Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Self-reported reproductive health in women with tuberous sclerosis complex

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Purpose: Little is known about sex-specific manifestations of tuberous sclerosis complex. Inactivating mutations in the *TSC1* and *TSC2* genes cause tuberous sclerosis complex, and recent evidence points to a crucial role for these genes in maintaining appropriate ovarian function. The main objective of this study was to estimate reproductive dysfunction in a sample of women with tuberous sclerosis complex.

Methods: We designed a three-part questionnaire that included demographic information, reproductive history, and tuberous sclerosis complex history, and developed strict criteria to assess patterns in menstrual cyclicity; we analyzed 182 responses from female adult members of the Tuberous Sclerosis Alliance.

Results: More than one-third of women in our sample displayed some degree of menstrual irregularity, and their reported miscarriage rate was 41%. More than 4% of women had reproductive histories suggestive of premature ovarian insufficiency, higher than the general population estimate of 1%.

Conclusion: Our data reveal an underappreciated aspect of tuberous sclerosis complex in affected women, suggesting that a further exploration of the role the tuberous sclerosis complex genes play in reproductive function is warranted.

Genet Med advance online publication 9 May 2013

Key Words: reproductive health; tuberous sclerosis complex

Tuberous sclerosis complex (TSC) is an autosomal-dominant tumor-suppressor disorder characterized by the development of hamartomas in many organ systems. The prevalence of TSC is estimated at 1 in 6,000 individuals, and up to 70% of cases are due to *de novo* mutation.[1,2]

Inactivating mutations in either the *TSC1* or *TSC2* gene, which encode the proteins hamartin and tuberin, respectively, cause TSC.[3,4] These proteins form a heterodimer involved in negative regulation of the mammalian target of rapamycin complex 1 (mTORC1) kinase.[5–7] mTORC1 is an important element in controlling cell proliferation and growth, insulin signaling, and protein translation.[8] Dysregulation of mTORC1 is an important aspect in the pathogenesis of TSC.[9] Somatic mutation of the second *TSC1* or *TSC2* allele leads to a loss of heterozygosity, upregulation of mTOR, and dysregulated cellular metabolism.[9]

Although virtually any organ can be affected in TSC, the brain, skin, kidneys, heart, and lungs are the principal sites of pathology. TSC shows no ethnic or sex predilection, but interestingly, sex-specific manifestations have been described. Almost 40% of women with TSC develop the pulmonary manifestation lymphangioleiomyomatosis, typically between the ages of 20 and 40 years; most men are unaffected.[10,11] Moreover, the most common renal manifestation, angiomyolipoma, impacts more than 70% of patients,[2] but females are affected an estimated three to four times more often than males.[12] Lymphangioleiomyomatosis and angiomyolipomas share histological features and express estrogen and progesterone receptors, considered important in development.[13] Other effects of sex hormones or reproductive manifestations in TSC are poorly understood.

Intriguingly, the role of the *TSC1* and *TSC2* genes in normal organ function and TSC-associated pathogenesis came to light from the creation of a variety of organ-specific *Tsc1*- or *Tsc2*-null animals. Recently, the use of conditional knockout models revealed a role for the murine *Tsc1* and *Tsc2* genes in the reproductive functioning of the ovary.[14,15] Specifically, these genes are crucial for the maintenance of primordial follicles in a quiescent state and appropriate activation throughout the murine reproductive period. Deletions of either the *Tsc1* or *Tsc2* gene in the ovaries of mice led to a premature activation of primordial follicles and subsequent depletion of the entire follicular pool, despite initial intact reproductive maturation. The mice essentially experienced what would be defined in humans as premature ovarian insufficiency (POI). POI represents a clear disruption in the normal female reproductive life span and is defined as the cessation of menses in a woman younger than 40 years. POI affects an estimated 1% of the females in the US

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Submitted 4 December 2012; accepted 28 March 2013; advance online publication 9 May 2013. doi:10.1038/gim.2013.60

population, and there is a 10-fold increase in the prevalence each decade, ranging from 0.1% at 30 years of age to 1% at 40 years.[16] The clinical presentation of POI may involve subfertility, oligomenorrhea, and menopausal symptoms.[17,18] Until recently, there was no evidence to suggest that the TSC genes were crucial for follicular recruitment, survival, maturation, or atresia; additional insight into reproductive function in women with TSC is equally lacking.

On the basis of the murine studies, we sought to determine whether there is a connection between the *TSC1* and *TSC2* genes and reproductive function in humans. We hypothesized that women with TSC might experience reproductive dysfunction at a higher rate than the general female population. The aim of this project was to evaluate self-reported menstrual history and general reproductive health in a cohort of women with TSC. Our results have yielded previously unreported insights into the mechanisms of TSC in the human reproductive system and have implications for the maintenance of reproductive health in women with TSC.

MATERIALS AND METHODS

Study design and population

This was a cross-sectional study under an IRB approved protocol assessing reproductive health and disease history in women with TSC aged older than 18 years. A diagnosis of TSC was assumed on the basis of membership in the National Tuberous Sclerosis Alliance support organization. A questionnaire was sent through the Tuberous Sclerosis Alliance to the addresses of 1,000 women listed in an organization database. Completion and return of the survey implied consent. Identifying information was available only to Tuberous Sclerosis Alliance staff, and surveys were returned in blank, preaddressed envelopes. Data from surveys were entered into a password-protected database, and no personal identifying information was used. Approximately three-fourths of women who received questionnaires were "independent" responders, completing the survey themselves. Approximately one-fourth of the women were "dependent," meaning they were entered in the Tuberous Sclerosis Alliance database as individuals living under the care of another individual; these surveys were completed by a caretaker or family member. We received 195 completed surveys, but 12 were excluded for underage respondents (younger than 18 years), and one was excluded for gender, for a final sample size of 182.

Questionnaire design

The questionnaire was developed through extensive literature review and with clinical expertise to encompass demographic information, reproductive health and history, and the individual's history of TSC (**Supplementary Data** online). The reproductive health section included age of menarche; pregnancy and miscarriage information; 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; family history (relation to individual and age of diagnosis) of fragile X syndrome, POI, and unspecified autoimmune disorders; current medications; and history and age at diagnosis of the following: POI, polycystic ovarian syndrome (PCOS), Addison's disease, autoimmune disease, anorexia, infertility, Turner syndrome, galactosemia, and amenorrhea. Reported TSC manifestations included specific lesions in dermatological, renal, cardiac, pulmonary, and neurological organ systems, and a free-text box was provided for respondents to indicate other diagnoses, medical issues, or concerns.

Menstrual classification

Menstrual history was reported as regularity of menstrual cycles for the majority of the time in 5-year increments (ages 16–35 years) and 1-year increments (ages 36–40 years). Menstrual regularity was defined as ≥10 periods per year. Menstrual irregularity was defined as ≤9 periods per year or none. Time frames in which women indicated contraceptive use (oral contraception or an intrauterine contraceptive device) were considered uninformative. For women reporting hysterectomy before 40 years, only time frames before the age of their hysterectomy were considered informative. Seven women reported oophorectomy without hysterectomy; these were considered to be bilateral, as all women ceased menstruation thereafter. Information gathered from the responses to the menstrual history was classified into the following six menstrual history categories: All Regular (at least one informative time frame, regular in all time frames); All Irregular (at least one informative time frame, irregular in all time frames); Regular to Irregular (at least two informative time frames, consistently regular in early time frame(s), changing to consistently irregular in later time frame(s)); Irregular to Regular (at least two informative time frames, consistently irregular in early time frame(s), changing to consistently regular in later time frame(s)); Alternating Regularity (at least three informative time frames, showing no consistent trend in regularity); and Unknown (no informative time frames).

Statistical analyses

All analyses were performed in Stata v.11 (Stata, College Station, TX). Descriptive analysis was performed for all variables (frequencies, means, median, range, and SD, as applicable). For statistical comparisons of significance, Fisher's exact *t*-test analysis was used with a P value of < 0.05.

RESULTS

Demographics

A final sample size of 182 comprised 140 independent (76.9%) and 42 dependent (23.1%) respondents. The average age was 42 years and ranged from 18 to 72 years. Demographic information are provided in **Table 1**. The majority of respondents were Caucasian; we saw a varied distribution of employment, household and marital status, and level of education.

Table 1 Demographic information

Ethnicity (*N* = 182)	*n* (%)	Marital status (*N* = 182)	*n* (%)	Respondent lives (*N* = 182)	*n* (%)
Caucasian	164 (90.11)	Single	74 (40.66)	Alone	27 (14.84)
African American	6 (3.30)	Married	86 (47.25)	With family	106 (58.24)
Hispanic	1 (0.55)	Divorced	17 (9.34)	With significant other	38 (20.88)
Asian	3 (1.65)	Widowed	5 (2.75)	In assisted living environment	7 (3.85)
Other	8 (4.40)			Other	4 (2.20)
Highest education (*N* = 181)	***n* (%)**	**Employment status (*N* = 181)**	***n* (%)**	**Annual household income (*N* = 182)**	***n* (%)**
Under 12th grade	21 (11.60)	Unemployed	49 (27.07)	<$10,000	21 (11.54)
Completed 12th grade	42 (23.20)	Part-time	20 (11.05)	$10,000–24,000	20 (10.99)
Some college	49 (27.07)	Full-time	68 (37.57)	$25,000–49,000	28 (15.38)
Bachelor's degree	35 (19.34)	Student	8 (4.42)	$50,000–74,999	23 (12.64)
Master's degree	34 (18.78)	Disabled	16 (8.84)	$75,000–99,999	23 (12.64)
		Retired	11 (6.08)	>$100,000	17 (9.34)
		Other	9 (4.97)	Decline to answer/blank	50 (27.47)

Table 2 Menstrual history

N = 182	*n* (%)
Always regular	88 (48.4)
Always irregular	24 (13.2)
Alternating regular and irregular	33 (18.1)
Uninformative	37 (20.3)

Table 3 Pregnancy and miscarriage rate

N = 182	*n* (%)
Pregnant at any time	92 (50.6)
Of those women reporting pregnancy:	
Average number of live births	1.7 ± 1.5
Average age at first live birth (years)	25.3 ± 5.4
Number who had preterm birth	8
Number who had voluntary termination	18
Miscarriage at any time	38 (41.3% of women reporting pregnancy)
Average number of miscarriages	1.6 ± 1.1
Average age at first miscarriage (years)	28.5 ± 5.9
Spontaneous miscarriage rate	17.1%

Menstrual history

The average reported age of menarche in our sample was 12.5 years, ranging from 7 to 18 years. Although some degree of pubertal irregularity is not uncommon, most cycles typically normalize by the beginning of the age ranges during which our data collection was initiated (16–20 years). Therefore, reported irregularity for the time frame of 16–20 years was still considered irregular, even if followed by consistently regular cycles. Using the stringent criteria discussed above, 182 women were further stratified into four categories of menstrual regularity (Table 2). Thirty-seven had an uninformative history, based on either unclear responses or reported oral contraceptive use. Almost half of responders reported a history of consistently regular cycles, whereas nearly one-third of women in our sample (57) experienced some irregularity in menstrual cyclicity between the ages of 18 and 40 years.

Pregnancy and miscarriage

Pregnancy and miscarriage data are presented in Table 3. Ninety-two women (50.6%) indicated that they had become pregnant at any time. The average number of pregnancies was 1.71, and average age of first pregnancy was 25.3 years. Of the 92 women reporting pregnancy, 38 (41.3%) reported a miscarriage. The ages at which women experienced their first miscarriage varied from 19 to 40 years, with a mean of 28.5 years. The number of miscarriages reported in these women ranged from 1 to 6, with an average of 1.6. Menstrual history for women reporting miscarriage was consistent with the overall sample population. Of all reported pregnancies, we observed a spontaneous miscarriage rate of 17.1%.

Reproductive symptoms

We asked women to report menopausal and other symptoms they were currently experiencing or had experienced in the past and the duration of these symptoms in years. Data for women younger than 40 years are presented in Table 4. Hot flashes and night sweats were common, present in 12.3 and 16.4% of women, respectively.

Reproductive disorders

Premature ovarian insufficiency. To assess for possible POI in the absence of clinical and laboratory data, we narrowed our focus to women who experienced a gradual progression from consistently regular cycles to consistently irregular cycles later in their reproductive lives, particularly after age the of 35 years.

Table 4 Current and previous reproductive symptoms in women younger than 40 years

Current or previous symptoms	*n* (%)	Average duration (years)
Hot flashes	9 (12.3), *N* = 73	4.9 ± 4.4 (range: 3 months–30 years)
Night sweats	12 (16.4), *N* = 73	1.5 ± 1.2 (range: 6 months–16 years)
Body hair	11 (15.1), *N* = 73	13.2 ± 6.0 (range: 2 years–48 years)
Milky breast discharge, excluding breastfeeding	8 (11.0), *N* = 73	1.3 ± 0.4 (range: 1 month–3 years)
Weight gain of 25 pounds, excluding pregnancy	26 (34.7), *N* = 75	NA

NA, not applicable.

Table 5 Self-reported reproductive disorders

Diagnosis	*n* (%)	Average age diagnosed (years)
POI	4 (2.3), *N* = 172	39.3 ± 4.9[a]
PCOS	11 (6.4), *N* = 171	32.4 ± 14.7
Amenorrhea	9 (4.9), *N* = 172	30.6 ± 11.5
Infertility	13 (7.1), *N* = 173	30.9 ± 6.0
Anorexia	6 (8.2), *N* = 173	18.5 ± 1.9

PCOS, polycystic ovarian syndrome; POI, premature ovarian insufficiency.

[a]One woman indicated an age at diagnosis of 45 years; this age was excluded.

We observed this pattern in 18 women, but elected to exclude 10 women for whom additional data were inconsistent or incomplete. In the entire sample, four women (2.3%) reported receiving a diagnosis of POI, but three were excluded for inconsistent information, which included history of radiation therapy at unknown site and age, age of POI diagnosis later than 40 years, and lack of supporting menstrual history data. After all exclusions, a total of eight respondents were deemed to have a "possible" diagnosis of POI, a prevalence of 4.4% in our sample. There were no statistically significant differences between women with and without possible POI in our sample.

PCOS. Eleven women in our sample (6.4%) self-reported a diagnosis of PCOS (Table 5). The average age of diagnosis was 32.4 years. The average body mass index for women with self-reported PCOS was 26.9, ranging from 20.2 to 40.3. Of the eleven women with PCOS, eight reported a history of hot flashes, night sweats, and excess body hair, seven a history of acne, and four a history of milky discharge from the breasts.

Amenorrhea. After excluding those with inconsistent data, nine women (4.9%) in our sample reported a history of amenorrhea (Table 5). The average age of diagnosis was 30.6 years. On further analysis of menstrual history, one of these women was included in the group of "possible POI."

Infertility. Thirteen women (7.1%) reported a history of infertility, with an average age of diagnosis at 31 years (Table 5). Reported comorbidities included one respondent with reported anorexia and three with reported amenorrhea. One of the 13 women reported having never been pregnant. The remaining had an average of 1.25 live births, with one reported preterm birth and six reported miscarriages.

Anorexia. Six women (8.2%) reported a history of anorexia, three of whom reported an age of diagnosis (Table 5). Anorexia can affect menstrual function and often results in amenorrhea. The menstrual history data for women reporting an age of diagnosis varied; one woman diagnosed at the age of 19 years reported irregular cycles in this time frame, as well as between 31 and 35 years of age. The second and third women reported an age at diagnosis of 22 and 20 years, respectively, and reported irregular cycles in these time frames followed by both regular cycles and uninformative data thereafter.

DISCUSSION

This study was designed to estimate the prevalence of self-reported reproductive dysfunction in a sample of women affected with TSC. Our analysis uncovered significant menstrual and reproductive dysfunction in women with TSC.

The age of menarche in our sample was consistent with the national average (12.5 years) and suggests intact menstrual functioning at the onset of menses.[19] Overall, 31% of women with TSC in our sample reported evidence of menstrual irregularity. A lack of data from missing information or data obfuscated by contraception could either underestimate or overestimate the true prevalence of menstrual function. We also observed a 41.8% miscarriage rate in women reporting a history of pregnancy. Estimates of miscarriage in the general population vary, particularly by age. It is estimated that one in four women will experience miscarriage of a recognized pregnancy.[20] The average age of miscarriage in our sample was 28.5 years. Given this average, and an average respondent age of 42 years, the year 1996 best represents an epidemiological comparison for these data. According to US Census data, the average fetal loss rates among women in 1990 and 2004 were 15 and 17%, respectively.[21] Therefore, women in our sample reported more miscarriages and that they occurred at ages younger than "advanced maternal age," when fecundity typically declines. Finally, 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.

Using self-reported menstrual history, we were able to infer the prevalence of specific reproductive disorders that might explain the data. For example, POI may be reflected in the women whose cycles went from consistently regular to consistently irregular. We applied stringent criteria in identifying these women. Eight of 182 women were in that category, which would give an estimate of possible POI of 4.4%. Reports

of additional reproductive problems varied across the eight women but did include reproductive symptoms of night sweats and hot flashes, infertility, and/or blood tests indicating a menopausal state. These would support a diagnosis of POI because women with POI experience perimenopausal symptoms at an earlier age than expected. Few studies have empirically assessed the prevalence of POI in the general population; most cite an estimate of 1% from a 1986 longitudinal study.[16] Therefore, our finding of possible POI in 8 of 182 women studied (4%) is significant.

The prevalence of PCOS in our sample was approximately 6%, comparable with national estimates in the general population, which range from 4.7 to 6.6% among US women.[22,23] To corroborate our assessment of PCOS, we considered menstrual histories that included irregularity in the 20s, unlike the pattern of regular to irregular cycles in POI; additional report of reproductive symptoms such as body hair and acne added to our evidence base.

Traditional mechanisms of TSC-associated manifestations involve loss of heterozygosity and haploinsufficiency,[9,24] but we do not know which if either of these are likely to be responsible for the effects we saw on reproductive function in the women studied. The mouse data suggested that loss of heterozygosity would be the likely mechanism of POI. Complete loss of either *Tsc1* or *Tsc2* in primordial follicles led to increased activation of mTORC1 and the entire population of oocytes.[14,15] The mutant mice were initially fertile with normal litter sizes; however, litter size progressively decreased until POI and infertility manifested. Our data suggest that similar events may occur in the ovaries of women with TSC, but in humans, haploinsufficiency may be a more likely explanation to account for some of the reproductive dysfunction observed in the human population.

Our study is the first to assess the presence of menstrual irregularities and reproductive dysfunction in a TSC population. We observed an average age of menarche consistent with the general population; nevertheless, one-third of women sampled had some form of menstrual irregularity between the ages of 16 and 40 years. We also observed a high rate of miscarriage, over 40%, at an average age of 28.5 years. Finally, we document self-report of several reproductive disorders such as amenorrhea, anorexia, possible POI, PCOS, and infertility. In particular, the possibility of a 4.4% prevalence of POI is four times as high as in the general population and deserves to be further investigated prospectively with laboratory data. This finding is supported by the data obtained from conditional mouse models of TSC.

On the basis of these data, a multidisciplinary management and treatment approach in women with TSC should address reproductive health and further corroborate the self-reported data presented here. A major strength of our study is the application of strict criteria for the categorization of menstrual function in the absence of definitive clinical data. Limitations include a small sample size biased to members of a large support organization and consisting of older members for whom rendering a detailed reproductive history may be difficult. We also encountered missing data and an absence of medical chart review, making it possible that we have underestimated reproductive dysfunction for the TSC population studied. Overall, our findings argue for a thorough evaluation and attention to menstrual history in women with TSC, and further molecular insights into genes important in reproductive function.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

ACKNOWLEDGMENTS

We sincerely thank the respondents and members of the Tuberous Sclerosis Alliance organization for their participation, and the Alliance staff for assisting in the distribution of these surveys. The University of Texas-Memorial Hermann Hospital TSC Center of Excellence generously provided financial support for the development of this survey.

DISCLOSURE

The authors declare no conflict of interest.

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