=42
Renal Cancer	3 (2.1) N=140	3 (7.1) N=42

Table 23. Pulmonary Involvement in Women With TSC		
	n (%)	
Manifestation	**Independent women**	**Dependent women**
Pulmonary Cyst	30 (21.4) N=140	8 (19.1) N=42
LAM	35 (25.0) N=140	8 (19.1) N=42
Shortness of Breath with Exertion	47 (33.6) N=140	14 (33.3) N=42

Table 24. Cardiac Involvement in Women With TSC		
	n (%)	
Manifestation	**Independent women**	**Dependent women**
Rhabdomyomas	10 (7.1) N=140	10 (23.8) N=42
Irregular Heartbeat	33 (23.6) N=	13 (31.0) N=42

POF

The reproductive histories of all women were looked at in detail. Women who had irregular cycles early in their lives that persisted were considered to have a different reproductive dysfunction. However, women who had regular cycles most of their life but later changed to irregular cycles later in their reproductive lives, particularly after age 35 were considered to have possible POF as this is a common pattern in women have been documented with this diagnosis. This pattern was observed in 18 women; 16 independent women, and two dependent women. The group was further refined to exclude those women whose other information that was inconsistent or incomplete. This resulted in a total of eight women who were deemed to have a "possible" diagnosis of POF. One of these women was dependent (discussed later), and the remaining seven were independent.

Of note, four women out of the total 182 participants reported receiving a diagnosis of POF, but two were excluded due to contradictory and/or insufficient information. One woman indicated receiving a diagnosis of POF at age 45, which would not be clinically accurate. She also reported a diagnosis of PCOS at age 42, a hysterectomy at age 32, and an oophorectomy at age 42. This history suggests she had other reproductive problems but is not consistent with a decline in ovarian reserve observed in POF. The second woman reported a diagnosis of POF at age 33. Her entire menstrual history was lacking with the exception of reported irregular periods without oral contraceptive use at age 40. Thus, we cannot assess her menstrual history for a progression from regular periods to irregular periods. Furthermore, this individual reported a miscarriage at age 40. The other two women reporting a diagnosis of POF are **included** in the group of seven women. One of these women reported a diagnosis of POF at age 39, and also reported a history of anorexia, a disease in which menstrual abnormalities are common. She did not, however, indicate an age of this diagnosis. The second women reported a diagnosis of POF at age 40. She indicated receiving radiation therapy at an unspecified location and age. This woman had no seizure history, no known history of SEGA, and therefore was unlikely to have received radiation to the head for TSC management. We cannot, however, exclude the possibility of pituitary failure due to disruption of the pituitary hypothalamic ovarian axis. In addition, her uterus and ovaries remained intact, making pelvic radiation – a more direct cause of POF - less likely.

Analysis was performed on the seven independent with presumptive POF. Because of the global differences between independent and dependent women in this study, further analysis was performed in comparison to independent women only. A statistical comparison of all variables was made between these women and 110 independent women not determined to

have possible POF over the age of 30. in an effort to use a population both similar to the seven women being studied, and to a typical presentation of POF itself. Information for the one dependent woman with possible POF is summarized in Appendix B.

The data for women with possible POF is presented in Tables 24 - 44. There were four statistically significant findings in the comparison between women with possible POF and independent women over the age of 30 without possible POF. These include: Lower frequency of hypopigmented macules in women with possible POF (Table 41); A higher frequency of reported high blood pressure in women with possible POF (Table 28); Report of blood tests revealing a menopausal state in women with possible POF as compared to independent women without possible POF between the ages of 30 and 40 (Table 29); A higher frequency of reported shortness of breath upon exertion in women with possible POF (Table 45).

Table 25. Demographic Information: Independent Women With Possible POF and Independent Women Without Possible POF over the age of 30

	Possible POF	Non-POF		Possible POF	Non-POF
	n (% of 7)	n (% of 110)		n (% of 7)	n (% of 110)
Age			**Marital Status**		
Median:	44 years	35 years	**Single**	1 (14.3)	20 (18.2)
Range:	18 – 77 years	18 – 68 years	**Married**	4 (57.1)	71 (64.6)
			Divorced	1 (14.3)	15 (13.6)
			Widowed	1 (14.3)	4 (3.6)
Employment Status			**Annual Household Income**		
Unemployed	1 (14.3)	23 (20.9)	**< $10,000**	0	6 (5.5)
Part-time	0	13 (11.8)	**$10,000 – 24,000**	1 (14.3)	10 (9.1)
Full-time	2 (28.6)	50 (45.5)	**$25,000 – 49,000**	0	24 (21.8)
Student	0	1 (0.9)	**$50,000 -74,999**	0	13 (11.8)
Disabled	1 (14.3)	9 (8.2)	**$75,000-99,999**	3 (42.9)	19 (17.3)
Retired	3 (42.9)	7 (6.4)	**> $100,000**	0	11 (10.0)
Other	0	7 (6.4)	**Decline / blank**	3	27 (24.5)
Household Status			**Highest Education Level**		
Alone	1 (14.3)	20 (18.2)	**Under 12th grade**	0	2 (1.9)
With family	5 (71.4)	63 (57.3)	**Completed 12th grade**	4 (57.1)	20 (18.2)
With S/O	1 (14.3)	27 (24.6)	**Some college**	1 (14.3)	36 (32.7)
Assisted living	0	0	**Bachelor / Master degree**	2 (28.6)	52 (47.3)
Ethnicity					
Caucasian	6 (85.7)	102 (92.7)			
African-American	1 (14.3)	2 (1.8)			
Hispanic	0	1 (0.9)			
Asian	0	1 (0.9)			
Other	0	4 (3.6)			

The majority of the seven women with possible POF were Caucasian, married, working full-time or retired, and had completed high school and/or some college (Table 24). Though they all displayed menstrual irregularity of consistently regular cycles to consistently irregular cycles, the average age of menarche (12.7 years) was comparable to both the other women

sampled and the general population (Table 25 and Figure 4). This suggests intact ovarian function at the start of puberty. Rates of pregnancy were also similar to that of the entire study population, and women on average had 1.6 children, with an average age of first pregnancy of 21.8 years. One of the seven women reported miscarriage at age 31, one reported preterm birth, and 0 reported a history of voluntary termination (Tables 26 and 27).

Table 26. Menstrual History

	No POF N=110		Possible POF N=7
Menstrual category	**n (%)**		**n (%)**
Always regular	59 (53.6)		0
Always irregular	15 (13.6)		0
Regular to irregular	6 (5.5)		7 (100)
Alternating regularity	12 (10.9)		0
Uninformative	18 (16.4)		0

Figure 4. Age of Menarche

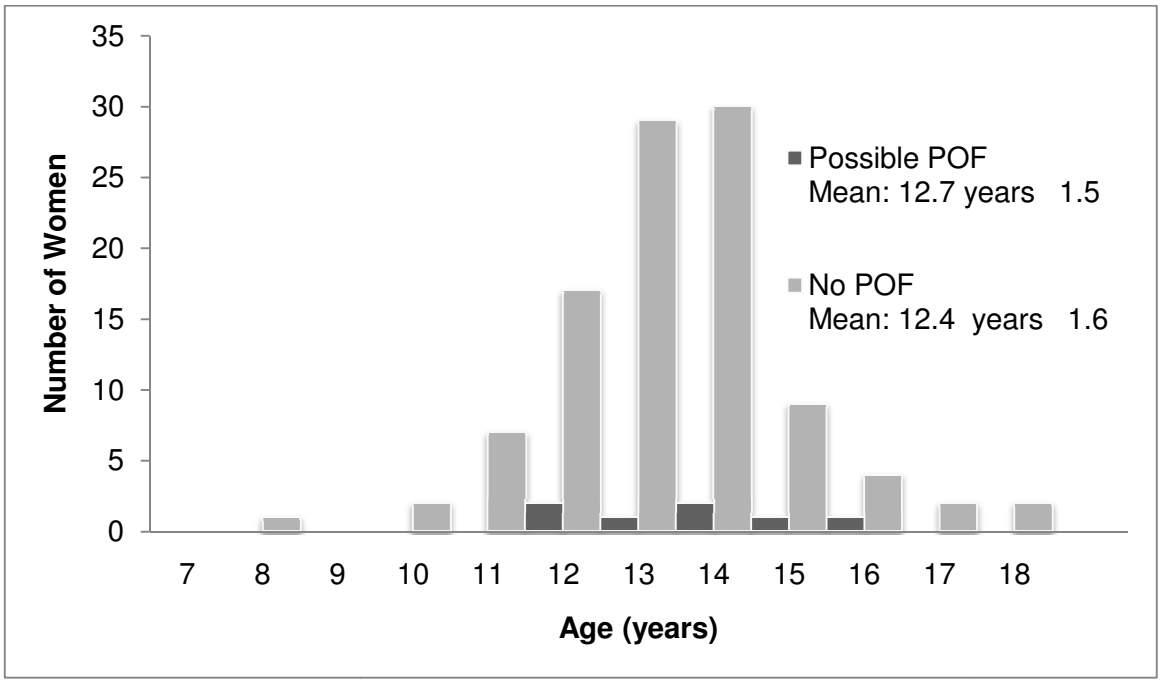


Table 27. Pregnancy			
	Non POF		**Possible POF**
Women pregnant at any time	75 (68.2 %) N=110		5 (71.4%) N=7
Average number of live births	1.8 ± 1.6		1.6 ± 0.5
Average age at first birth	25.6 ± 5.7		21.8 ± 2.9
Number of women who had a preterm birth	5 (8.4%) N=68		1 (20%) N=5
Women who had a voluntary termination	15 (20.0%) N=75		0

Table 28. Miscarriage History		
	Non POF	**Possible POF**
Women who had a miscarriage	32 (43.2%) N=74	1 (20.0%) N=5
Average age of miscarriage	28.6 ± 6.2	31
Average number of miscarriages	1.7 ± 1.1	1

The most commonly reported current or previous reproductive symptoms were hot flashes, night sweats, high blood pressure, and weight gain, each present in over half of women with possible POF (Table 28). Two women indicated receiving a blood test indicating a menopausal state (Table 29). Three had a hysterectomy, and two reported oophorectomy. These occurred at an average age of 43.5 and 44 years, respectively (Table 30). Of the reproductive disorders, diagnoses of infertility, amenorrhea, and autoimmune disease were reported in three women with possible POF; frequencies of all reproductive disorders are provided in Table 31. There were, on average, 4 medications taken by women with possible POF. Categories and frequencies of medications are shown in Table 32.

Table 29. Reproductive Symptoms				
	Non POF Under the Age of 40		POF	
Symptom	Current or previous n (%)	Average duration (years)	Current or previous n (%)	Average duration (years)
Hot Flashes	4 (15.4) N=26	4.5 ± 4.8	5 (83.3) N=6	Unknown
Night Sweats	8 (30.8) N=26	1.3 ± 0.5	5 (83.3) N=6	Unknown
Body Hair	6 (23.1) N=26	12.5 ± 10.7	0 N=6	Unknown
High Blood Pressure*	8 (30.8) N=26	3 ± 0	5 (83.3) N=6	Unknown
Milky breast discharge excluding breastfeeding	6 (23.1) N=26	1.1 ±1.3	1 (16.7) N=6	Unknown
Weight gain over 25 pounds, excluding pregnancy	11 (42.3) N=26	N/A	4 (66.7) N=6	N/A
* p=0.027				

Table 30. Reproductive Blood Tests		
	Non POF Under the Age of 40	Possible POF
Blood Test Indicating:	**n (%)**	**n (%)**
Menopausal State*	0 N=12	2 (28.6) N=7
Thyroid Disease	3 (25) N=12	1 (14.3) N=7
Elevated Testosterone Levels	1 (8.3) N=12	0 N=7
*p = 0.009		

Table 31. Surgical History in Independent Women with TSC

	Non POF		Possible POF	
Surgery	**N (%)**	**Average Age**	**N (%)**	**Average Age**
Hysterectomy	31 (28.7) N=108	35.4 ± 9.8	3 (57.1) N=7	43.5 ± 7.8
Oophorectomy	17 (16.2) N=105	41.2 ± 7.7	2 (28.6) N=7	44 ± 7.1
Radiation therapy	2 (1.9) N=106	Unknown	1 (14.3) N=7 *	Unknown
Chemotherapy	1 (1.0) N=106	45	0 N=7	N/A

*site of radiation unknown

Table 32. Reproductive Diagnoses

	Non POF		Possible POF	
Diagnosis	**N (%)**	**Average Age Diagnosed**	**N (%)**	**Average Age Diagnosed**
POF	2* (1.9) N=108	39 ± 8.5	2 (33.3) N=6	39.5 ± 0.71
PCOS	10 (9.4) N=107	34.7 ± 14.2	0	N/A
Amenorrhea	9 (8.4) N=107	30.6 ± 11.5	1 (16.7) N=6	Unknown
Infertility	12 (11.1) N=108	31.2 ± 6.3	1 (16.7) N=6	28
Anorexia	4 (3.7) N=108	20.3 ± 1.5	1 (16.7) N=6	N/A
Addison disease	1 (0.9) N=107	41	0	N/A
Autoimmune Disease	13 (12.2) N=107	36.6 ± 15.0	0	Unknown

* Excluded from possible POF group due to inconsistent/contradictory information

Table 33. Current Medications (Non-seizure) in Independent Women with TSC		
Type of Medicine	**Non-POF**	**Possible POF**
Number of women reporting medication	87 (79.8%)	4 (57.1%)
Average number of medications used	5.2 ± 4.2	4
Analgesic	36	1
Anti-histamine	18	0
Anti-microbial	8	2
Asthma-related	21	0
Bisphosphonate	8	0
Contraceptives/HRT	20	1
High Blood Pressure	26	1
Multivitamin	35	0
Other	40	4
PPI/GERD	12	2
Psychotropic	43	2
Statin	25	2
Steroids	14	1
Supplement	104	0
Thyroid hormone	21	0

Overall, our data for the entire study population indicated a later age of TSC diagnosis, a higher prevalence of mood disorders, and a less severe course of TSC disease. The seven independent women with possible POF did not appear significantly different in regard to their clinical severity. The average age of TSC diagnosis was 23.7 years (Figure 4). A family history of TSC was reported by six of the women, indicating a lower rate of apparent *de novo* disease. Frequencies of affected relatives with respect to the survey participants are shown in Table 33.

Genetic testing results are shown in Table 34. Three women with possible POF reported receiving testing, but two did not know the result. Unknown mutation testing results was also seen in the entire sample population, providing little data to utilize genotype in hypothesizing a correlation with women's' reproductive history.

Figure 5. Ages of TSC Diagnosis

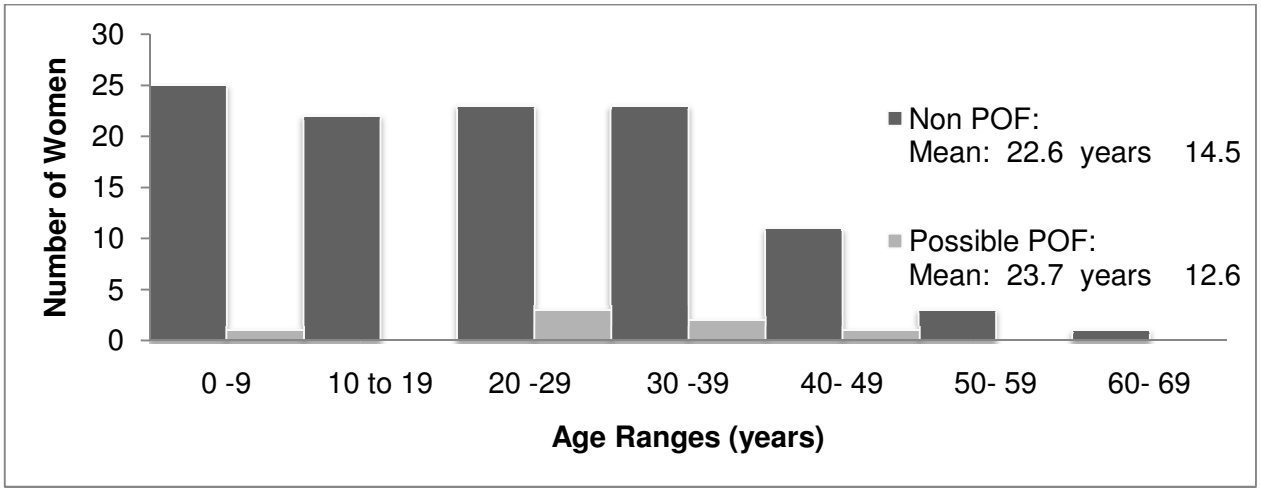


Table 34. Family History Reported in Women with TSC		
	Non POF	**Possible POF**
	n (%)	
Women reporting family history	51 (46.8) N=109	6 (85.7) N=87
Average number of affected relatives	2.33	1.33
Mother	7	2
Father	8	0
Sister	14	1
Brother	10	1
Daughter	28	0
Son	21	2
Grandmother	2	0
Grandfather	3	0
Aunt	3	0
Uncle	4	0
Cousin – Female	4	0
Cousin – Male	1	0
Niece	8	0
Nephew	2	0
Other	4	1
Women with unknown family history	16 (14.7%)	1 (14.3%)
Women with no family history (*de novo*)	42 (38.5%)	0

Table 35. Genetic Testing Results in Independent Women with TSC		
	n (%)	
Women reporting genetic Testing	**Non POF**	**Possible POF**
	61 (55.5) N=110	3 (42.9) N=7
TSC1	6 (9.8)	0
TSC2	10 (16.4)	1 (33.3)
No mutation identified	20 (32.8)	0
Other	2 (3.3)	0
Don't Know result	23 (37.7)	2 (66.7)

Data on seizure history, age of onset, and frequency is shown in Table 35. Four of the seven women reported a history of seizures, with a mean age of onset of two and a half years. All of these women reported a seizure frequency of less than once a year. One women currently uses seizure medication, and the four mentioned above provided information on previous seizure medication use. Data on seizure medication are presented in Tables 36-38. The possible relationship between seizure history, anti-epileptic drug use, and menstrual irregularities is provided in the discussion section.

Table 36. Seizure History in Independent Women with TSC		
Seizure History	**Non POF**	**Possible POF**
Number reporting seizures	55 (50.0%) N=110	4 (57.1%) N=110
Mean age of onset (years)	11.6 ± 12.7	2.5 ± 2.4
Range in age of onset (years)	0.3 – 45	0 – 5
Frequency n (%)	N=49	N=4
At least once a day	4 (8.2)	0
At least once a week, but not daily	4 (8.2)	0
At least once a month, but not weekly	3 (6.1)	0
At least once every six months, but not every month	8 (16.3)	0
Less than once a year	30 (61.2)	4 (100)
Number reporting surgery for seizures	5 (9.1)	0

Table 38. Seizure Medication History in Independent Women with TSC		
	Non POF	Possible POF
	N (% of women with seizure history)	
Reporting current seizure medications	30 (27.3) N=110	1 (25) N=4
Average number of medications	1.6 ± 0.9	1
Reporting previous seizure medications	41 (37.3)	4 (100)
Average number of medications	1.8 ± 1.3	1.4 ± 0.55

Table 37. Current Seizure Medications in Independent Women with TSC

	Name	Number of women	Median dosage (mg)	Median frequency (X / day)	Median years taken	Median Effectiveness
Non POF N=30	Depakote	4	375	3	19	2.5
	Dilantin	2	300	1.5	27.5	2.5
	Felbatol	1	600	4	15	2
	Gabapentin	1	600	2	4	2
	Keppra	9	750	2	3.5	3
	Klonopin	2	.75	2.5	6.5	3
	Lamictal	7	300	2	4	3
	Lorazepam	1	1	1	13	1
	Phenobarbital	6	60	1	36	2.5
	Tegretol	8	350	2	20	3
	Topamax	4	175	2	2	2
	Trileptal	1	1800	1	9	3
	Valium	1	5	1	6	3
	Zonegran	2	75	2	4	2
Possible POF N=1	Neurontin	1	300	3	10	3

Table 39. Previous Seizure Medications in Independent Women with TSC

	Name	Number of women	Number years taken	Average Effectiveness
Non POF N= 40	ACTH	1	1	3
	Carbimazole	1	6	2
	Cortizone	1	1	3
	Depakote	7	1	1.5
	Dilantin	24	3.75	2.5
	Keppra	1	2	1
	Lamictal	1	1	1
	Mogadon	1	-	3
	Mysoline	1	0.5	1
	Neberil	1	-	1
	Neurontin	6	1	1
	Phenobarbital	15	6.5	3
	Tegretol	11	5.5	2
	Topamax	1	-	-
	Trileptal	1	-	-
	Unknown	2	-	-
Possible POF N=4	Dilantin	2	14	2
	Phenobarbital	1	2	2
	Tegretol	1	-	1

Depression was the most common psychiatric manifestations of TSC in these seven women, present in five women (Table 39), with an average duration of 27 years. This adds to the high numbers of depression observed in the entire study sample.

Table 40. Diagnosis of Neuropsychiatric Disorder in Independent Women with TSC					
Non POF Independent Women			**Possible POF Independent Women**		
Disorder	**Current or previous diagnosis N (%)**	**Average age diagnosed (years)**		**Current or previous diagnosis N (%)**	**Average age diagnosed (years)**
Learning Disability	25 (23.4) N=107	8.4 ± 4.6		1 (16.7) N=6	18
ADHD	7 (6.6) N=106	15.6 ± 7.2		0	-
Autism	0	-		0	-
MR	0	-		0	-
Dyslexia	6 (5.6) N=107	14 ± 5.7		1 (16.7) N=6	Unknown
OCD	6 (5.7) N=106	23.3 ± 9.9		0	-
Depression	49 (45.0) N=109	28.2 ± 10.8		5 (71.4) N=7	27
Bipolar Disorder	5 (4.7) N=106	33.2 ± 10.9		0	-
Other	14 (28.6) N=49	28.7 ± 11.9		1 (33.3) N=3	14

Lastly, the manifestations of specific organ systems affected by TSC are presented in Tables 40 through 44. Aside from the differences in the aforementioned dermatological manifestations a comparable number of women indicated the presence of renal, pulmonary, and cardiac issues in both groups.

Table 41. Overall Organ System Involvement in Independent Women with TSC			
	n (%)		
Organ System	**Non POF N=110**		**Possible POF N=7**
Dermatological	106 (96.4)		5 (71.4)
Renal	65 (59.1)		4 (57.1)
Pulmonary	58 (52.7)		4 (57.1)
Cardiac	28 (25.5)		4 (57.1)

Table 42. Dermatological Involvement in Independent Women with TSC			
	n (%)		
Manifestation	**Non POF N=110**		**Possible POF N=7**
Angiofibromas	91 (82.7)		4 (57.1)
Hypopigmented Macules*	97 (88.2)		5 (74.1)
Fingernail / Toenail Tumors	68 (61.8)		3 (42.9)
* This finding was statistically significant, with a p value of 0.021			

Table 43. Renal Involvement in Independent Women with TSC			
	n (%)		
Manifestation	**Non-POF N=110**		**Possible POF N=7**
Renal Cysts	51 (46.4)		4 (57.1)
Angiomyolipoma	52 (47.7)		2 (28.6)
Renal Cancer	3 (2.7)		0

Table 44. Pulmonary Involvement in Independent Women with TSC			
	n (%)		
Manifestation	**Non-POF N=110**		**Possible POF N=7**
Pulmonary Cyst	26 (23.6)		2 (28.6)
LAM	29 (26.4)		3 (42.9)
Shortness of Breath with Exertion*	41 (37.3)		5 (71.3)
* p = 0.024			

Table 45. Cardiac Involvement in Independent Women with TSC			
	n (%)		
Manifestation	**Non-POF N=110**		**Possible POF N=7**
Rhabdomyomas	4 (3.6)		1 (14.3)
Irregular Heartbeat	27 (24.5)		4 (57.1)

Table 46. Women with Possible POF: Statistically Significant Findings			
	n (%)		(p<0.005)
Finding	**Possible POF**	**Non-POF**	**p value**
Shortness of Breath with Exertion	5 (71.3) N=7	41 (37.3) N=110	0.024
High Blood Pressure	5 (83.3) N=6 *	3 (25) N=12	0.024
Blood Test Indicating Menopausal State	0 *	2 (28.6) N=7	0.009
Hypopigmented Macules	5 (74.1) N=110	97 (88.2) N=7	0.021
* Comparison was limited to independent, non-POF women between the ages of 30 and 40			

Overall, our data for women with possible POF is based on clinical refinement of self-reported menstrual histories and trends. The limited sample size makes a comparison to women whose histories were not suggestive of POF difficult. However, the small differences noted and other observations in the overall population do warrant further discussion.

Discussion

This study was designed to estimate the prevalence of premature ovarian failure in a sample of women affected with TSC. It is the first study to highlight significant menstrual dysfunction in women with TSC. The hypothesis was based on evidence from conditional knock-out mice models that indicated the importance of the TSC genes and mTORC regulation in murine ovarian function. The aim of the study was to determine what, if any, association may exist between the development of POF and TSC in women. Questionnaires were sent to 1000 adult female members of the Tuberous Sclerosis Alliance, a national support group, providing self-reported data on demographics, reproductive history, and TSC manifestations. A total of 182 qualifying surveys were informative and used in the analysis, and 18 surveys were excluded due to insufficient information or underage respondents. Demographic analysis demonstrated a high socio-economic status for this group of 182 women. TSC clinical data suggested milder disease than typically observed in TSC patients. As variable expressivity is a well-established feature of TSC, we did not anticipate that milder disease would underestimate the presence of reproductive issues in these women. In fact, given this caveat, our study would be predicted to underestimate reproductive problems in the overall TSC female population.

POF

Our data suggest that there is an increase in the prevalence of POF in women with TSC compared to the general population. Analysis of 182 women categorized 9 with “possible POF” (4.95%). Two of these women reported receiving a diagnosis of POF. The selection of women from which to consider the possibility of POF was created through application of stringent reproductive and menstrual criteria. Since the diagnosis of POF involves the cessation of menses before the age of 40, self-reported data of menstrual history for time frames between 18 and 40 were used as an initial filter to categorize women. The natural history of POF typically involves regular menses, followed by irregular cycles, before the complete cessation of menses. Therefore, women considered for a categorization of “possible POF” were identified by menstrual trends indicating consistently regular cyclicity progressing to consistently irregular cyclicity as previously defined in the Methods. Our refinement resulted in

a sample of 18 women. Of these, nine were excluded upon further analysis of clinical features, as their histories were consistent with other reproductive disorders, such as PCOS. The remaining eight women included seven independent respondents and one dependent respondent. Independent women were those that completed the questionnaire themselves, whereas dependent women were those for whom a questionnaire was completed by a caretaker, suggesting more severe TSC manifestation or cognitive disability. Because our initial comparisons of independent and dependent women in the entire sample of women revealed differences between these groups, the dependent woman was excluded from further analysis of the group with possible POF. Furthermore, it is less likely that women would present with POF in their 20s, so comparison between the seven independent women with possible POF was limited to independent women over the age of 30 years. Choosing this age constraint allowed for a more direct comparison of groups.

Few studies have empirically assessed the prevalence of POF in the general population. The literature primarily quotes an estimate of 1% that comes from a longitudinal study from 1983 [Coulam]. Previously, estimates of POF came from cohort studies analyzing specific and narrow age ranges. Coulam, et al. followed a cohort of 1858 women and found an incidence of 0.9%. They also reported that the risk to develop POF increases from 0.1% at age 30 to 1% by 40 years of age; a ten-fold increase over a ten year period. Thus, our finding of possible POF in nine (4%) of women studied is a significant finding, particularly as only two of these women reported receiving a diagnosis of POF. As discussed earlier, there is often a delay in the diagnosis of POF, especially for women who are not struggling with conception. Other causes for delayed diagnosis are varied symptomatology and other etiologies for isolated menstrual irregularity. In women with a genetic condition that affects multiple organ systems, attention to additional symptoms or medical problems may be misconstrued as related to TSC or overlooked entirely. No studies have previously attempted to assess if there is an association between TSC and menstrual irregularities such as POF.

Because we were not able to obtain medical data from women, we do not know if the women with possible POF would have had elevations in FSH levels, necessary for a clinical diagnosis. Thus our results fit with a broader possibility of premature ovarian insufficiency, or POI, as was introduced earlier. This term encompasses declining ovarian reserve, which we observed in analyzing women's menstrual trends. The distinction is an important one, as we lack clinical data to confirm diagnoses in these women, and the accuracy of "insufficiency" better reflects a condition marked by reversibility and many unknowns.

Analysis of women with possible POF was performed by comparing all variables for significant associations. There was no statistically significant association between women with or without possible POF with respect to age, demographic information, BMI, medication use, seizure history, age at menarche, number of pregnancies or miscarriages, genetic testing results, neuropsychiatric diagnoses, or TSC-related manifestations in cortical, pulmonary, cardiac, or renal systems. The only statistically significant findings among these women in comparison to the independent women without possible POF were related to reproductive symptoms, report of receiving a blood testing indicating a menopausal state, and the presence of facial angiofibromas, which were present in a smaller percentage (57.1%) of women with possible POF compared to those without POF (82.6%). These findings could represent a true association, or may be due to limitations of a small sample size or statistical chance. High blood pressure and shortness of breath are both prevalent in the general population, particularly in women at older ages. It may be expected that women experiencing menstrual irregularity would have a blood test indicating a menopausal state, which may not necessarily lead to a diagnosis of POF. As for the dermatological finding, additional manifestations of these women were consistent with the rest of the sample, making it more likely that these women were at increased risk for POF due to specific manifestations of TSC. The absence of significant differences between women is consistent with the theoretical possibility that TSC can manifest in any organ system, and does not suggest an increased likelihood in women with more severe TSC disease from our specific sample. There was little data on genetic testing results to uncover genotype/phenotype correlations, and thus further exploration of reproductive issues in women with TSC would be useful in conjunction with molecular information. It is noteworthy that there were no differences between the POF in mice with a *Tsc1* specific ovarian deletion compared to *Tsc2* [Adhikari, 2010]. Other reproductive problems revealed in these women suggest implications for ovarian function from mutations in the TSC genes and warrant further study.

Additional data about reproductive history were considered in the eight women with possible POF. Two women reported receiving radiation therapy, though we do not know the ages when the treatment occurred. One woman indicated the therapy was for SEGA treatment, while the other did not indicate a location. Radiation to the brain could disrupt the hypothalamic-pituitary-ovarian axis, indirectly disrupting ovarian function. Alternatively, it is known that pelvic irradiation can lead to POF, and shows an age and dose-dependent effect [Nippita]. From the information given we cannot exclude either possibility for these respondents, nor can we discern the likely effects of radiation on menstrual function without knowing the ages when therapy occurred. Next, one woman did indicate a diagnosis of

anorexia from the list of reproductive disorders. Anorexia is a known contributor to menstrual irregularity, but the respondent did not specify an age at which the diagnosis of anorexia occurred, negating a clear interpretation of any potential effects on the menstrual history. Anorexia is more common in adolescence (ages 14-18), though recent evidence suggests it may also be common among older women [American Psychiatric Association; Parker-Pope]. The study participant was 57 at the time the survey was completed, and her menstrual irregularity began at age 37, though she did report use of oral contraceptives between the ages of 21 and 30. She has symptoms of high blood pressure, takes Metformin, and has a BMI over 32; thus it is unlikely her diagnosis of anorexia was recent given her history. Without further information, we cannot depend on the respondent's history of anorexia alone as a route to menstrual irregularity. Report of additional reproductive problems varied across the 8 women but did include reproductive symptoms of night sweats and hot flashes, infertility, and/or blood tests indicating a menopausal state. These would be consistent with a diagnosis of POF, as women with POF experience perimenopausal symptoms at an earlier age than is expected, and may be evaluated for reproductive dysfunction by infertility specialists or as they near the age of menopause. Our reproductive and menstrual data indicate the criteria used for considering women for POF are reliable, as most women with a consistent menstrual history lacked contradictory information and instead contributed additional characteristics pointing to a possibility of POF

Lastly, we analyzed if there were associations between POF and a history of seizures, as well as current /previous seizure medications. Reproductive endocrine disorders and infertility are more common among women with epilepsy than the general population, observations that have been attributed to both epilepsy itself and the use of anti-epileptic drugs (AED)s. for treatment [Isojarvi]. Epilepsy may directly affect reproductive function through disrupted regulation of hypothalamic hormone release [Pimentel]. Additionally, many women with epilepsy have catamenial seizures, presenting during menses and menopause when the levels of steroid ovarian hormones change. The risk from AEDs is thought to originate from their pharmacological induction of liver enzymes that gradually decrease the bioactivity of ovarian hormones and androgens [Isojarvi]. Additionally, valproic acid has been reported to have a possible effect on fertility, while limited data exists for the effects of gabapentin and lamotrigine on reproductive function or fertility [Kaplan 2004].

We assessed if the use of AED or seizure history could be a possible explanation for the etiology of possible POF in the sample of eight women. Two women reported use of current seizure medications. These were lamotrigine, topirimate, and gabapentin. The two former

medications were reported as current from one woman and were used for 16 years. The time frame when the study participant reported an onset of irregularity is that immediately following the start of her medication use. She also had the highest frequency of seizures of the women with possible POF, occurring no greater than twice per month. Current use of gabapentin was reported by one woman, for ten year duration. She reported the onset of menstrual irregularity at age 38, approximately three years following the first use of this medication; she also reported her last seizure occurred at age 28. Previous seizure medication use was reported for five of the eight women with possible POF, and included phenytoin, valproic acid, phenobarbital, and carbamazepine. None of these medications were used more than 14 years, and the majority were ceased at least ten years prior to the onset of menstrual irregularity. We cannot completely exclude the possibility that these medications had an effect on the menstrual function of these women. However, there were also three women in the group with possible POF that reported no history of seizures, and three women who reported an early childhood onset of seizures and cessation of AED use several years prior to the onset of menstrual irregularity. Our data suggest that neither seizures nor seizure medications alone would be sufficient to account for the onset of POF in all women.

There were nine women who were excluded upon further refinement of the group showing a history of menstrual regularity to irregularity. Clinical guidance from a reproductive endocrinologist suggested other reproductive disorders in these women, including PCOS (one subject reported a diagnosis of PCOS). We also found that overall, 31% of women with TSC experienced some sort of menstrual irregularity. A number of women in the entire sample reported a diagnosis of amenorrhea and infertility, mostly diagnosed by gynecologists and infertility specialists. But a greater number with various forms of menstrual irregularities did not report a diagnosis of any reproductive disorder. Furthermore, only a handful of these women indicated having a blood test showing a menopausal state, thyroid disease, or elevated testosterone levels. Overall our data suggest there is a lack of awareness of reproductive issues in women with TSC. In light of missing data and an absence of medical chart review, it is likely we have underestimated reproductive dysfunction in general and POF in particular for the TSC population studied. Thus, thorough evaluation and attention to menstrual history, as well as proper hormonal assessment of women with TSC is indicated, and further research will aid in determining specific risk factors and characteristics of this issue.

Additional Findings from the entire sample of women

Reproductive History

Although not originally intended as part of the study design, several other findings are notable. The age of menarche in our sample was consistent with the national average (12.3 years), and points to intact menstrual functioning at the onset of menses [Anderson]. In general, it is not uncommon for young women to display pubertal irregularities of abnormal uterine bleeding during the initial 19 months of menstruation, typically reported with anovulatory cycles due to an immature hypothalamic-pituitary-ovarian axis [Lemarchand-beraud]. The duration of time that it takes to establish regular ovulatory cycles may to be related to age at the time of menarche, and in most girls who begin menses between the ages of 12 and 13, 50% of cycles are ovulatory by three years [Apter]. As the women in our survey had an average age of menarche of 12, with a 75th percentile of 13, it would be expected that any pubertal irregularities would normalize by the age at which data collection began, 16 years. Thus, irregularities in the first time frame surveyed (16-20 years) were not considered to be due to pubertal irregularity and were instead counted as irregular. Initial normal menstruation is consistent with a gradual progression to later development of POF.

Another metric for reproductive health is a history of pregnancy and miscarriage. We found that 41.8% of women who reported ever being pregnant experienced a miscarriage, higher than observed in the general population. The approximate distribution of miscarriage rate estimates in the general population varies with maternal age; below 35 years, it is estimated that women have a 15% risk to experience miscarriage; between 35-39 years, a 20-25% risk; between 40-42 years, 35% risk, and over 42 years, a 50% risk [American Pregnancy Association]. We also observed that the average age when these miscarriages took place in our sample (28.5 years) was younger than the above ranges. A higher miscarriage rate at younger ages may point to separate or additive effects related distinctly to TSC and not seen in the general population. Lastly, 50% of the women who experienced miscarriage had a menstrual history of always regular cycles, suggesting that menstrual irregularity alone is not a sufficient predictor of miscarriage risk in women with TSC. Furthermore, as dependent women generally became pregnant less often, we have limited data to assess whether severity of TSC symptoms might play any role in the risk for miscarriage. Of 33 women reporting miscarriage, 17 (42.5%) also reported a history of seizures, while 16 (45.7%) reported no history of seizures. Studies of pregnancy women with epilepsy have not observed significantly elevated rates of fetal loss, though it does occur. Similar to the effects of epilepsy on menstrual functioning previously discussed, it is difficult to differentiate between risks from seizures and

AED use [Kaplan 2007]. A prospective study of pregnant women with epilepsy from 1994 observed 21% rate of fetal loss in a group of 119 women receiving AEDs, and a 12.2% rate of fetal loss in 106 women not receiving medication [Steegers-Theunissen]. The elevated rates of miscarriage at young ages suggest a distinct finding for women with TSC, for whom there is little information on predictive factors or breadth of this issue.

Of the reproductive diagnoses reported and menstrual histories consistent with specific reproductive dysfunction, PCOS was self-reported in 11 (6.04%) of the 182 women. We also identified four women whose information was more consistent with that seen in PCOS from our initial group of 18 women with a menstrual history of regular. PCOS is among the most common menstrual irregularities in women today, with an estimated prevalence between 6 and 8% [Knochenhauer]. Risk factors and/or associations include infertility, insulin resistance, diabetes mellitus, and family history [Allen; Hartz; Conn; Legro]. Of note, studies also indicate the use of valproic acid to be associated with PCOS, and the drug may increase androgen biosynthesis in theca cells [Bilo; Nelson-DeGrave]. Many healthcare providers monitor the length of the menstrual cycles in women with epilepsy after commencement of treatment with valproate [Isojarvi; Kaplan 2004]. A total of 14 women reported current use of valproate, and of these, one indicated a diagnosis of PCOS, at age 45. Twenty-one women reported previous use of valproate, one of whom indicated a diagnosis of PCOS at age 48.

Another common cause of menstrual irregularity, particularly oligomenorrhea and menorrhagia, is hypothyroidism. A study reported such irregularities in approximately 23.4% of women with hypothyroidism [Krassas]. The prevalence of overt hypothyroidism may vary from 0.1 to 2 percent in the population, while subclinical hypothyroidism is higher, ranging from four to ten percent of adults, and is 5-8 times more common in women [Tunbridge; Kajantie]. We observed 43 women (24.0%) reporting a blood tests indicating thyroid disease. Of the group of 18 women progressing from regular to irregular periods, four reported receiving a blood test indicating thyroid disease, and one of these women is included in our group of possible POF. Of the women with alternating cycles, six indicated thyroid disease, one of whom specified a diagnosis of Hashimoto thyroiditis. Lastly, six of the 24 women whose menstrual history was consistently irregular indicated thyroid abnormalities. Though we do not know the specific type of thyroid disease we did observe menstrual abnormalities in approximately half of the 43 women with self-reported blood tests indicating thyroid disease, higher than that reported in hypothyroidism.

Menstrual and reproductive problems specific to the TSC population remain unreported in the general population and thus all of these novel findings are relevant to TSC. The true

menstrual function of the women whose menstrual history was deemed uninformative is unknown, whether obscured by the use of oral contraceptives or intrauterine contraceptive device, terminated with reproductive surgery, or simply missing from the analysis. The data presented above points to an increased risk for a number of menstrual irregularities in women with TSC in addition to the study question of interest (POF). Furthermore these irregularities appear to occur with or without a history of seizures or AED use, and outside of correlations to TSC severity or genetic testing results. At the very least, further attention to reproductive health is necessary for women with TSC, and further research is needed to further identify both risk factors and more accurate estimates of the prevalence of such reproductive disruptions.

TSC Diagnosis

Review of the data characterizing the TSC of these women revealed some noteworthy results. The mean age of diagnosis of TSC was 15 years, ranging from prenatally to 62 years. These ages are later than expected, particularly when considering the number of features present in infancy or early childhood. However, because of variable expressivity of TSC, it is not uncommon for individuals to have a very mild disease and thus subtle presentation. Adults are often diagnosed when more severely affected child or family member is ascertained. Additionally, 103 of women were age 40 or older at diagnosis, and it is plausible that many went undiagnosed prior to increased access and knowledge of genetic services in medicine and awareness among practitioners. We analyzed these two groups for significant differences among those diagnosed at a younger age (less than 18 years), and those diagnosed later in life (after the age of 18). First, as might be expected, there was a greater severity of TSC in those individuals diagnosed at a younger age, indicated by a higher prevalence of intellectual disability, learning disabilities, and autism. Furthermore, there were higher percentages of angiofibromas, hypopigmented macules, angiomyolipomas, cardiac rhabdomyomas, and seizures in the individuals who were diagnosed at a younger age. These results are not surprising as many of these clinical features are clues to the diagnosis of TSC. Additionally, of the 99 individuals who were diagnosed at a younger age, over 70% of them indicated they did not have a family history of TSC (this is consistent with the *de novo* mutation rate), or did not know. A lack of awareness of a dominant genetic condition in one's family might also impede a definitive diagnosis. In contrast, women diagnosed with TSC after the age of 18 had an increased frequency of blood tests indicating a menopausal state, a higher pregnancy history, higher report of reproductive menopausal symptoms including hot flashes and night sweats, higher diagnoses of autoimmune disease, and a higher numbers of reported hysterectomies.

Some of these findings would be expected in older respondents, and accordingly, the average age of 82 respondents who were diagnosed at age 18 years or older was 51.1 years (±10.6). Thus, the findings discussed are more likely to be found in a population of older women. Despite these possible confounders, the later age of diagnosis in a sample of mostly independent women who belong to a national support group likely underestimates a more widespread lack of awareness and diagnosis of affected individuals nationwide, especially those with mild disease.

Mood Disorders

Another important finding from the data is the high prevalence of mood disorders in women affected with TSC. Detailed data is presented in Table 18. The diagnoses of anxiety, depression, obsessive compulsive disorder (OCD), and bipolar disorder (BPD), taken together, were observed in 93 of women (51.1%). Studies examining psychological distress in TSC have found anxiety and depression to be the most common, particularly in women [Pulsifer]. Previous studies have found a prevalence of anxiety of up to 56% and depression of up to 43% in adults with TSC [Lewis; Raznahan]. A large contributing factor to this prevalence of psychiatric illness is the individual's history of seizures. Individuals with epilepsy in the general population have higher rates of depression, anxiety, and other mood disorders, and particularly those individuals with a higher frequency of seizures [Thompson; Kanner]. This background risk makes medication difficult as individuals are often taking additional medication to reduce their seizures. In our sample, 57 women (50%) of those with an apparent mood disorder reported a history of seizures. About 50% of these women reported a seizure frequency of less than once a year, while about 40% reported frequencies ranging from once every six months - once a week. Next, as previously discussed, the rates of depression are also higher in women with a diagnosis of POF [Schmidt], and may present prior to the actual time of diagnosis. In the general population mood disorders are estimated to affect about 9.5% of adult individuals, at an average onset of 30 years [NIMH]. Women are more likely to be affected, particularly those of Caucasian ethnicity, and it is thought that 1 in 8 women will develop clinical depression in some time during her life [NIMH, 1999]. Therefore, it is likely that a woman affected with TSC experiencing menstrual irregularities has an even higher risk to develop mood disorders when compared to any three of these considerations alone. Risk of psychological problems warrants further attention by clinical providers for women with TSC, and they should be counseled accordingly and/or referred to appropriate specialists to optimize their well-being. When we analyzed women reporting a history of mood disorder, we found statistically significant differences in comparison to women without illness. Provided in

Table 18, these included higher percentages of hot flashes and night sweats, consistent with a possible presentation of POF. A higher frequency of reported High blood pressure may be related to side effects of medication, or found commonly in the general adult US female population, but this same finding was also a significant difference among our sample of seven women with possible POF. Regarding mood disorders, we did not observe any differences directly related to manifestations specific to TSC. It may be that women who have a baseline risk for mood disorders from gender alone, increased through the presence of a genetic disorder involving epilepsy, could face a higher risk from problems related to menstrual dysfunction or reproductive issues. It would be prudent for healthcare providers to address these issues with women while managing their TSC. Forty-four women (24.2%) reported use of psychotropic non-seizure medication, and women took an average of 1.5 of these classes of medicine A significant portion of women who reported mood disorder are not receiving medication (49 women, or 52.7% of those reporting a mood disorder). Secondly, as multiple medications are needed and likely contribute to additional side effects which must be considered in light of the number of medications these women are prescribed for the management of TSC alone. Perhaps these women need additional psychiatric support, and would possibly benefit from the use of other services attending to psychiatric illness, including psychotherapy. It also suggests that not all psychiatric manifestations arise from TSC alone, and women may be unaware of additional, unrecognized menstrual problems contributing to their psychiatric health.

Strengths and Limitations

The present study is strengthened by the use of thorough personal reproductive history, and numerous questions aimed at addressing other known causes of POF, and menstrual irregularity. The questionnaire was detailed and thorough. The majority of participants filled it out sufficiently enough for inclusion in the study.. Furthermore, the data are considered reliable, reflecting personal histories of mostly independent women of higher socio-economic status involved in their medical care. Our sample size of 182 presents a good representative number of women affected with TSC, and allows for the identification of a reproductive disorder reported to be present in only 1% of women. The prevalence determined from our study may be an underestimate. A larger sample size would allow the research to identify whether even more women are affected.

Limitations include those common to self-reported questionnaire data. A number of women were old enough to make recollection of menstrual history in the 30s more difficult. Additionally, the use of oral contraceptives could mask other women who may have had unrecognized menstrual dysfunction. Infertility specialists often diagnose women with POF as part of an infertility evaluation. As over 40% of women remained single in our sample, and a number stopped having children by their 30s, fewer women may be aware of underlying menstrual irregularities. We were also limited in our inability to verify diagnosis of either TSC or POF in these women. However, when considering dermatological, renal, cardiac, pulmonary manifestations of TSC and the presence of seizures, we observed the presence of at least one affected organ system or seizure history in 100% of women. Ninety-three percent of women had more than one affected organ system or a seizure history, over two-thirds have manifestations in at least three areas, and 9.34% had manifestations in all five. We would thus expect these women were indeed affected with TSC. The second issue was addressed by applying stringent criteria to evaluate menstrual history for the presence of POF. Menstrual trends of regular cycles to irregular cycles were an initial filter, and women with inconsistent or contradictory information were further excluded. Additionally, a diagnosis of POF requires corresponding values of LH and FSH, which we were not able to obtain. These issues and other missing data may contribute to an underestimate of other women with an unrecognized progression to menstrual irregularity or POF. Our sample population reflects a bias not uncommon in study populations ascertained from national support groups, and may be less reflective of TSC, particularly as our analysis of mostly independent women appearing to have milder disease was evidenced by the lower percentages of women reporting a history of seizures, and fewer indications of cognitive or psychiatric impairment. It is expected that 90%

of individuals with TSC will have or have had seizures, and about half are expected to have some cognitive impairment or psychiatric disturbance [Prather]. Finally, TSC shows no ethnic predilection, but our study was primarily conducted on Caucasian women. Additional risk factors that may or may not be present in other ethnic populations were essentially unstudied in this research

Future Directions

Data from the present study unveils previously unrecognized gender-specific manifestations of TSC and highlights a need for future studies assessing the reproductive health of women with TSC. More work is needed to elucidate the risk of reproductive dysfunction in women with TSC. It will be important to assess longitudinal FSH and LH levels in women with TSC from age 30 to 40. Such laboratory studies along with better reproductive histories would make the diagnosis of POF more definitive and provide a better prevalence in the TSC population. Women receiving radiation therapy in the brain may need to be monitored for ovarian function following treatment and recognition of possible effects on the hypothalamic-pituitary axis. Lastly, referral to reproductive specialists may be important, particularly to bring attention to other disease separate from more familiar manifestations of TSC.

Traditional mechanisms of TSC associated manifestations involve loss of heterozygosity and haploinsufficiency [(Castro; Wilson]. It is unknown which, if either, of these are likely responsible for the effect on ovarian function observed in the women studied. The original data from the mouse models suggested that LOH would be the likely mechanism of POF. Complete loss of either *Tsc1* or *Tsc2* in primordial follicles led to increased activation of mTORC1 and the entire population of oocytes [Adkihari 2009, 2010]. The mutant mice were initially fertile with normal litter sizes; however litter size progressively decreased until POF and infertility occurred. Our data suggests that similar events may occur in the ovaries of women with TSC. However, in humans, haploinsufficiency may be a more likely explanation to account for the gradual and magnitude of decline necessary to deplete ovarian reserve. The data suggest that it may be useful to study women with TSC for signs of premature ovarian failure and other reproductive disorders. These data also raise the intriguing possibility that the use of mTORC1 inhibitors may be a potential treatment for TSC-related menstrual dysfunction. Trials of the compound rapamycin, a potent and specific mTORC1 inhibitor, have shown great promise for SEGAs, angiomyolipomas, and facial angiofibromas. If up-regulated mTORC1 is demonstrated to be a main mechanism of POF in women with TSC, then perhaps rapamycin might be a feasible treatment for this newly appreciated manifestation of TSC. Unfortunately

the potential teratogenicity of rapamycin might present a problem in its use as a treatment for POF. Rapamycin is registered as Class C and animal studies have indicated embryotoxicity and fetotoxicity, which include intrauterine fetal demise, reduced weights, and delayed ossification. No adequate studies of pregnant women exist, though successful use of this drug during pregnancy has been reported [Jankowska].

Conclusion

Our study is the first to assess the presence of menstrual irregularities and reproductive dysfunction in a TSC population. We uncovered a significantly elevated prevalence of POF in a sample of 182 women belonging to the Tuberous Sclerosis Alliance. The majority of women were independent and mildly affected with TSC. They responded to a thorough questionnaire and self-reported history of menstrual health and reproductive function, as well as trends in menstrual cyclicity. These data were filtered through stringent criteria to determine women who progress from regular cycles to irregular cycles, consistent with the development of POF. Additional data on reproductive and menstrual health was used to support the possibility of or exclude a categorization of possible POF. Nine women were deemed to have possible POF. This figure is higher than that previously suggested in the general population, and parallels molecular research reported in conditional knock-out mice models of TSC. The research suggested dysregulation of mTORC leading to problems in murine ovarian function, and indeed, this may also be implicated in women's health. When comparing these 9 women with possible POF to independent women in the sample population over the age of 30, no connections to other variables, overall TSC severity, or a history of seizures / anti-epileptic drugs were found. These results suggest that TSC per se is likely predisposing these women to POF.

In addition to specific results of POF in this population, we identified other notable findings, also previously unreported. First, we observed that the women in our sample experienced a higher rate of miscarriage, and at an earlier age than is expected for the general population. In our analysis of menstrual trends, we found that over one-third of women had some sort of irregularity, and may be an underestimate as we could not analyze data for women using oral contraceptives or those that did not recall their prior menstrual regularity. Outside of menstrual health, we also found that the women studied had a later age of diagnosis than is typical for TSC. As would be expected, individuals who were diagnosed earlier were more likely to have seizures and be dependent. Lastly, the presence of mood disorders such as depression, anxiety, OCD, and BPD were elevated in our population, signifying a possible additive effect of gender, the genetic disorder TSC, and menstrual

irregularities on mental health. Furthermore, not all of these women were receiving psychotropic medication. More attention is needed to resolve some of these issues with women's well-being, in addition to increased awareness of other reproductive issues in women with TSC. In the absence of clinical data but with stringent menstrual trend criteria, supporting reproductive data, and reliable self-reporting, our findings present a reasonable possibility of POF or ovarian insufficiency in women with TSC. However, a lack of reporting and a sample population with some ascertainment bias calls for further work to elucidate this risk and bring attention to other issues in these women. The use of clinical laboratory information will further the research suggesting the possible connection with TSC and POF.

Appendix A. Survey Questionnaire

Tuberous Sclerosis Complex / Reproductive Health Questionnaire

This questionnaire is designed to learn if Tuberous Sclerosis Complex (TSC) affects a woman's reproductive health. Specifically, we are interested in a condition known as Premature Ovarian Failure (POF), in which the ovaries stop working normally before the age of 40. Currently very little is known about how TSC might affect ovarian function, but more information will be helpful to people with TSC. We have designed this questionnaire to learn a little about you or your affected family member (demographics), about the severity of your TSC, and about your past and current reproductive health. Please keep in mind no personal information will be used. We greatly appreciate your help with this research project.

Instructions:

The enclosed envelope includes return postage for one survey. If there is more than one female with TSC over the age of 18 in your household we ask that you complete a questionnaire for each individual. You may make a copy of this survey and provide your own postage to return it. Or you may request another one, either through the TS Alliance, by emailing tscstudy@yahoo.com, or by phone to Emily Gabitzsch (713 500 5760). We will not keep or share any of your personal information.

Please answer the questions (circle the numbers, responses, or write in as requested) about you or your affected family member as best as possible and return the questionnaire in the enclosed addressed envelope.

If you are unsure about an answer or do not wish to respond, please mark "Don't Know (D/K)" or leave it blank. Should you have any specific questions about the survey, you can send an email to tscstudy@yahoo.com for assistance.

These questionnaires are anonymous. Please do not include your name, address or any other information that would allow us to identify you. Please be aware these questions are for research purposes only and are not related to the medical supervision by your primary, or specialty healthcare providers. We thank you very much for sharing this information. Results of this study will be available through the Tuberous Sclerosis Alliance.

Sincerely,

Michael J. Gambello, MD, PhD
Associate Professor of Pediatrics
UT Health

Emily Gabitzsch, BS
Genetic Counseling Student
UT Health

*******Is the person filling out this questionnaire affected with TSC?*******

☐ Yes: please continue to Part 1
☐ No: **please answer all of the questions <u>with respect to the individual with TSC</u>**

Part 1 – Demographics:

Age: _____ ***Height*** _____ ***Weight*** _____

1) What is your ethnicity?

☐ Caucasian, non-Hispanic

☐ African-American

☐ Hispanic

☐ Asian

☐ Other, please specify _____

2) What is your current marital status?

☐ Single
☐ Married
☐ Divorced

☐ Widowed

3) Who do you currently live with?

☐ Alone
☐ With family members

☐ With a significant other

☐ With unrelated individuals in an assisted living environment

☐ Other, please specify _____

4) What is the highest level of education you've completed?

☐ Under 12th grade

☐ Completed 12th grade

☐ Some college

☐ Associate's degree

☐ Bachelor's degree

☐ Master's degree or higher

5) What is your current employment status?

☐ Unemployed

☐ Employed part-time

☐ Employed full-time

☐ Student

☐ Other _____

6) Optional: Which of the following best describes your approximate household yearly income?

☐ Less than $10,000

☐ $10,000 – $24,999

☐ $25,000 – $49,999

☐ $50,000 – $74,999

☐ $75,000 – $100,000

☐ More than $100,000

☐ Prefer not to answer

Part 2 – General and Reproductive Health:

7a) Have you ever had a period? (circle one)

Yes No (please skip to question 9)

b) If yes, how old were you when you had your first period? _____ years

8a) Have you ever been pregnant? (circle one)

Yes No *(please skip to question 9)*

b) How many live births have you had? _____

c) How many preterm births have you had, if any? _____

d) Please indicate your age at each delivery, in years

Pregnancy #	My age at delivery, in years
1	
2	
3	
4	
5	
6	
7	

e) Have you ever had a miscarriage? (circle one)

Yes No Don't Know

f) If yes, please indicate your age at each miscarriage

Miscarriage #	My age, in years
1	
2	
3	
4	
5	
6	

g) Have you ever had a voluntary termination? (circle one)

Yes No Don't Know

h) If yes, how many? _____

9) The chart below is about your menstrual history, since you first got your period. Please <u>circle the appropriate response in each column for each range</u>.

*****PLEASE FILL OUT THE CHART FOR EACH AGE RANGE <u>UP TO AND INCLUDING YOUR CURRENT AGE</u>.**For example, if you are 22, please only complete rows 1 and 2. If you are 36, please complete rows 1 through 5, etc.***

. Row #	Age range (in years)	*For the <u>MAJORITY</u> of this age range, my periods were :*		*For the <u>MAJORITY</u> of this age range, I took :*	
		Regular: *10 or more periods per year*	**Irregular:** *9 or less periods per year, or 0* **OR**	*Oral contraceptive pills or had an intrauterine contraceptive device (IUD) in place:*	
1	**16-20**	Regular	Irregular	Yes	No
2	**21-25**	Regular	Irregular	Yes	No
3	**26-30**	Regular	Irregular	Yes	No
4	**31-35**	Regular	Irregular	Yes	No
5	**36**	Regular	Irregular	Yes	No
6	**37**	Regular	Irregular	Yes	No
7	**38**	Regular	Irregular	Yes	No
8	**39**	Regular	Irregular	Yes	No
9	**40**	Regular	Irregular	Yes	No

10) Please indicate what medications, supplements, and/or vitamins you are <u>currently</u> taking. Please specify the name and dose, if known.

Name	***Dose***	***How often you take this medicine***

11) Please choose the appropriate response in EACH row to indicate if you have experienced any of the following symptoms, within the past year or at any time, and indicate how long those symptoms continued, in years.

Symptom	**Ever**		**Within the past year**		**Overall duration (in years)**
Hot Flashes	Yes	No	Yes	No	
Night Sweats	Yes	No	Yes	No	
Excess body hair	Yes	No	Yes	No	
Acne	Yes	No	Yes	No	
High blood pressure	Yes	No	Yes	No	
Milky discharge from the breasts (when not breastfeeding)	Yes	No	Yes	No	
Weight gain over 25 pounds, not including gain during pregnancy	Yes	No			

12) Have you ever been diagnosed with any of the following? Please circle the appropriate response in EACH row. To indicate who diagnosed you, please select from:

<u>General physician, Infertility specialist, endocrinologist, Ob-Gyn, Other, specify:</u>

Name	**Diagnosed**			**Age (years)**	**Diagnosed By**
Premature Ovarian Failure	Yes	No	Don't Know		
Polycystic Ovarian Syndrome	Yes	No	Don't Know		
Amenorrhea (no menstrual period)	Yes	No	Don't Know		
Infertility	Yes	No	Don't Know		
Anorexia	Yes	No	Don't Know		
Galactosemia	Yes	No	Don't Know		
Turner Syndrome	Yes	No	Don't Know		
Addison Disease	Yes	No	Don't Know		
Autoimmune Disorder (i.e., Lupus)	Yes	No	Don't Know		

13) Have you ever had a blood test showing the following? Please circle the appropriate response for EACH row.

Menopausal state	Yes	No	Don't Know
Thyroid disease	Yes	No	Don't Know
High testosterone levels	Yes	No	Don't Know

14) Have you had any of the following surgeries/procedures? Please circle the appropriate response in EACH row.

Procedure	Performed			Age when performed (in years)
Hysterectomy (removal of uterus)	Yes	No	Don't Know	
Oophorectomy (removal of ovaries)	Yes	No	Don't Know	
Radiation therapy	Yes	No	Don't Know	
Chemotherapy	Yes	No	Don't Know	

15)Please indicate if you have any family members who have also been diagnosed with any of the conditions below.
Please indicate their relationship to you, and the age in years or decade of life (teens, 30s, 40, etc.) when they were diagnosed.

Diagnosis			Relationship to you	Age at diagnosis
Premature Ovarian Failure (menopause before age 40)	**No one in my family**	**Don't Know**	**1.**	
			2.	
			3.	
Autoimmune disorders (i.e. Lupus, or under/overactive thyroid, rheumatoid arthritis)	**No one in my family**	**Don't Know**	**1.**	
			2.	
			3.	
Fragile X syndrome	**No one in my family**	**Don't Know**	**1.**	
			2.	
			3.	

Part 3 – Tuberous Sclerosis Complex (TSC):

16) How old were you when you were diagnosed with TSC (age, in years)? _____

17a) Do you have any family members who also have TSC? (circle one)

Yes No Don't Know

b) If yes, please indicate their relationship to you. Please provide their specific age in years, or decade of life (teens, 20s, 30s, etc.) when they were diagnosed.

Relationship to you	Age at diagnosis
1.	
2.	
3.	
4.	

18a) Have you or a family member ever had genetic testing? (circle one)

Yes No Don't Know

b) If yes, what were the results? (check one)

- ☐ Mutation in *TSC1*
- ☐ Mutation in *TSC2*
- ☐ No mutation identified
- ☐ Other mutation, please specify: _____
- ☐ Don't Know

19a) Do you have (or have you ever had) a subependymal giant cell astrocytoma (SEGA)? (circle one)

Yes No (*skip to #20)* Don't Know

b) If yes, have you received treatment for your SEGA? (circle one)

Yes No Don't Know

c) If yes, what type of treatment did you receive? (check all that apply)

- ☐ Surgery
- ☐ Radiation
- ☐ Medication/chemotherapy (including rapamycin)
- ☐ Other, please specify: _____

20a) Have you ever had seizures? (circle one)

Yes No *(skip to #21)*
Don't Know

b) If yes, please indicate the age (in years) at which they first started _____

c) How often do you have seizures? (please check only one)

- ☐ At least once a day
- ☐ At least once a week, but not daily
- ☐ At least once a month, but not weekly
- ☐ At least once every six months, but not every month
- ☐ Less than once a year

d) Have you ever had surgery to control your seizures? (circle one)

Yes No Don't know

e*) Please indicate the medications you <u>currently take</u> for your seizures and how long (in years) you've been taking them. Using a scale from 1 – 3, please indicate how effective you believe the medicines are:*

1 (did not work well) 2 (neutral) 3 (worked well)

Medication	# years	Effectiveness (circle one)		
1.		1	2	3
2.		1	2	3
3.		1	2	3
4.		1	2	3
5.		1	2	3

f) Please indicate the medications you <u>have taken in the past</u> for your seizures and how long (in years) you took them for. Using a scale from 1 – 3, please indicate how effective you believe these medicines were:

1 (did not work well) 2 (neutral) 3 (worked well)

Medication	# years	Effectiveness (circle one)		
1.		1	2	3
2.		1	2	3
3.		1	2	3
4.		1	2	3
5.		1	2	3

21) Have you ever been diagnosed with the following? Please circle the appropriate response for each row and indicate your age of diagnosis or when symptoms began.

Name	Currently		Ever		Don't Know	Age diagnosed (years)
Learning disability	Yes	No	Yes	No	Don't Know	
ADHD/ADD	Yes	No	Yes	No	Don't Know	
Autism	Yes	No	Yes	No	Don't Know	
Bipolar Disorder	Yes	No	Yes	No	Don't Know	
Depression	Yes	No	Yes	No	Don't Know	
Dyslexia	Yes	No	Yes	No	Don't Know	
Mental Retardation	Yes	No	Yes	No	Don't Know	
Obsessive Compulsive Disorder (OCD)	Yes	No	Yes	No	Don't Know	
Other, please specify _____	Yes	No	Yes	No	Don't Know	

22) Do you have or have you ever had the following? Please circle the appropriate response in each row.

Name	Currently		Ever		Don't Know
Facial angiofibromas/adenoma sebaceum/ red spots on the face	Yes	No	Yes	No	Don't Know
Ash leaf spots/white birth marks or patches	Yes	No	Yes	No	Don't Know
Fingernail/toenail tumors	Yes	No	Yes	No	Don't Know

23) Have you ever been diagnosed with the following? Please circle the appropriate response for each row and indicate your age at diagnosis.

Name	Currently		Ever		Don't Know	Age diagnosed (years)
Renal (kidney) cysts	Yes	No	Yes	No	Don't Know	
Angiomyolypoma	Yes	No	Yes	No	Don't Know	
Kidney (renal) cancer	Yes	No	Yes	No	Don't Know	

24) Have you ever been diagnosed with the following? Please circle the appropriate response for each row and indicate your age of diagnosis or when symptoms began.

Name	Currently		Ever		Don't Know	Age of diagnosis or symptom onset (years)
Pulmonary (lung) cysts	Yes	No	Yes	No	Don't Know	
Lymphangioleiomyomatosis (LAM)	Yes	No	Yes	No	Don't Know	
I experience shortness of breath going up one flight of stairs	Yes	No	Yes	No	Don't Know	

25) Have you ever been diagnosed with the following? Please circle the appropriate response for each row and indicate your age when each was noticed.

Name	Currently		Ever		Don't Know	Age noticed (years)
Rhabdomyomas/ Heart tumor	Yes	No	Yes	No	Don't Know	
Irregular heartbeat	Yes	No	Yes	No	Don't Know	

26) Are there any other medical problems related to TSC you would like to mention?

_____

_____

_____

_____

Conclusion

Congratulations! You are finished with the Questionnaire. Please place it in the addressed, stamped envelope and mail it back to us. We thank you again for sharing your time and health information. We hope the results of this study will benefit women with TSC.

Michael J. Gambello, MD, PhD Emily Gabitzsch, BS

Appendix B

Characteristics of the dependent woman with possible POF

Briefly, the one dependent woman whose menstrual cycles demonstrated a progression from regular to irregular will be described. As mentioned above, she was not included in the group of seven women as we chose to compare to independent women only. This eighth woman was 46 years old when she had her questionnaire completed by another individual. She is African-American and single, lives with family, and is unemployed; she has completed some college. Her reported age of menarche was not provided to us in the questionnaire. This woman has no reported pregnancies. She did experience normal cyclicity between the ages of 16 and 35, but after age 36 irregular cycles were reported; she has never used oral contraceptives. Of note, she did have a hysterectomy and oophorectomy at age 37. Our participant was also reported to have radiation therapy at age 24, but a specific

We do not have menstrual reporting for ages Her reproductive history is negative for blood tests, diagnoses, symptoms, or surgeries, per report. This woman does have a history of seizures, with an onset of 6 months. She experiences seizures approximately once per month. In the past, this woman used levetiracetam and topiramate for seizure control, each for one month. Her current medications include valproic acid, phenobarbital, (taken for 31 years), and clonazepam (taken for 16 years). She did receive genetic testing and her results were reported as no mutation identified. Interestingly, the only clinical manifestations of TSC reported for this woman were angiofibromas and hypopigmented macules. She also has diagnoses of autism and mental retardation, both at age 2. This woman meets the menstrual history criteria set for a categorization of possible POF. She does lack additional reproductive data to solidify this possibility; however, she does not have contradictory information. It is possible that her current use of medication for seizures could contribute to her menstrual irregularity as discussed above. As a general comparison to other dependent women between the ages of 30 and 40, she appears to differ only in the small number of TSC manifestations, as she does not exhibit findings in the cardiac, renal, or pulmonary systems. Our choice to exclude this woman from the analysis was based on her differences due to her being dependent, but at a glance, she does not appear to exhibit significant differences.

Bibliography

Abbott, G. F., M. L. Rosado-de-Christenson, A. A. Frazier, T. J. Franks, R. D. Pugatch, and J. R. Galvin. "From the Archives of the AFIP: Lymphangioleiomyomatosis: Radiologic-Pathologic Correlation." *Radiographics* 25.3 (2005): 803-28. Print.

ACOG. *Early Pregnancy Loss: Miscarriage and Molar Pregnancy*. Rep. no. AP090. 2002. Web. http://www.acog.org/publications/patient_education/bp090.cfm

ACOG. Committee Opinion. No 338: *Screening for Fragile X Syndrome*. Obstet Gynecol. 107.6 (2006): 1483-5.

Adhikari, D., G. Flohr, N. Gorre, Y. Shen, H. Yang, E. Lundin, Z. Lan, M. J. Gambello, and K. Liu. "Disruption of TSc2 in Oocytes Leads to Overactivation of the Entire Pool of Primordial Follicles." *Molecular Human Reproduction* 15.12 (2009): 765-70. Print.

Adhikari, D., W. Zheng, Y. Shen, N. Gorre, T. Hämäläinen, A. J. Cooney, I. Huhtaniemi, Z. J. Lan, and K. Liu. "Tsc/mTORC1 Signaling in Oocytes Governs the Quiescence and Activation of Primordial Follicles." *Human Molecular Genetics* 19.3 (2010): 397-410. Print.

Altchek, Albert, Liane Deligdisch, and Nathan G. Kase. *Diagnosis and Management of Ovarian Disorders*. San Diego: Academic, 2003. Print.

Alzubaidi, N. H., H. L. Chapin, V. H. Vanderhoof, K. A. Calis, and L. M. Nelson. "Meeting the Needs of Young Women with Secondary Amenorrhea and Spontaneous Premature Ovarian Failure." *Obstetrics and Gynecology* 99.5 (2002): 720-25. Print.

American Pregnancy Association . “Miscarriage.” Last update July 2007. Retrieved January 20, 2011, from http://www.americanpregnancy.org/pregnancycomplications/miscarriage.html

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision, American Psychiatric Association, Washington, DC, 2000.

Au, K.S., A. T., Williams,, E. S. Roach, L. Batchelor,,S.P. Sparagana, M.R. Delgado, J.W. Wheless, J.E. Baumgartner, B.B. Roa, C.M Wilson, T. K. Smith-Knuppel, M.Y. Cheung, V. H Whittemore, T. M. King, and H. Northrup. “Genotype/phenotype correlation in 325

individuals referred for a diagnosis of tuberous sclerosis complex in the United States." *Genet Med* 9 (2007):88–100.

Benvenuto, G., S. Li, S. J. Brown, R. Braverman, W. C. Vass, J. P. Cheadle, D. J. Halley, J. R. Sampson, R. Wienecke, and J. E. DeClue. "The Tuberous Sclerosis-1 (TSC1) Gene Product Hamartin Suppresses Cell Growth and Augments the Expression of the TSC2 Product Tuberin by Inhibiting Its Ubiquitination." *Oncogene* 19.54 (2000): 6305-316. Print.

Boixeda, P., E. Sánchez-Miralles, J. M. Azaña, J. M. Arrazola, R. Moreno, and A. Ledo. "CO2, Argon, and Pulsed Dye Laser Treatment of Angiofibromas." *The Journal of Dermatologic Surgery and Oncology* 20.12 (1994): 808-12. Print.

Bombardieria, R., M. Pincia, R. Moaveroa, C. Cerminara, and P. Curatulo. "Early Control of Seizures Improves Long-term Outcome in Children with Tuberous Sclerosis Complex." *European Journal of Pediatric Neurology* 14.2 (2010): 146-49. Print.

Bourneville, D. "Sclérose Tubéreuse Des Circonvolutions Cérébrales: Idiotie Et épilepsie Hemiplégique." *Archives De Neurologie* 1 (1880): 81-91. Print.

Campen, C.J., and B.E. Porter, B.E. “Subependymal Giant Cell Astrocytoma (SEGA) Treatment Update.” *Current Treatment Options in Neurology.* Epub (2011). DOI: 10.1007/s11940-011-0123-z

Cao, J., L. Gong, D. C. Guo, U. Mietzsch, S. Q. Kuang, C. S. Kwartler, H. Safi, A. Estrera, M. J. Gambello, and D. M. Milewicz. ". Thoracic Aortic Disease in Tuberous Sclerosis Complex: Molecular Pathogenesis and Potential Therapies in Tsc2± Mice." *Human Molecular Genetics* 19.10 (2010): 1908-920. Print.

Carsillo, T., A. Astrinidis, and E. P. Kenske. "Mutations in the Tuberous Sclerosis Complex Gene TSC2 Are a Cause of Sporadic Pulmonary Lymphangioleiomyomatosis." *Proceedings of the National Academy of Sciences* 97.11 (2000): 6085-090. Print.

Castrillon, D. H., L. Miao, R. Kollipara, J. W. Horner, and R. A. DePinho. "Suppression of Ovarian Follicle Activation in Mice by the Transcription Factor Foxo3a." *Science* 301.5630 (2003): 215-18. Print.

Castro, A. F., J. F. Rebhun, G. J. Clark, and L. A. Quilliman. "Rheb Binds Tuberous Sclerosis Complex 2 (TSC2) and Promotes S6 Kinase Activation in a Rapamycin- and

Farnesylation-dependent Manner." *Journal of Biological Chemistry* 278.35 (2003): 32493-2496. Print.

Chao, D. "Congenital Neurocutaneous Syndromes in Childhood. Tuberous Sclerosis." *The Journal of Pediatrics* 55.4 (1959): 447-59. Print.

Chonchaiya, W., A. Schneider, and R. J. Hagerman. "Fragile X: a Family of Disorders." *Advances in Pediatrics* 56 (2009): 165-85. Print.

Corrigan, E., M. Raygada, V. Vanderhoof, and L. Nelson. "A Woman with Spontaneous Premature Ovarian Failure Gives Birth to a Child with Fragile X Syndrome." *Fertility and Sterility* 84.5 (2005): 1508.e5-508.e8. Print.

Coulam, C.B., S.C. Adamson, and J.F. Annegers. “Incidence of Premature Ovarian Failure.” Obstetrics and Gynecology. 67.604 (1986) : 604-606.

Crino, P. B., K. L. Nathanson, and E. P. Henske. "The Tuberous Sclerosis Complex." New England Journal of Medicine 355.13 (2006): 1345-356. Print.

Curatolo, P. Tuberous Sclerosis Complex: From Basic Science to Clinic Phenotypes. London: Mac Keith, 2003. Print.

DeVos, M., P. Devroey, and B. C. Fauser. "Primary Ovarian Insufficiency." Lancet 376 (2010): 911-21. Print.

Dixon, B. P., J. C. Hulbert, and J. J. Bissler. "Tuberous Sclerosis Complex Renal Disease."

Nephron Experimental Nephrology 2011th ser. 118.1: E15-20. Print.

European Chromosome 16 Tuberous Sclerosis Consortium. "European Chromosome 16 Tuberous Sclerosis Consortium. Identification and Characterization of the Tuberous Sclerosis Gene on Chromosome 16." *Cell* 75 (1993): 1305-315. Print.

Faddy, M. J., R. G. Gosden, A. Gougeon, S. J. Richardson, and J. F. Nelson. "Accelerated Disappearance of Ovarian Follicles in Mid-life: Implications for Forecasting Menopause." *Human Reproduction* 7.10 (1992): 1342-346. Print.

Farooq, A., L. J. Walker, J. Bowling, and R. A. Audusui. "Cowden Syndrome." *Cancer Treatment Reviews* 36.8 (2010): 577-83. Print.

Finlay, G. "The LAM Cell: What Is It, Where Does It Come From, and Why Does It Grow?" *AJP: Lung Cellular and Molecular Physiology* 286.4 (2003): 690L-93. Print.

Fitzpatrick, Thomas B. "History and Significance of White Macules, Earliest Visible Sign of Tuberous Sclerosis." *Annals of the New York Academy of Sciences* 615.1 Tuberous Scle (1991): 26-35. Print.

Franz, D. N. "Non-neurologic Manifestations of Tuberous Sclerosis Complex." *Journal of Child Neurology* 19 (2004): 690-98. Print.

Fryer, A. E., A. Chalmers, J. M. Connor, I. Fraser, S. Povey, A. D. Yates, J. R. Yates, and J. P. Osborne. "Evidence That The Gene For Tuberous Sclerosis Is On Chromosome 9." *The Lancet* 329.8534 (1987): 659-61. Print.

Gallardo, T. D., G. B. John, K. Bradshaw, C. Welt, R. Reijo-Pera, P. H. Vogt, P. Touraine, S. Bione, D. Toniolo, L. M. Nelson, A. R. Zinn, and D. H. Castrillon. "Sequence Variation at the Human FOXO3 Locus: a Study of Premature Ovarian Failure and Primary Amenorrhea." *Human Reproduction* 23.1 (2007): 216-21. Print.

Gomez, M. R. *Criteria for Diagnosis in Tuberous Sclerosis*. 2nd ed. New York: Raven, 1988. Print.

Groff, A., S. Covington, L. Halverson, O. Fitzgerald, V. Vanderhoof, K. Calis, and L. Nelson. "Assessing the Emotional Needs of Women with Spontaneous Premature Ovarian Failure." *Fertility and Sterility* 83.6 (2005): 1734-741. Print.

Gunther, M., and L. S. Penrose. "The Genetics of Epiloia." *Journal of Genetics* 31.3 (1935): 413-30. Print.

Hagerman, P., and R. Hagerman. "The Fragile-X Premutation: A Maturing Perspective." *The American Journal of Human Genetics* 74.5 (2004): 805-16. Print.

Han, S., T. M. Santos, A. Puga, J. Roy, E. A. Thiele, M. McCollin, A. Stemmer-Rachamimov, and V. Ramesh. "Phosphorylation of Tuberin as a Novel Mechanism for Somatic Inactivation of the Tuberous Sclerosis Complex Proteins in Brain Lesions." *Cancer Research* 64.3 (2004): 812-16. Print.

Hansen, K. R., N. S. Knowlton, A. C. Thyer, J. S. Charleston, M. R. Soules, and N. A. Klein. "A New Model of Reproductive Aging: the Decline in Ovarian Non-growing Follicle Number from Birth to Menopause." *Human Reproduction* 23.3 (2008): 699-708. Print.

Hay, Nissim. "The Akt-mTOR Tango and Its Relevance to Cancer." *Cancer Cell* 8.3 (2005): 179-83. Print.

Henske, Elizabeth Petri. "Tuberous Sclerosis and the Kidney: from Mesenchyme to Epithelium, and beyond." *Pediatric Nephrology* 20.7 (2005): 854-57. Print.

Huang, J., C. C. Dibble, M. Matsuzaki, and B. D. Manning. "The TSC1-TSC2 Complex Is Required for Proper Activation of MTOR Complex 2." *Molecular and Cellular BIology* 28.12 (2008): 4104-115. Print.

Hundscheid, R., E. Sistermans, C. Thomas, D. Braat, H. Straatman, L. Kiemeney, B. Oostra, and A. Smits. "Imprinting Effect in Premature Ovarian Failure Confined to Paternally Inherited Fragile X Premutations." *The American Journal of Human Genetics* 66.2 (2000): 413-18. Print.

Inoki, Ken, Michael N. Corradetti, and Kun-Liang Guan. "Dysregulation of the TSC-mTOR Pathway in Human Disease." *Nature Genetics* 37.1 (2005): 19-24. Print.

Jansen, F. E., O. Braams, K. L. Vincken, A. Algra, P. Anbeek, A. Jennekens-Schinkel, D. Halley, B. A. Zonnenberg, A. Van Den Ouweland, A. C. Van Huffelen, O. Van Nieuwenhuizen, and M. Nellist. "Overlapping Neurologic and Cognitive Phenotypes in Patients with TSC1 or TSC2 Mutations." *Neurology* 70.12 (2008): 908-15. Print.

Johannessen, C., B. Johnson, S. Williams, A. Chan, E. Reczek, R. Lynch, M. Rioth, A. Mcclatchey, S. Ryeom, and K. Cichowski. "TORC1 Is Essential for NF1-Associated Malignancies." *Current Biology* 18.1 (2008): 56-62. Print.

Józwiak, S., R. A. Schwartz, C. K. Janniger, R. Michałowicz, and J. Chmielik. "Skin Lesions in Children with Tuberous Sclerosis Complex: Their Prevalence, Natural Course, and Diagnostic Significance." *International Journal of Dermatology* 37.12 (1998): 911-17. Print.

Kandt, R. S., J. L. Haines, M. Smith, H. Northrup, R. J. M. Gardner, M. P. Short, K. Dumars, E. S. Roach, S. Steingold, S. Wall, S. H. Blanton, P. Flodman, D. J. Kwiatkowski, A. Jewell, J. L. Weber, A. D. Roses, and M. A. Pericak-Vance. "Linkage of an Important Gene Locus for Tuberous Sclerosis to a Chromosome 16 Marker for Polycystic Kidney Disease." *Nature Genetics* 2.1 (1992): 37-41. Print.

Kandt, R. "Tuberous Sclerosis Complex and Neurofibromatosis Type 1: the Two Most Common Neurocutaneous Diseases." *Neurologic Clinics* 20.4 (2002): 941-64. Print.

Kanner, A.M. “Psychiatric issues in epilepsy: the complex relation of mood, anxiety disorders, and epilepsy.” *Epilepsy & Behavior* 15.1 (2009): 83-87. Print.

Kaplan, P.W. “Reproductive health effects and teratogenicity of antiepileptic drugs.” *Neurology* 63.10.4 (2004): S13-S23.

Kaplan, P.W., E.R. Norwitz, E. Ben-menachem, P.G. Pennell, M. Druzin, J.N.Robinson, and J.C. Gordon. “Obstetric risks for women with epilepsy during pregnancy.” *Epilepsy & Behavior* 11.3 (2007): 283-291. Print.

Kim, T. J., J. N. Anasti, M. R. Flack, L. M. Kimzey, R. A. Defensor, and L. M. Nelson. "Routine Endocrine Screening for Patients with Karyotypically Normal Spontaneous Premature Ovarian Failure." *Obstetrics and Gynecology* 89 (1997): 777-79. Print.

Kirpicznik, J. "Ein Fall Von Tuberoser Skelrose Und Gleichzeitigen Multiplem Nieren Gesch Wuel Stern." *Virchow Archives of Pathology and Anatomy* 202.3 (1910): 358. Print.

Knauff, Erik A.H., Hendrika E. Westerveld, Angelique J. Goverde, Marinus J. Eijkemans, Olivier Valkenburg, Evert J.P. Van Santbrink, Bart C.J.M. Fauser, and Yvonne T. Van Der Schouw. "Lipid Profile of Women with Premature Ovarian Failure." *Menopause* 15.5 (2008): 919-23. Print.

Koenig, M. K., I. J. Butler, and H. Northrup. "Regression of Subependymal Giant Cell Astrocytoma With Rapamycin in Tuberous Sclerosis Complex." *Journal of Child Neurology* 23.10 (2008): 1238-239. Print.

Kronenberg, F. "Menopausal Hot Flashes: a Review of Physiology and Biosociocultural Perspectives on Methods of Assessment." *The Journal of Nutrition* 140.7 (2010): 1380S-5S. Print.

Kwiatkowski, D. J. "Animal Models of Lymphangioleiomyomatosis (LAM) and Tuberous Sclerosis Complex." *Lymphatic Research and Biology* 8.1 (2010): 51-57. Print.

Kwiatkowski, David. "Cancer Genetics: TSC1, TSC2, TSC3? or Mosaicism?" *European Journal of Human Genetics* 13.6 (2005): 695-96. Print.

Kwiatkowski, David J. *Tuberous Sclerosis Complex Genes, Clinical Features and Therapeutics*. Weinheim: Wiley-Blackwell, 2010. Print.

LaBarbera, A.R., Miller, M.M., Ober, C., Rebar, R.W. Autoimmune etiology in premature ovarian failure. *Am J Reprod Immunol Microbiol* 16 (1988): 115. Print.

LaCroix, A.Z., R.T. Chlebowski, J.E. Manson, A.K. Aragaki, K.C. Johnson, L. Martin, K.L. Margolis, M.L. Stefanick, R. Brzyski, J.D. Curb, B.V. Howard, C.E. Lewis, J Wende-Wactawski, and WHI Investigators. “Health Outcomes After Stopping Conjugated Equine Estrogens Among Postmenopausal Women with Prior Hysterectomy: A Randomized Controlled Trial.” *JAMA* 305.13 (2011): 1305-1314. Print.

Lagos, J. C., and M. R. Gomez. "Tuberous Sclerosis: Reappraisal of a Clinical Entity." *Mayo Clinic Proceedings: Mayo Clinic* 42.1 (1967): 26-49. Print.

Lamb, R. F., C. Roy, T. J. Diefenbach, H. V. Vinters, M. W. Johnson, D. G. Jay, and A. Hall. "The TSC1 Tumour Suppressor Hamartin Regulates Cell Adhesion through ERM Proteins and the GTPase Rho." *Nature Cell Biology* 2.5 (2000): 281-87. Print.

Laml, T., O. Preyer, W. Umek, M. Hengstschlager, and H. Hanzal. "Genetic Disorders in Premature Ovarian Failure." *Human Reproduction Update* 8.4 (2002): 483-91. Print.

Ledig, S., A. Ropke, and P. Wieacker. "Copy Number Variants in Premature Ovarian Failure and Ovarian Dysgenesis." *Sexual Development* 4.4-5 (2010): 225-32. Print.

Leiden Open Variation Database (LOVD). Tuberous Sclerosis 1. http://chromium.liacs.nl/LOVD2/TSC/home.php

Leiden Open Variation Database (LOVD). Tuberous Sclerosis 2. http://chromium.liacs.nl/LOVD2/TSC/home.php?select_db=TSC2

Leung, A., and W. Robson. "Tuberous Sclerosis Complex: A Review." *Journal of Pediatric Health Care* 21.2 (2007): 108-14. Print.

Lewis, J.C., H.V., Thomas, K.C. Murphy, and J.R. Sampson. “Genotype and Psychological Phenotype in Tuberous Sclerosis.” *J Med Genet.* 41 (2004):203-207.

Liao, K. L., N. Wood, and G. S. Conway. "Premature Menopause and Psychological Well-being. Journal of Psychosomatic Obstetrics and Gynaecology." *Journal of Psychosomatic Obsetrics and Gynecology* 21.3 (2000): 167-74. Print.

Lourenco, D., R. Brauner, L. Lin, A. De Perdigo, G. Weryha, M. Muresan, R. Boudjenah, G. Guerra-Junior, A. T. Maciel-Guerra, J. C. Achermann, K. McElreavey, and A.

Bashamboo. "Mutations in NR5A1 Associated with Ovarian Insufficiency." *New England Journal of Medicine* 360.12 (2009): 1200-210. Print.

McDonough, Paul G. "Molecular Abnormalities of FSH and LH Action." *Annals of the New York Academy of Sciences* 997.1 (2003): 22-34. Print.

Moolten, S. E. "Hamartial Nature of Tuberous Sclerosis Complex and Its Bearings on the Tumor Problem: Report of a Case with Tumor Anomaly of the Kidney and Adenoma Sebaceum." *Archives of Internal Medicine* 69 (1942): 589-623. Print.

Moss, J., N. Avila, P.M. Barnes, R.A. Litzenberger, J. Bechtle, P.G. Brooks, C. J. Hedin, S. Hunsberger, and A.S. Kristof. “Prevalence and Clinical Characteristics of Lymphangioleiomyomatosis (LAM) in Patients with Tuberous Sclerosis Complex.” *American Journal of Respiratory and Critical Care Medicine* 164.4 (2001): 668-671.

Nelson, LM. Autoimmune ovarian failure: comparing the mouse model and the human disease. *J Soc Gynecol Investig* 8 (2001):S55. Print.

Nelson, L. M. "Primary Ovarian Insufficiency." *New England Journal of Medicine* 360.6 (2009): 606-14. Print.

Nelson, L., S. Covington, and R. Rebar. "An Update: Spontaneous Premature Ovarian Failure Is Not an Early Menopause." *Fertility and Sterility* 83.5 (2005): 1327-332. Print.

Niida, Y., A. Stemmerrachamimov, M. Logrip, D. Tapon, R. Perez, D. Kwiatkowski, K. Sims, M. Maccollin, D. Louis, and V. Ramesh. "Survey of Somatic Mutations in Tuberous Sclerosis Complex (TSC) Hamartomas Suggests Different Genetic Mechanisms for Pathogenesis of TSC Lesions." *The American Journal of Human Genetics* 69.3 (2001): 493-503. Print.

O’Callaghan, F., A. Shiell, J. Osborne, and C. Martyn. "Prevalence of Tuberous Sclerosis Estimated by Capture-recapture Analysis." *The Lancet* 351.9114 (1998): 1490. Print.

O'Callaghan, F J K., C. N. Martyn, S. Renowden, M. Noakes, D. Presdee, and J. P. Osborne. "Subependymal Nodules, Giant Cell Astrocytomas and the Tuberous Sclerosis Complex: a Population-based Study." *Archives of Disease in Childhood* 93.9 (2008): 751-54. Print.

O'Hagan, A. R., R. Ellsworth, M. Secic, A. Rothner, and B. H. Brouhard. "Renal Manifestations of Tuberous Sclerosis Complex." *Clinical Pediatrics* 35.10 (1996): 483-89. Print.

Osborne, J. P., J. Merrifield, J. Finbar, and K. O'Callaghan. "Tuberous Sclerosis—what's New?" *Archives of Disease in Childhood* 93.9 (2008): 728-31. Print.

Osborne, John P., Alan Fryer, and David Webb. "Epidemiology of Tuberous Sclerosis." *Annals of the New York Academy of Sciences* 615.1 Tuberous Scle (1991): 125-27. Print.

Parker-Pope, T. (2011, March 28). An Older Generation Falls Prey to Eating Disorders. *New York Times*, Retrieved April 4, 2011, from http://well.blogs.nytimes.com/2011/03/28.

Persani, L., R. Rossetti, and C. Cacciatore. "Genes Involved in Human Premature Ovarian Failure -- Persani Et Al. 45 (5): 257 -- Journal of Molecular Endocrinology." Journal of Molecular Endocrinology, including Cell Signalling, Cytokines and Protein Structure-function Relationships. 28 July 2010. Web. 27 Mar. 2011. <http://jme.endocrinology-journals.org/cgi/content/abstract/45/5/257>.

Prather, P., and P. J. De Vries. "Behavioral and Cognitive Aspects of Tuberous Sclerosis Complex." Journal of Child Neurology 19.9 (2004): 666-74. Print.

Pringle, J. J. "A Case of Congenital Adenoma Sebaceum." British Journal of Dermatology 2 (1890): 1-14. Print.

Pulsifer, M.B., E.B. Winterkorn, and E.A. Thiele. "Psychological Profile of Adults with Tuberous Sclerosis Complex." Epilepsy & Behavior. 10.3 (2007): 402-406.

Qin, W., P. Kozlowski, B. E. Taillon, P. Boufarrd, A. J. Holmes, P. Janne, S. Camposano, E. Thiele, D. Franz, and D. J. Kwiatkowski. "Ultra Deep Sequencing Detects a Low Rate of Mosaic Mutations in Tuberous Sclerosis Complex." Human Genetics 127.5 (2010): 573-82. Print.

Raznahan, A., C. Joinson, F. O'Callaghan, J.P. Osborne, and P.F. Bolton. "Psychopathology in Tuberous Sclerosis: An Overview of Findings in a Population-Based Sample of Adults with Tuberous Sclerosis." J Intellect Disabil Res. 50 (2006): 561 – 569.

Rebar, R. "Premature Ovarian Failure." Obstetrics & Gynecology 113.6 (2009): 1355-363.

Rebar, R. W. "Premature Ovarian "failure" in the Adolescent." Annals of the New York Academy of Sciences 1135 (2008): 138-45. Print.

Reddy, P., D. Adhikari, W. Zheng, S. Liang, T. Hamalainen, V. Tohonen, W. Ogawa, T. Noda, S. Volarevic, I. Huhtaniemi, and K. Liu. "PDK1 Signaling in Oocytes Controls Reproductive Aging and Lifespan by Manipulating the Survival of Primordial Follicles." *Human Molecular Genetics* 18.15 (2009): 2813-824. Print.

Reddy, P., L. Liu, D. Adhikari, K. Jagarlamudi, S. Rajareddy, Y. Shen, C. Du, W. Tang, T. Hamalainen, S. L. Peng, Z.-J. Lan, A. J. Cooney, I. Huhtaniemi, and K. Liu. "Oocyte-Specific Deletion of Pten Causes Premature Activation of the Primordial Follicle Pool." *Science* 319.5863 (2008): 611-13. Print.

Roach, E. S., and S. P. Sparagana. "Diagnosis of Tuberous Sclerosis Complex." *Journal of Child Neurology* 19.9 (2004): 643-49. Print.

Roach, E.S., M. R. Gomez, and H. Northrup. "Tuberous Sclerosis Complex Consensus Conference: Revised Clinical Diagnostic Criteria." *Journal of Child Neurology* 13.12 (1998): 624-28. Print.

Rohr, J., E.G. Allen, K. Charen, J. Giles, W. He, C. Dominguez, and S.L. Sherman. "Anti-Mullerian Hormone Indicates Early Ovarian Decline in Fragile X Mental Retardation (FMR1) Premutation Carriers: a Preliminary Study." *Human Reproduction* 23.5 (2008): 1220-225. Print.

Romanelli, P., M. Verdecchia, R. Rodas, S. Seri, and P. Curatolo. "Epilepsy Surgery for Tuberous Sclerosis." *Pediatric Neurology* 31.4 (2004): 239-47. Print.

Rose, V., K. Au, G. Pollom, E. Roach, H. Prashner, and H. Northrup. "Germ-Line Mosaicism in Tuberous Sclerosis: How Common?" *The American Journal of Human Genetics* 64.4 (1999): 986-92. Print.

Rosner, M., M. Hanneder, N. Siegel, A. Valli, and M. Hengstschlager. "The Tuberous Sclerosis Gene Products Hamartin and Tuberin Are Multifunctional Proteins with a Wide Spectrum of Interacting Partners." *Mutation Research/Reviews in Mutation Research* 658.3 (2008): 234-46. Print.

Sampson, J. R., J. R. Yates, L. A. Pirrit, P. Fleury, I. Winship, P. Beighton, and J. M. Connor. "Evidence for Genetic Heterogeneity in Tuberous Sclerosis." *Journal of Medical Genetics* 26.8 (1989): 511-16. Print.

Sancak, Ozgur, Mark Nellist, Miriam Goedbloed, Peter Elfferich, Cokkie Wouters, Anneke Maat-Kievit, Bernard Zonnenberg, Senno Verhoef, Dicky Halley, and Ans Van Den Ouweland. "Mutational Analysis of the TSC1 and TSC2 Genes in a Diagnostic Setting: Genotype %u2013 Phenotype Correlations and Comparison of Diagnostic DNA Techniques in Tuberous Sclerosis Complex." *European Journal of Human Genetics* 13.6 (2005): 731-41. Print.

Sarbassov, D. D., S. M. Ali, and D. M. Sabatini. "Growing Roles for the MTOR Pathway." *Current Opinions in Cell Biology* 17.6 (2005): 596-603. Print.

Schmidt, P. J., G. Cardoso, J. Ross, N. Haq, D. Rubinow, and C. Bondy. "Shyness, Social Anxiety, and Impaired Self-esteem in Turner Syndrome and Premature Ovarian Failure." *JAMA: The Journal of the American Medical Association* 295.12 (2006): 1374-376. Print.

Sparagana, S. P., and E. P. Roach. "Tuberous Sclerosis Complex." *Current Opinion in Neurology* 13 (2000): 115-19. Print.

Steegers-Theunissen, R.P.M., W.O. Renier, G.F. Borm, C.M.G. Thomas, H.M.W.M. Merkis, D. Op de Coul, P. DeJong, H. van Geijn, M. Wouters, and T. Eskes. "Factors influencing the risk of abnormal pregnancy outcome in epileptic women: A multi-centre prospective study." *Epilepsy Research* 18.3 (1994): 261-269. Print.

Tee, A. R., B. D. Manning, P. P. Roux, L. C. Cantley, and J. Blenis. "Sclerosis Complex Gene Products, Tuberin and Hamartin, Control MTOR Signaling by Acting as a GTPase-activating Protein Complex toward Rheb." *Current Biology* 5.13 (2003): 1259-268. Print.

Thompson, A.W., J.W. Miller, W. Katon, N. Chaytor, and P. Ciechanowski. "Sociodemographic and clinical factors associated with depression in epilepsy." *Epilepsy & Behavior* 14.4 (2009): 655-660. Print.

Van Den Ouweland, A. M., P. Efferich, B. A. Zonnenberg, W. F. Arts, T. Kleefstra, M. D. Nellist, and J. M. Millan. "Characterisation of TSC1 Promoter Deletions in Tuberous Sclerosis Complex Patients." *European Journal of Human Genetics* 19.2 (2011): 157-63. Print.

van der Schouw, Y., Y. Van Der Graaf, E. Steyerberg, J. C. Eijkemans, and J. D. Banga. "Age at Menopause as a Risk Factor for Cardiovascular Mortality." *The Lancet* 347.9003 (1996): 714-18. Print.

Verhoef, S., L. Bakker, A. Tempelaars, A. Hesselingjanssen, T. Mazurczak, S. Jozwiak, A. Fois, G. Bartalini, B. Zonnenberg, and A. Vanessen. "High Rate of Mosaicism in Tuberous Sclerosis Complex." *The American Journal of Human Genetics* 64.6 (1999): 1632-637. Print.

Verhoef, S., L. Bakker, A. Tempelaars, A. Hesselingjanssen, T. Mazurczak, S. Jozwiak, A. Fois, G. Bartalini, B. Zonnenberg, and A. Vanessen. "High Rate of Mosaicism in Tuberous Sclerosis Complex." *The American Journal of Human Genetics* 64.6 (1999): 1632-637. Print.

Vivanco, I., and C. L. Sawyers. "The Phosphatidylinositol 3-Kinase AKT Pathway in Human Cancer." *Nature Reviews Cancer* 2.7 (2002): 489-501. Print.

Von Baal, J. G., N. J. Smits, J. N. Keeman, D. Lindhout, and S. Verhoef. "The Evolution of Renal Angiomyolipomas in Patients with Tuberous Sclerosis." *The Journal of Urology* 152.1 (1994): 35-38. Print.

Wang, B., Y. Mu, and F. Ni. "Analysis of F0X03 Mutation in 114 Chinese Women with Premature Ovarian Failure." *Reproductive BioMedicine Online* 20 (2010): 499-503. Print.

Wang, X., C. Chen, L. Wang, D. Chen, W. Guang, and J. French. “Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study.” *Fertil Steril.* (2003) 79.3: 577.

Wang, X., and C. G. Proud. "The MTOR Pathway in the Control of Protein Synthesis." *Physiology* 21 (2006): 362-69. Print.

Watkins, W., A. Umbers, K. Woad, S. Harris, I. Winship, K. Gersak, and A. Shelling. "Mutational Screening of FOXO3A and FOXO1A in Women with Premature Ovarian Failure." *Fertility and Sterility* 86.5 (2006): 1518-521. Print.

Webb, D.W., A. Clarke, A. Fryer, and J.P. Osborne. "The Cutaneous Features of Tuberous Sclerosis: a Population Study." *British Journal of Dermatology* 135.1 (1996): 1-5. Print.

Weiss, E. T., and R. G. Geronemus. "New Technique Using Combined Pulsed Dye Laser and Fractional Resurfacing for Treating Facial Angiofibromas in Tuberous Sclerosis." *Lasers in Surgery and Medicine* 42.5 (2010): 357-60. Print.

Welt, Corrine K. "Primary Ovarian Insufficiency: a More Accurate Term for Premature Ovarian Failure." *Clinical Endocrinology* 68.4 (2008): 499-509. Print.

Weston, M. "Tuberose Sclerosis Complex: Analysis of Growth Rates Aids Differentiation of Renal Cell Carcinoma from Atypical or Minimal-fat-containing Angiomyolipoma." *Clinical Radiology* 60.6 (2005): 663-64. Print.

Wilson, C., C. Bonnet, C. Guy, S. Idziaszczyk, J. Colley, V. Humphreys, J. Maynard, J. R. Sampson, and J. P. Cheadle. "Tsc1 Haploinsufficiency without Mammalian Target of Rapamycin Activation Is Sufficient for Renal Cyst Formation in Tsc1 /- Mice." *Cancer Research* 66.16 (2006): 7934-938. Print.

Yu, J., A. Astrinidis, S. Howard, and E. P. Henske. "Estradiol and Tamoxifen Stimulate LAM-associated Angiomyolipoma Cell Growth and Activate Both Genomic and Nongenomic Signaling Pathways." *AJP: Lung Cellular and Molecular Physiology* 286.4 (2003): 694L-700. Print.

Vita

Emily Gabitzsch was born in Greenville, Texas to Kurt W. Gabitzsch and Elizabeth A. Gabitzsch on April 9, 1985. She graduated from Greenville High School and received a Bachelors of Science in Human Biology in 2007 from the University of Texas in Austin. After graduation, Emily did human embryonic stem cell research at the National Institute of Drug Abuse, part of the National Institutes of Health. Prior to her graduate education at the Graduate School of Biomedical Sciences in Genetic Counseling, Emily worked as an environmental consultant at Abt Associates, Inc., and lived in Washington, D.C.