STUDYING THE DIAGNOSTIC PROCESS OF WOMEN WITH PREMATURE OVARIAN INSUFFICIENCY

by

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Premature Ovarian Insufficiency (POI) is a condition diagnosed in women who experience cessation of menses prior to age forty (secondary amenorrhea), or never initially begin menses (primary amenorrhea). Both primary and secondary amenorrhea lead to infertility, and can increase the risk for osteoporosis and cardiovascular disease. Typically having an idiopathic cause, POI is diagnosed in 1% of women before age 40, and 0.1% before age 30. In this study, the diagnostic process of women with POI was studied through the use of a questionnaire. Twenty-three women with POI were recruited through Magee Womens Hospital and other sites, nineteen of whom were eligible for analysis. Using logistic regression analysis, factors such as age of diagnosis, months of cessation, and type of amenorrhea were studied to determine the role they play in a participant's opinion regarding helpfulness of providers. Odds ratios of 0.87, 0.99, and 3.63 were calculated for each of these factors, respectively. The most striking trend was the difference between women with primary amenorrhea and women with secondary amenorrhea; the odds of a reported helpful outcome was found to be 3.63 times more likely in those with secondary amenorrhea than those with primary. In addition, the results showed that most women find their physician provider and the internet essentially equivalently helpful with respect to providing health-related information about POI. Studies on the diagnostic process of women with POI have public health significance because this condition affects 1% of the population in the United States. More needs to be done in terms of providing educational and emotional support after a diagnosis is made. As awareness is increased, referrals to genetic counselors can

be made. Genetic counselors are a resource that could be utilized for these patients, not only for educational purposes, but also for the emotional and psychosocial aspects of a POI diagnosis.

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PREFACE

First and foremost I would like to thank my family for supporting me not just in the past two years, but for always being so proud and supportive of my schooling. I couldn't ask for a better family, and I am so thankful to be able to share in this with them.

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1.0 INTRODUCTION

Premature Ovarian Insufficiency (POI), also known as Premature Ovarian Failure (POF), is the condition when women experience early menopause. Clinical presentation includes amenorrhea and two follicle-stimulating hormone (FSH) levels in the menopausal range. Amenorrhea is considered primary if there is absent menarche altogether, in contrast to secondary amenorrhea when there is premature depletion of follicles in the ovaries prior to 40 years old [1]. The term secondary amenorrhea is used when menses have been absent for more than 6 months. Early menopause in POI patients is in contrast to the typical age of 51 in which most women experience menopause[8]. POI affects approximately 1% of women by age 40[1, 8]. POI leads to infertility and menopause symptoms. Infertility is not universal however, as 5-10% of women with POI are able to conceive [14]. POI can be identified as having a genetic cause in some women, yet the cause of POI remains unknown in 90% of cases[14]. POI occurring spontaneously can be part of syndrome, due to a single gene defect, or even result from a structural abnormality in the X chromosome. The aim of this project was to study the responses given in a questionnaire administered when a woman is recruited to be in an ongoing study regarding genetic causes of POI. The medical and emotional needs experienced during the diagnosis process were explored – focusing on the benefit a genetic counselor could provide.

2.0 BACKGROUND AND SIGNIFICANCE

2.1 OVERVIEW

2.1.1 Typical ovarian function

Women have their maximum number of follicles in the ovaries around 20 weeks gestation[16, 21]. The process of atresia starts at this time. When a female is born, her oocytes have been reduced in number to 700,000 down from 6-7 million[16]. By the time she reaches puberty, a female has only 400,000 follicles remaining[16]. Monthly ovulation and continued atresia lead to few follicles remaining by the time menopause arrives[16, 22]. In a normal development scenario, a graafian follicle is developed in the ovary each month. It is a new secretory structure with each monthly cycle, arising from a microscopic primordial follicle. This continues until menopause, when menses permanently ceases. This results due to the depletion of functional primordial follicles. The typical age for menopause in most women is around age 50. If menopause occurs before age 40, it is considered to be premature [14]. The constant oocyte atresia mentioned can lead to early menopause in someone who is either starting out with fewer follicles or loses them at a more rapid rate[16]. In order for normal ovarian function to proceed, two functioning X chromosomes are required[8].

2.1.2 Ovarian function and clinical presentation in women with POI

While 1 out of 100 women under age 40 are affected with POI, the numbers reduce as the upper age limit is decreased. 1 in 10,000 women are affected by age 20, and 1 in 1,000 women are affected by age 30[1]. Primary amenorrhea presents clinically as pubertal delay and absent menarche, whereas women with secondary amenorrhea often have no idea they have aberrant ovarian function until they experience early menopause. POI presents in a similar manner to natural menopause, in that the women experience heat intolerance, flushes, decreased libido, depression and anxiety, vaginal dryness, fatigue, etc.[1] Hormone levels are characteristic as well. Low levels of estrogen and inhibin (the gonadal hormones) are seen, along with high levels of LH and FSH (the gonadotropins). This is where the name "hypergonadotropic amenorrhea" stems from[1]. An FSH value of over 40IU/L is suggestive of ovarian failure. The condition can come on quite suddenly for some women, while others experience signs and symptoms for years before a diagnosis is made. Osteoporosis and heart disease are secondary health concerns for women with POI[1].

Ultrasound findings during the diagnosis of POI can include small ovaries, typically without any growing follicles. Women with POI who have ovarian cysts may have mutations in *LHR*[1]. These women experience secondary amenorrhea and LH serum levels that are higher than serum FSH; this is contrary to typical POI patients[1]. Karyotype and Fragile X testing are done in conjunction with ultrasound and hormone levels, in attempt to determine a cause for the amenorrhea. Karyotype and Fragile X testing help depict if the woman has normal chromosomes, as abnormal chromosomes can be causal for POI in certain circumstances.

Half of the women that present with primary amenorrhea are found to have ovarian dysgenesis[1]. Ovarian dysgenesis is determined by a pelvic ultrasound depicting absent ovaries

and uterine hypoplasia. Other patients may be seen with follicles under 10 mm. This is the typical case in patients with more normal pubertal development and *FSHR* mutations[2]. The hormone FSH is necessary for the initiation of follicular growth at the antral stage. In one patient studied, complete FSH resistance led to infertility yet numerous small follicles were present[2]. FSHR is a G protein-couple receptor, and was linked to POI after a study in Finnish families with primary amenorrhea.

The women that present with secondary amenorrhea are in the majority of women with POI. These cases are most likely due to premature follicular depletion secondary to multiple factors, including a genetic predisposition and environmental exposures.

2.1.3 Diagnosis

The first step in confirming a diagnosis of POI in a symptomatic woman is to rule out the possibility of pregnancy. Other reasons for amenorrhea should be considered, including but not limited to excessive exercise, emotional stress, inadequate caloric intake, and chemo/radiation therapy. The majority of cases of secondary amenorrhea are due to polycystic ovary syndrome, hypothalamic amenorrhea, primary ovarian insufficiency, or hyperprolactinemia[15]. Women have karyotype testing, as well as Fragile X carrier testing, in order to determine if a potential chromosome problem could be the cause.

Initial tests to be run include a physical exam, measurement of serum prolactin, FSH and thyrotropin levels as well as a pelvic ultrasound. Low FSH may indicate hypothalamic amenorrhea (caused by stress), while high FSH can indicate menopause and prompt a repeat sample in one month with a serum estradiol measurement at that time[14].

To be considered for POI, women have cessation of menses prior to age 40, symptoms of ovarian failure, as well as FSH levels measured on two separate blood draws greater than 40 IU/liter (i.e. menopausal levels). Symptoms of ovarian failure can include hot flashes, night sweats, trouble sleeping, vaginal dryness and decreased sex drive, and mood swings[14].

Testing for adrenal antibodies can also be considered in those women that have POI not associated with a syndrome. Indirect immunofluorescence or 21-hydroxylase immunoprecipitation can be used. Positive results are obtained in an estimated 4% of women that have POI[14]. In these women, there is steroidogenic cell autoimmunity with lymphocytic autoimmune oophoritis as the mechanism[14].

2.1.4 Management of women with POI

"The diagnosis of primary ovarian insufficiency affects a woman's physical and emotional well-being, and the management of the condition should address both"[14]. This statement highlights a goal in managing women with POI. The diagnosis of POI comes with the unexpected shock of infertility for many women. Lowered self-esteem, depression, anxiety, lack of support and severe emotional distress are all more common in this population of women. An unfortunate fact is that while many women want emotional guidance and support, they seldom ask for such support. It would potentially be beneficial to ensure that sources for emotional support are discussed with the patient whether it be friends, family, or a mental health professional (i.e. including genetic counselors).

Hormone replacement therapy is often used in the management of POI. Women with POI are at an increased risk for health conditions including osteoporosis (increased risk of fractures) and ischemic heart disease. A prolonged lack of estrogen is the culprit for early onset of osteopenia and osteoporosis in women with POI[1]. It is typically recommended that a combined (estrogen and progestin) hormone therapy regime be used for young women who have POI until they reach the normal age of menopause[14, 16]. As far as established guidelines, estrogen-replacement therapy is recommended by the American Society for Reproductive Medicine and the International Menopause Society[14, 15, 17]. Oral contraceptives typically have more steroid hormone than is needed and are thus not recommended for management purposes. The goal is simply for levels of physiologic replacement.

To establish the degree of osteopenia/osteoporosis, bone mineral density test should be evaluated. As it is reduced in women with POI, education should be offered on ways to maintain bone health. Intake of calcium and vitamin D, along with weight-bearing exercise (jogging, walking, resistance exercises, stair climbing) are some of the strategies[14, 18].

Additional recommendations may be advised depending on the underlying cause for the premature menopause in the patient.

2.1.5 Fertility concerns

Infertility is frequently the most devastating consequence for patients with POI. Infertility is irreversible, and it is often discovered late in a woman's potential child-bearing years. Some patients have children early, only to discover the diagnosis of POI later on. However, many women postpone having children, for a variety of reasons, missing the small window of

opportunity to naturally conceive. However, 5-10% of women are able to conceive and deliver a child after receiving the diagnosis of POI[14].

While various options have been tried, the only true known solution for allowing women with POI to have a child involves ovum donation[1]. This assumes there is a completely absent follicular reserve. There is the possibility for the woman's own eggs to be preserved if her diagnosis of POI is made early enough. Other options if parenthood is desired include adoption, fostering, and embryo donation[14].

Some women with POI do not wish to conceive, and as mentioned above, it is possible – though rare – to conceive with this condition. If the wish is to avoid pregnancy, contraception should be used whether it is the barrier method or an intrauterine device. This is an important discussion to have with women after being diagnosed.

2.2 CAUSES

2.2.1 Genetic causes

A first step in the diagnostic process for women with POI is chromosomal analysis. This includes having blood drawn and a karyotype performed. X-chromosome abnormalities are responsible for the majority of POI cases with ovarian dysgenesis[1, 23-26]. Specifically, chromosomal regions Xq21.3-Xq27 (for POF1) and Xq13.3-q21.1 are of interest. The former has been seen in patients and families showing deletions in this area, while the latter has been seen in individuals with balanced X/autosome translocations. Several candidate genes have been identified [27-32].

Well-established X chromosome defects include Turner syndrome and Fragile X syndrome (*FMR1* gene premutation). Females with Turner syndrome have only one X chromosome, leading to gonadal dysgenesis, short stature, and congenital lymphedema[5-7]. Other genetic causes of Turner syndrome can include duplication of the q arm in one X chromosome. Mosaicism can occur as well. One in 2500 women have Turner syndrome (this number would be higher but affected fetuses often spontaneously abort) [4]. Other features can be seen in those with Turner syndrome, including developmental delay, congenital heart disease, hypothyroidism, and ocular findings such as strabismus. Turner syndrome is a known cause for POI – there are fewer primordial follicles in the ovaries *in utero*, and these follicles commit premature apoptosis and are no longer present at adulthood[4]. A smaller uterus and vagina can be seen as well, or an atrophic vaginal lining. Initial ovarian function is present (known due to the presence of gonadotropin levels that are normal for the first months of life) but does not progress[4]. Hormone replacement therapy is typically used in these patients. Just as with women with idiopathic POI, spontaneous fertility is a rare event.

In Fragile X syndrome, the full mutation results in affected males who have mental retardation and autism. The trinucleotide repeat occurs in the 5' untranslated region of the *FMR1* gene. The normal repeat length of *FMR1* is less than 40 repeats[16]. The full mutation has more than 200 CGG repeats[16]. The full mutation results after the gene is methylated and silenced[16]. The premutation, containing 60-199 repeats, is problematic. It was discovered in the 1980s and 1990s that females with the premutation can have early menopause (i.e. ovarian failure) [3]. It was noted prior that POI occurs for 1% of women in the general population. That number is increased to 21% for women that are premutation carriers[3]. An additional striking figure is that women

who have sporadic and idiopathic POI are premutation carriers 2% of the time; moreover, women with the rare form of familial POI are premutation carriers 14% of the time[3].

Monogenic defects can include both those that are syndromic and those that are isolated. Table 1 below outlines such disorders involved[1]. Despite genes being identified, the phenotype may still vary. For example, *FSHR* mutations can either be associated with primary or secondary amenorrhea, depending on nature of the mutation[1].

Table 1: Monogenic Causes of POI

Monogenic defect		**Inheritance**
Syndromic defects	Congenital disorders of glycosylation	Recessive
	Galactosemia	Recessive
	Blepharophimosis-ptosis-epicanthus inversus syndrome	Dominant, female-limited
	Pseudohypoparathyroidism type Ia	Maternal inheritance/parental imprinting
Isolated defects	Follicle stimulation hormone receptor mutations (*FSHR*)	Recessive
	Luteinizing hormone receptor mutations (*LHR*)	Recessive
	FOXL2 mutations	Dominant, female-limited
	Bone morphogenetic protein 15 (*BMP15*) mutations	Female-limited, heterozygous mutation

2.2.2 Non-genetic causes

In most cases of POI, the cause has yet to be determined. The questionnaire used for this research study is part of an ongoing study that is focused on idiopathic cases of POI. Both sporadic and familial appearing histories are seen in idiopathic POI. Causes can include those of iatrogenic origin (such as radiation, surgery, chemotherapy), autoimmune, infections, etc.

Cigarette smoke is one of the main environmental toxins known to be associated with a decreased age at menopause. There is a dose-dependent effect of smoking on fertility, with the relationship starting at half a pack per day[16]. A component of cigarette smoke called dimethyl benzantracene binds the aromatic hydrocarbon receptor on oocytes and granulosa cells. Nicotine inhibits aromatase which results in lowered oestradiol production[16]. Several studies have found similar results where female smokers have lower endogenous estrogen levels when compared to non-smokers[19]. History of cigarette smoking is therefore something that should certainly be asked of women who are experiencing early menopause.

An association is seen with menopausal age among *FMR1* premutation carriers and cigarette smoking[33]. It has been noted prior that premutation carriers are at risk for premature menopause, and later studies show smoking leads to an even earlier age of menopause among this group of women. A hazard ratio of 1.34 was determined from the study, indicating smoking indicates a risk factor for reducing the age of menopause. A cohort of 1,068 women was studied[33].

2.2.3 Mechanisms of POI

As discussed above, causes can be both genetic and non-genetic. The mechanism by which POI can occur can be divided into two categories. The first is follicle dysfunction. In this instance, follicles are in the ovary but do not have normal function. A pathologic process is preventing folliculogenesis to progress and follicles arrest their development. Examples include FSH-receptor mutations, LH-receptor mutations, G-protein mutations, enzyme deficiencies, autoimmune lymphocytic oophoritis, and insufficient follicle number[14].

The second mechanism is follicle depletion. In this instance, primordial follicles in the ovary are significantly diminished. It is possible there was never an adequate amount of primordial follicles *in utero* initially, or there was an accelerated rate at which follicles were expended, or even a destruction of follicles via an autoimmune disease or toxic exposure. Examples include Blepharophimosis-ptosis-epicanthus inversus syndrome, Turner syndrome, and exposure to cleaning solvents such as industrial 2-bromopropane[14].

2.3 PSYCHOSOCIAL CONSIDERATIONS

2.3.1 Depression in women with POI

As found with several medical conditions, "POI is associated with an increased lifetime risk for major depression"[9]. One study at the National Institutes of Health Clinical Research Center found that women with POI have depression more often than women with Turner syndrome. The percentages determined were 54.5% and 36%, respectively[9, 10]. The onset of depression was

typically found to be before the diagnosis of POI, but after menstrual irregularity had begun. Since women with Turner syndrome endure a longer duration of ovarian insufficiency yet tend to have lower depression rates, there must be additional factors contributing to the relationship between POI and depression besides the ovarian failure alone[9]. One of those factors may be the age at which the diagnosis is learned. For example, women with Turner syndrome often learn of this diagnosis at a young age in a supportive environment, compared to women with POI who are often diagnosed as a result of their inability to conceive[9]. The second scenario may have more emotions involved and less potential support/options.

Studies have been conducted that look at women with POI and Axis I disorders, disorders that include depression, anxiety, mood, etc. to determine a correlation. The subject population included women with spontaneous POI, and normal female karyotypes of 46,XX. While Axis I disorders have a lifetime prevalence of 50% in community-based studies, a lifetime prevalence of 69.5% was found in women with POI[9, 11]. Rates specific to depression were also found to be higher in those with POI. Specifically, major depression had a lifetime occurrence of 54.5% in women with POI compared with 20% in women without POI[9, 11].

In regards to the timing of onset, 68.4% of the women – in the study described above who reported at least one major depression – reported that the onset was before being diagnosed with POI[9]. This is by far the majority of women in the study. However, when menstrual cycle irregularity (MCI) was taken into account, it was found that the women reporting at least one episode of major depression typically had MCI before the onset of depression. While 26.4% reported depression before the onset of MCI, 73.6% reported depression after the onset of MCI[9]. It was concluded that depression and POI are linked, with the menstrual irregularity (and thus depression) usually preceding a diagnosis of POI. Timing varies from woman to woman,

shedding light on possible reasons for the variation of depression. Depression in women with POI can stem from several sources, including the knowledge of infertility and her role possibly changing within the family. Menstrual irregularity can be a significant stressor for some women, as well as the menopause symptoms that are paired with POI.

2.4 ROLE OF GENETIC COUNSELING

2.4.1 Genetic Counseling for POI with Genetic Etiology

Genetic counseling can play an important role when POI is due to a known genetic syndrome. For example, if the cause for a woman's POI is determined to be Fragile X syndrome, the genetic issues are broader than just POI. Fragile X syndrome is caused by expansion of CGG repeat in the *FMR1* gene located at Xq27.3. Not only are the women at risk for POI, but there is intellectual disability in the affected males[1's 8]. This condition is X-linked. Families would benefit from speaking with a genetic counselor, who could address issues such as inheritance, who is at risk in the family, medical implications for those affected, and genetic testing.

Fragile X syndrome has a phenomenon known as expansion. The repeat number can grow, or expand, upon transmission to the fetus through a female premutation carrier[16]. A female premutation carrier would thus be at risk to have a son with Fragile X syndrome, and should be appropriately counseled. When the premutation is passed through the father, the length remains stable or contracts[16]. Fragile X syndrome is a complex genetic disorder, and families could benefit from the expertise of a genetic counseling professional.

There are additional circumstances where POI has a genetic etiology, as mentioned in the section Genetic Causes of POI. Different defects can have varying patterns of inheritance and different concerns that may need to be addressed. A genetic counselor would be able to explain the different risks associated depending on the genetic cause, and what other features may be associated with the condition.

2.4.2 Familial POI and Implications for family members

As with several conditions, a POI diagnosis of one person can have implications on the medical histories of other family members who may be unaware that they are carriers or possibly affected. If familial POI is established, early menopause can be predicted and watched for in other female relatives. This creates the opportunity for discussion regarding reproductive choices such as having children earlier or freezing embryos for the future.

Not only can measures be taken in regards to fertility, but for other areas that POI affects as well. The heart and bones are important body systems to include in management. Women with POI should be offered symptom management, emotional support, and risk reduction strategies as early as possible in order to maximize benefits[1]. To date, overall ways a genetic counselor could benefit a patient with POI could not be found in the literature. Research has mainly examined how POI is inherited; genetic counselors can play a role in educating families about the inheritance of POI.

The familial form of POI is considered to be rare. The exact figures are unknown, but the familial form is estimated to be responsible for 4-31% of all POI cases[1]. Inheritance patterns can vary, including autosomal dominant sex-limited and x-linked, with some families showing reduced penetrance. A genetic counselor would be able to assess the family history in order to

determine which inheritance pattern is most likely. Once this information is known, which females might be at risk could be better understood. For those families where only one female is affected and the case appears to be sporadically occurring, her female relatives could be quoted to be at the general population risk for developing POI[1].

2.4.3 Advocacy for the patient

In regards to medical care, a genetic counselor could ensure that the patient with POI was getting all of the surveillance and resources she needs. Hormone replacement therapy, monitoring of osteoporosis and heart disease, as well as fertility concerns are all areas in which the patient may need an advocate and/or referral. Patients, no matter the condition in question, are not always aware of what management guidelines they should be following. This is seen in every area of genetic counseling. Genetic counseling could be a valuable resource to patients with POI, providing a service that offers patients both the emotional and medical support they need.

Mental health is an additional, and very important, area that often needs to be addressed. Depression is not always a main discussion point, yet it should be as it is under-diagnosed and undertreated[9]. An estimated 40% of individuals with major depression do not receive any form of treatment[9, 13]. While that number is not specific to those with POI, it certainly sheds light on the fact that mental health is not advocated for as needed in this country. Similar research found 6-8 years can pass before a depressed individual receives medical help[9, 14].

Depression and general infertility is a topic that has been studied in the literature. Infertility is estimated to affect 10-15% of couples that are of reproductive age[35]. The loss of fertility can result in several emotions including anger, depression, marital problems, anxiety,

social isolation, and sexual dysfunction. Diminished self-esteem, stigma and sense of loss have also been described for these couples[35]. These are areas where a genetic counselor could help ensure the patient has access to resources, whether it is a support group or a referral to a mental health specialist.

The psychosocial issues present may reflect not only those present in a patient with POI, but also in her partner or family members. If a patient was having difficulty communicating with her family, a genetic counselor could aid in this process. Ways in which a genetic counselor could make a difference include helping the patient think about ways to start the conversation with family, role play exercises, writing family letters the patient could distribute, and prepare for the different reactions family members might have. There are many ways to be an advocate for psychosocial concerns, and a genetic counselor is trained to serve in that role.

3.0 MATERIALS AND METHODS

3.1 SPECIFIC AIMS

Aim 1: To administer a questionnaire to women recruited to be a research study regarding genetic causes in women with idiopathic POI.

Aim 2: To study the responses in the diagnosis of POI section of the questionnaire to determine if a factor is present that has significant impact on a woman's response to if she feels providers are helpful at the time of diagnosis.

Aim 3: To demonstrate the need for an increased awareness of POI among primary care physicians.

3.2 RECRUITMENT

The recruitment of participants for this study was approved by the University of Pittsburgh Institutional Review Board (IRB) (PRO09080427). The most recent letter of approval can be found in Appendix C. Three groups of patients were enrolled in the study: (1) the subject in whom Premature Ovarian Insufficiency has been diagnosed, (2) family members of the subject

with POI, and (3) healthy fertile women to be used as controls. For the purposes of this project, only those in the first group were focused on. Subjects were identified through routine clinical evaluation for female fertility in the Reproductive Endocrinology and Infertility clinic at Magee Womens Hospital in Pittsburgh, PA. Subjects were also referred to Magee by physicians at Children's Hospital of Pittsburgh. Additional recruiting was made through The International Premature Ovarian Failure Association website (pofsupport.org), where interested and eligible women emailed the study coordinator and a blood kit was mailed to them to have their blood drawn and sent back to Pittsburgh. One family was recruited through the NIH. Nuclear family members were asked to participate at the time of the subject's recruiting, with the primary focus being on the subject's parents and unaffected sisters, if any. Controls were recruited through the Midlife Center, also at Magee Womens Hospital. All participation was voluntary.

Inclusion criteria included cessation of menses prior to age 40, symptoms of ovarian failure (hot flashes, etc.), FSH levels measured on two separate occasions that are greater than 40 IU/liter, unclear etiology for their POI, and normal karyotype. Exclusion criteria included those women with premature menopause due to surgery or known exposure to radiation and chemotherapy. Inclusion criteria for the control women included natural menopause after age 46, and at least one liveborn child.

When recruited, the participant filled out an informed consent form (see Appendix A), signed a medical records release such that her medical records related to her diagnosis of POI could be accessed, a family history was taken, and her blood drawn. Lastly, the subject filled out a questionnaire (see Appendix B). Part of the questionnaire is designed to ascertain the diagnostic process for females with POI. The questions in this section ask if it took a lot of time and/or providers before a diagnosis was made and the information known and/or presented to

them regarding POI. The data generated from this section of the questionnaire are the focus of this study.

3.3 ANALYSIS OF QUESTIONNAIRE

Analysis was performed using questionnaire data to examine whether certain factors influenced the question dealing with if the women felt providers are aware of POI and helpful in the diagnostic process. Factors studied included the age at which the woman was diagnosed, the type of amenorrhea, and the number of months of menses cessation before a diagnosis was made.

Preliminary analysis included verifying that no bias was introduced to the data. Women were mainly recruited through the Magee clinic, but an additional few were also recruited through the aforementioned POI website as well as the NIH. To check for potential biases in the factors listed by recruitment type, t tests and tests of proportions were calculated. To assess whether the means of two groups were statistically different from one another, as with the age of diagnosis and months cessation, a t-test was used. To assess categorical data, as with the type of amenorrhea and the variable of interest (if the provider was helpful), a test of proportions was used. If the woman said the provider was helpful, her response was scored as a 1. If the woman said the provider was unhelpful, was neutral in her response, or left the question blank, her response was scored as a 0. Four women were removed from this set of calculations. Two (ID-17 and ID-28) were removed because they had a sister who had been previously diagnosed with POI and thus her diagnostic experience would be quite different from someone who is an isolated family member. The other two were removed (ID-29 and ID-31) because they were never

officially diagnosed with POI, but rather reported early menopause after her daughters/granddaughters were diagnosed.

Once it was confirmed no bias was present, logistic regression analysis was performed. The predictor variable was if the provider was reported helpful. The variables to be factored in included the age of diagnosis, months cessation, and type of amenorrhea (primary vs. secondary). A p-value was determined for each factor, and if significant, would lead to the conclusion that there was an effect of that factor on the predictor variable. Odds ratios were calculated for each factor as well, to determine if trends are present.

4.0 RESULTS

Table 2 shown below depicts a summary of the questionnaire responses in the diagnosis section. A total of twenty-three women were recruited (table 2), although not all were included in all calculations (tables 3 and 4). Please see Appendix B for a copy of the questionnaire.

Table 2: Diagnosis Section of Questionnaire – Complete Version

Subject with POI identifier	Type of Amen.	Age of diagnosis	Type of provider who made diagnosis	Time menses had stopped before seen by provider and diagnosed	Number of providers seen before diagnosis made	Are most providers aware of POI and/or helpful in providing information	Been told a reason why have POI
PPOF-1	2°	36	Reproductive endocrinologist	3 months	2 (OB/GYN referred to endocrinologist)	-	Pre-menopausal
PPOF-2	1°	16	Reproductive endocrinologist	n/a	4	No; some have been more knowledgeable than others	Not specifically; something with malformation of the ovaries
PPOF-5	2°	27	Primary physician	4 months	No. she was just testing my thyroid and one of the hormone levels told her I was going through menopause	Yes. She wanted me to have genetic testing so felt she very helpful	No
PPOF-6	2°	30	OB/GYN	Never stopped	did not go to several providers before dx made	-	-
PPOF-8	1°	17	Reproductive endocrinologist	4 years	2: PCP and OB/GYN. No tests were done, told was a late bloomer	No. I think age plays a role and also at age 13 I began having migraines w/ aura. This should	I'm also Triple XXX and was told at a study at NIH that this could be a factor

Table 2: Continued

						have been an indicator something was wrong.	
PPOF-9	2°	18	OB/GYN	1 year	3 or 4	No, not then, maybe presently they are more familiar, but my OB/GYN had to consult several specialists	Possibly autoimmune disorder
PPOF-10	2°	22	Endocrinologist	11 months	2	Yes	No
PPOF-14	1°	16	OB/GYN and genetics specialist	n/a	3	Just found out	Just now
PPOF-15	2°	33	General endocrinologist	Approximately 18 months	I saw my normal OB/GYN who didn't really know why it stopped, then saw an endocrinologist 3x over 9 month period. He ran a few tests and wanted to monitor my FSH and LH levels before making a dx	I don't think normal providers are (obviously, endocrinologists have a little more experience with it). My OB/GYN recommended putting me on pills that would cause me to menstruate, but didn't seem concerned or knowledgeable about why I stopped in the first place.	No, but at one point, the endocrinologist told me its sometimes caused by autoimmune disorders. Within the last 2 months, I have been dx with lichen plantis (in my mouth), which I think is an autoimmune disorder so I (not the doctors) wonder if it is related.
PPOF-16	1°	15	OB/GYN	n/a	Yes, 2	Yes they are aware, and inform treatment options	No
PPOF-17	1°	11	POF investigator in Bethesda	n/a	No	Since her sister has POF and she was seen by POF investigator, we went directly with him and he made the dx	No
PPOF-21	2°	17	Reproductive endocrinologist	Less than 1 year	No	OB/GYN providers, yes	No
PPOF-24	2°	27	OB/GYN, fertility and reproductive endocrinologist	5 or 6 months	2	No. I went to an OB/GYN for regular pap. Nurses were completely unaware of POF	Deletion on X chromosome

Table 2: Continued

						and doctor did not know much.	
PPOF-27	2°	34	OB/GYN, reproductive endocrinologist	1 month	3 or 4	No. The first doctor was able to dx but unaware of additional info. Referred to reproductive endocrinologist who was helpful	-
PPOF-36	2°	23	Reproductive endocrinologist	n/a	No, but afterwards I saw 4-5 different doctors due to differences in treatment	Some are, some aren't	No, but it probably has something to do with genetics.
PPOF-28	2°	26	Reproductive endocrinologist	Hadn't stopped completely; was seen for menopause-like symptoms	2: one reproductive endocrinologist and one gynecologist	OBs are aware I think but I don't think they push for early detection as much as they should. I think if the chance is there, girls should be encouraged to get oocyte preservation as soon as possible	I was told that no one knew why, it could be a fluke
PPOF-29	2°	Never officially diagnosed	OB/GYN	-	-	No. My doctor did not believe me when I started with menopause symptoms – told me I was too young. He was also extremely hesitant to dx my daughter	-
PPOF-31	2°	Never officially diagnosed					
PPOF-34	2°	37	OB/GYN	2.5 years	Yes, 2 other OB/GYN, 2 PCPs	No, the other OB/GYNs never mentioned the possibility or did testing. PCP ordered the blood work and told me it was menopause	No
PPOF-37	2°	39	Reproductive endocrinologist	4-5 months	2	No, they keep thinking I am pre-menopausal.	No

Table 2: Continued

						They tell me they don't understand how to manage it and defer management to the reproductive endocrinologist	
PPOF-39	1°	16	Medical endocrinologist	n/a	No	Very rare, so no	No, possible genetics
PPOF-40	2°	18	Reproductive endocrinologist	4-5 months	No	-	No
PPOF-42	2°	17	Internist followed by endocrinologist and OB/GYN	About 4 months	No	I was lucky my pediatrician was educated but no, not enough know especially about treatment options. There needs to be better advice on preventative measures for future health related problems.	No, I wish

"-" symbolizes that the person left question blank in the questionnaire

n/a for "time menses had stopped before seen by provider and diagnosed" if primary amenorrhea

Table 3 below depicts the four participants from Table 2 who were removed, leaving a new total of 19 women.

Table 3: Participants Removed before Analysis

POI ID	Reason Participant was Removed from Analysis
17	Had sister who had been previously diagnosed with POI (diagnostic experience would be different in comparison to someone who has not had family member diagnosed with POI before them)
28	Had sister who had been previously diagnosed with POI (diagnostic experience would be different in comparison to someone who has not had family member diagnosed with POI before them)
29	Not officially diagnosed with POI
31	Not officially diagnosed with POI

Table 4 below depicts the data to be used in the logistic regression analysis.

Table 4: Diagnosis Section of Questionnaire - Data to be used for Analysis

POI ID number	How Recruited	Age of Diagnosis	Type of Amen.	Months Cessation before Diagnosis	If Provider Helpful (1=yes; 0=no, neutral, blank)
1	Magee	36	2°	3	0
2	Magee	16	1	0	0
5	Magee	27	2	4	1
6	Magee	30	2	0	0
8	Magee	17	1	48	0
9	Magee	18	2	12	0
10	Magee	22	2	11	1
14	Magee	16	1	0	0
15	Magee	33	2	18	0
16	NIH	15	1	0	1
21	Magee	17	2	11	1
24	Website	27	2	5.5	0
27	Magee	34	2	1	0
36	Magee	23	2	0	0
34	Website	37	2	30	0
37	Magee	39	2	4.5	0
39	Magee	16	1	0	0
40	Magee	18	2	4.5	0
42	Website	17	2	4	0
TOTALS	**Magee: 15 women** **Other: 4 women**	**Magee: Mean of 24.13 yrs** **Other: Mean of 24 yrs**	**Magee: 4 with 1°, 11 with 2°** **Other: 1 with1°, 3 with 2°**	**Magee: Mean of 7.8 months** **Other: Mean of 9.9 months**	**Magee: 3 with "1" (helpful) 12 with "0"** **Other: 1 with "1" (helpful) 3 with "0"**

A preliminary analysis was conducted to confirm that location of recruitment did not introduce bias into the data. The following summarizes this analysis:

Age of diagnosis had a t-value of 0.027490316022591624. The probability of this result, assuming the null hypothesis (that there is not a statistical difference between the 2 groups recruited on average), is 0.98.

Months cessation had a t-value of -0.291. The probability of this result, assuming the null hypothesis, is 0.77.

Type of amenorrhea: Determined at 95% confidence that there is not a significant difference between the two observed proportions. Of the 15 women recruited through Magee, 26.67% had primary amenorrhea. Of the 4 women recruited through "other" (i.e. NIH or the POF website), 25% had primary amenorrhea.

Reported provider being helpful: Determined at 95% confidence that there is not a significant difference between the two observed proportions. Of the 15 women recruited through Magee, 20% reported her provider being helpful. Of the 4 women recruited through "other" (i.e. NIH or the POF website), 25% reported her provider not being helpful or gave a neutral response.

Logistic Regression Analysis lists whether the participant reported the provider being helpful as the variable of interest. Each factor is also listed (age of diagnosis, type of amenorrhea, months cessation) with a β that represents the associated odds ratio.

(provider helpful) = (age of diagnosis)β + (type of amenorrhea)β + (months cessation)β

Table 5: Results of Logistic Regression Analysis

Factor/Variable	Average	P-value (less than 0.05 as significant)	Odds Ratio (β)
Age of diagnosis (yrs)	24.1053	0.2176	0.8722
Type of Amenorrhea	1.7368	0.4004	3.6262
Months Cessation	8.2368	0.8287	0.9868

No p-values were found to be significant.

Table 6 lists the type of resource from which the recruited women felt they learned the most about POI. The percentage of each type of resource is shown in figure 1.

Table 6: Responses Regarding Education of POI

	Where learned the most about Premature Ovarian Failure:					
Subject with POI identifier	**Physician provider**	**Internet**	**POF support group in local area**	**Medical library**	**Research study**	**Other**
PPOF-1	X	X				
PPOF-2	X	X				
PPOF-5						REI clinic at Magee
PPOF-6	X					
PPOF-8	X	X	X		X	
PPOF-9	X					
PPOF-10	X	X				
PPOF-14	X					
PPOF-15		X				
PPOF-16	X					
PPOF-17					POF investigator	
PPOF-21	X					
PPOF-24		X				
PPOF-27	X					
PPOF-36		X				X
PPOF-28	X	X				
PPOF-29		X				
PPOF-31	No response					
PPOF-34	X	X				
PPOF-37	X	X				
PPOF-39	X					
PPOF-40	X	X			X	
PPOF-41		X				
TOTALS	**15**	**13**	**1**	**0**	**3**	**3**

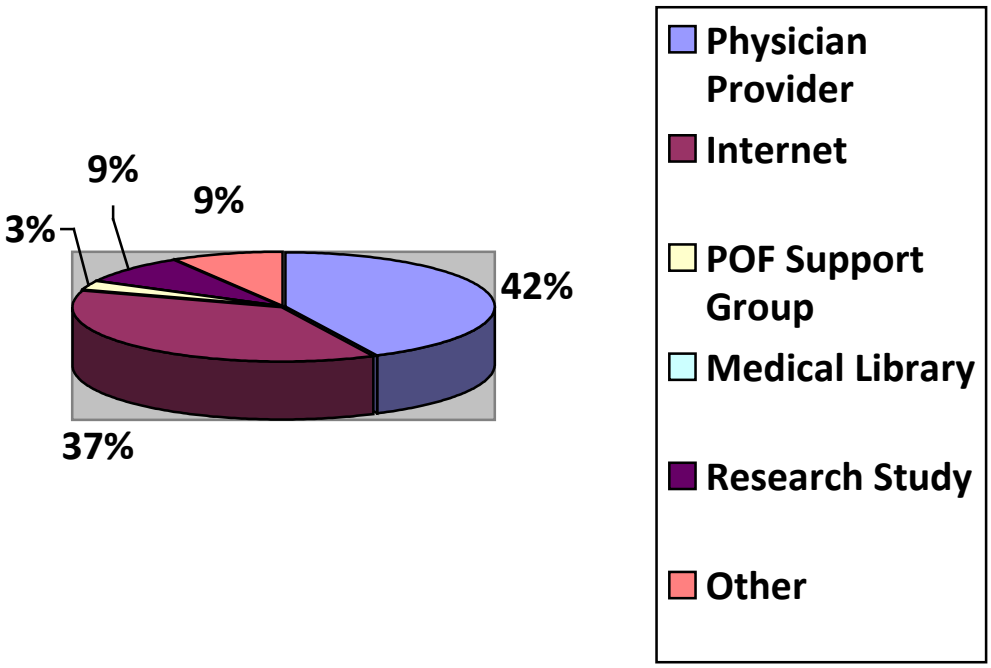

Figure 1: Pie Chart of the Reported Education of POI

5.0 DISCUSSION

5.1 ANALYSIS OF QUESTIONNAIRE

The data from the logistic regression analysis is presented in Table 5 of the results section. Three variables were studied to determine if they influenced a woman's answer to the question of whether her provider was helpful during her diagnosis and provided her with information about POI and treatment. The factors studied were the age of diagnosis, the type of amenorrhea (primary vs. secondary), and the months cessation for her menses before a diagnosis was made. Prior to the regression analysis, it was determined that bias was not introduced based on the recruitment site – participants were recruited through Magee and recruited outside Magee (through either NIH or the POF website).

After the regression analysis, no p-values were found to be significant for any of the three variables. It can thus not be concluded, at a statistically significant level, that these variables have an effect on the outcome of whether the woman thought the provider was helpful. However, trends were seen in the odds ratio values. In regards to the age of diagnosis, the odds ratio was 0.8722. This indicates this factor is associated with slightly lower odds of the participant finding her provider helpful. As the age of women increases, the rated helpfulness decreases slightly. This could be due to a variety of reasons, including older women having higher expectations for providers, and there being less options available regarding having children.

In regards to the months of cessation before a diagnosis was made, the odds ratio was 0.9868. The value is very close to 1, indicating this factor likely does not play a major role affecting the odds of the outcome (if the provider was reported helpful).

Finally, with regards to the type of amenorrhea, an odds ratio of 3.6262 was calculated. This variable has the most significant trend of all three variables studied. With an odds ratio of over 1, the type of amenorrhea is associated with higher odds of the participant feeling the provider was helpful. Specifically, the odds of the helpful outcome was found to be 3.6262 times more likely in those with secondary amenorrhea compared to those with primary amenorrhea. More fertility options exist for those with secondary amenorrhea in comparison to those with primary amenorrhea.

The second main analysis examined where each woman recruited felt that she learned the most about Premature Ovarian Failure (table 6). Fifteen women learned the most from her physician provider, thirteen from the internet, one from a POI support group in her local area, none from a medical library, three from a research study, and three from "other." It is interesting that the numbers for physician provider and the internet were almost equivalent. Research from the Pew Research Center published a survey from September 2012 that reported 72% of internet users report looking online for health information within the last year[34]. Most (77%) start their search at a search engine, while only 13% of online health users start at a website specializing in health information[34]. These results highlight some interesting points. Physicians should be made more aware of POI, including the emotional and psychosocial issues that women often experience with POI. Physicians should be prepared to refer their patients to a genetic counselor or other appropriate health care providers who can serve as an advocate, and determine if there are implications for other family members. It could also be suggested that medical staff should

provide internet references and sources to their patients – such that patients are using reliable sources to build their knowledge base.

Few women reported learning much from support groups, medical libraries, research studies, and other sources. Perhaps these areas could be explored and publicized more to patients. A genetic counselor would be knowledgeable in these areas and be able to direct patients to such sources. Knowledge coming from a variety of places is often the most helpful, and would allow patients to tailor their needs by having more resources and options available to them.

5.2 ADDITIONAL TRENDS SEEN IN THE QUESTIONNAIRE

Additional trends noted include the presence of cigarette smoking in a few of the participants. As mentioned in the Background and Significance section, cigarette smoking is a known environmental toxin that can lead to a decreased age of menopause[16]. Participants with ID numbers 5, 24, 31, 34, and 37 all have a history of smoking. The range varied from considering themselves a "social smoker" (i.e. smoking when they drink) to smoking every day for 42 years. All of those who noted a smoking history on their questionnaire had secondary amenorrhea. This is interesting, as their smoking history could certainly be a factor as to why they have POI.

Other information of note includes a history of depression in women with ID numbers 1 and 28. Additionally, subject ID number 8 noted that she "did not receive emotional support." Women diagnosed with POI who are experiencing depression could benefit (and desire) psychological support. A genetic counselor could either be that source of support, or provide a referral if necessary.

Some of the women recruited had a family history of POI. Whether or not the specific genetic cause can be identified, a genetic counselor could work with these types of families to help them understand whether there may be an underlying genetic cause. In one of the families, two sisters (ID numbers 16 and 17) both had primary amenorrhea and were diagnosed with POI. In another family, there was a history of POI in 2 sisters, their mother, and their maternal grandmother. While the mother and maternal grandmother were never officially diagnosed, this family appears to show a dominant inheritance pattern for early menopause. While POI is isolated in many families, there is certainly evidence to support a genetic cause in other families. A genetic counselor would be an appropriate health care provider to determine which families might fall into which category.

A final interesting trend noted was the reproductive history of several participants. One woman (ID number 10) shared that she has delivered a baby since being diagnosed with POI. This is a rare example of the statistic that 5-10% of women with POI are able to conceive [14]. Another, ID number 27, had three children before being diagnosed. This is evidence in support of women who are diagnosed later in life after they have already had children. This is pointed out in support of the fact that a genetic counselor could help the patient talk to her family about timing of family planning. If other women are at risk of POI, then they could benefit from such knowledge as they would be able to consider starting their family sooner.

5.3 LIMITATIONS OF STUDY AND FUTURE DIRECTIONS

There are several limitations to the current study. First, only twenty-three women with POI were recruited, and only nineteen of these women were eligible for the analysis portion. Few eligible

women were seen though the Magee REI clinic, and those that were seen did not always agree to participate. A larger sample size would have increased the likelihood of obtaining statistically significant data, and further conclusions could have been made. For those women that were successfully recruited, not all of them fully completed the questionnaire. This limited the strength of the calculations. To improve this aspect of the study, participants could be encouraged to take the questionnaire home if having more time would allow them to completely fill it out.

Future directions for the study could include gathering more women who have POI to participate in the study. With more women recruited, there would be more questionnaires available for analysis of trends and conclusions. Other future studies may include adding questions to the questionnaire to have more specific data in regards to the genetic and genetic counseling aspect of POI. It could be asked if women would be interested in genetic counseling, and ways they feel they would benefit from having a genetic counselor as an advocate and resource.

5.4 PUBLIC HEALTH SIGNIFICANCE

Premature Ovarian Insufficiency affects 1% of women by age 40[1, 8]. POI can come as an emotionally stressful diagnosis for women, and it can have implications for more than just fertility. Osteoporosis and cardiovascular disease are increased risks for women with POI. Thus, three conditions – infertility, osteoporosis, and cardiovascular disease – are intertwined in one diagnosis. Over time, it is expected that more will be learned about this public health issue and the diagnosis recognized earlier. This has potential to diminish the diagnostic odyssey. With

better education, women can reduce their risk for the co-morbid conditions and improve the likelihood of having children. Depression is a major concern with POI as discussed, and as a public health issue, it should be an important component of management options.

Not only can POI be devastating for the patient, but to her family as well. While the majority of POI is idiopathic, or due to an unknown cause, 4-31% of cases are thought to be familial[1]. Having an explanation for the diagnosis, whether it be inherited or not, can serve as a consolation to patients and families. Other family members can be educated about what their risks are for POI, and couples can be informed with regards to their options for family planning and recurrence risk. Genetic counselors are an appropriate advocate in this regard – not only can genetic counselors provide resources and explanations, but help the family work through who might be at risk and educate at risk members about various medical management options. Additionally, better technology may allow for improved detection. Family planning is one of the main concerns for couples and families, and earlier detection often allows for greater reproductive options.

5.5 CONCLUSIONS

The diagnostic process of women with Premature Ovarian Insufficiency was studied, specifically in regards to if they thought their provider was helpful during this process. Her age of diagnosis, type of amenorrhea, and months of cessation for her menses were examined in order to determine if these factors influenced a response of providers being helpful. None of the factors were found to be significantly associated with rated helpfulness. Odds ratios were calculated for each of these factors and trends were noted. As the age of diagnosis increased, the provider being

reported as helpful was 0.87 times less likely. It can be concluded that months of cessation does not affect the women's decision of whether the provider was helpful since the odds ratio was calculated to be so close to the value 1. The type of amenorrhea proved to be the best indicator; women with secondary amenorrhea were 3.62 times more likely than women with primary amenorrhea to feel their provider was helpful during their diagnosis. While both groups of women – those with primary amenorrhea and those with secondary amenorrhea – could benefit from seeing a genetic counselor, perhaps those with primary amenorrhea are slightly more in need.

When asked where women learned the most about POI, the two most common answers were the internet and her physician provider. This research study suggests the lack of satisfaction and knowledge being gained from a provider, and it is possible to conjecture that other health care providers may be beneficial to this population such as a genetic counselor. Genetic counselors are educators, provide resources, serve as advocates, and are a source for psychosocial support. A genetic counselor would be able to address the genetic and emotional sides of a POI diagnosis, and provide information regarding medical management of POI. Increased awareness of POI and the value of a genetic counseling referral for women with this condition could help ensure that the patient is receiving the most complete care possible.

APPENDIX A

POF PATIENT CONSENT FORM

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: Genetic Basis of Female Fertility and Premature Ovarian Failure

University of Pittsburgh IRB # PRO09080427

PRINCIPAL INVESTIGATOR: Aleksandar Rajkovic, M.D.

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Judith Balk, M.D.

SOURCE OF SUPPORT: Department of Genetics, UPMC

Why is this research being done?

Many cases of premature ovarian failure and infertility have an unknown cause. By studying the unique genetic makeup of women with this disease, we may gain insights into the disease process and in the future be able to offer better diagnostics and therapies to women with ovarian failure and infertility. You are being asked to participate in a research study in which we will obtain a blood sample from you in order to test for certain genetic aberrations.

Who is being asked to take part in this research study?

You are being invited to take part in this research study because you have been diagnosed with unexplained Premature Ovarian Failure (POF). People invited to participate in this study must have a diagnosis of POF, have a family member with POF, or be a postmenopausal woman with at least one child. Patients with a known cause of POF, such as surgery to remove pelvic organs, chemotherapy, radiation or known genetic disorders, such as Fragile X or Turner ’s syndrome, are excluded.

What procedures will be performed for research purposes?

A total of 1,160 subjects will be asked to participate in our study. You will be one of approximately 1,160 subjects to be asked to participate at this location. We are providing you with the information so you know that you are not the only one that is being asked to participate in the research study. The research will be conducted at the following location(s): University of Pittsburgh, Magee Womens Hospital. If you decide to participate, you will read and sign an informed consent form and return it to the study coordinator. Once this form is received, you will be sent the materials related to the research study. You will provide the study doctor with your medical and family history, information about premature ovarian failures or other gynecologic problems. You will also provide the study doctor with one blood sample or a tissue sample, a questionnaire, and a pedigree of your family.

We would like to get approximately 20cc or 4 teaspoons of blood from you to possibly find out if the cause of ovarian failure or infertility is due to genetic changes. You can come into our office and have a nurse draw blood from your vein in your arm. You can also have your primary care physician or their nurse draw the blood and mail the sample to our office. If you would like to do this then we will be able to provide you with a laboratory order to have your primary doctor's office obtain the blood sample. Also, we will provide you with a blood collection kit, instruction sheet that tells you what to do with the sample once it is obtained and a FEDEX envelope with a pre-paid return mailer to send the sample to our office. This blood will be used only to get genetic material (DNA). The blood sample is for research purposes only. We will look at genes thought to be related to infertility and POF, but we may also look at all of the genes in your body, as there may be many other genes that are not known to contribute to POF today but may cause your POF. We may also ask you for another sample of your blood if we are unable to obtain an adequate DNA sample or if we find new genetic information that may be important to the study, and requires more DNA.

The tissue sample will be a sample that has already been removed by a past surgery or biopsy. We would like to use a small amount of tissue that is not needed by the pathologist (the doctor that studied your tissue). We will only be examining tissue that would have otherwise been destroyed. Only if excess tissue from any surgical specimen is available will it be used for this study. No surgery will be performed solely for the purpose of obtaining surgical specimens. Tissue samples used for the study will only be those obtained at an earlier time for previous testing. If you would like to provide us with a sample of your tissue then you will need to give us the name of the hospital where surgery or biopsy was performed. We will also need you to provide us with a medical release form. The medical release form is a form that you sign that

grants us permission to allow us to obtain the tissue from the pathologist.

Once we receive your blood and/or tissue specimen it will be sent to the Magee Womens Research Institute for analysis. Specimens will be stored in a locked freezer located in the principal investigator's laboratory. The specimen will be provided a case number and no personal information will be linked to the specimen. The results of your DNA analysis will be entered into a computerized database and reviewed by the principal investigator and his staff. Only the study doctor and their research assistant will have access to the original research data. Data will be completely confidential, and will not be revealed to family members, insurance companies, employers, or other individuals or organizations.

Your DNA may be analyzed in the future as new tests become available that are related to premature ovarian failure. The DNA will be analyzed only to search for genes (functional unit of hereditary material) that may cause premature ovarian failure. No additional research (i.e., research not related to POF) will be performed on your DNA without your written permission. You may revoke your authorization for DNA research analyses at any time. In addition, if any DNA remains, you can request that it be destroyed and discarded at any time. All aspects of this study will be kept strictly confidential.

PEDIGREE CHART: You will complete a pedigree chart. A pedigree is like a family tree chart. It will identify family members that may have had problems with infertility or POF. We will give you a pedigree worksheet for you to complete. This form must be completed and returned to the study doctor or their coordinator.

QUESTIONNAIRE: You will complete a brief questionnaire. The questionnaire will take no longer than fifteen minutes to complete. The questionnaire will ask you questions about your menstrual history, how you were diagnosed with POF, medical and family history. Please answer

the questions as completely as possible.

FAMILY MEMBERS: We are interested in studying your family regardless of whether they can or cannot have children to help us find out if genetic changes are related to infertility or POF. We will be asking you to provide information about this study to your family members in order to test their DNA. We will provide you with a separate form for you to provide to them so they can read and possibly participate in the study.

The duration of your participation is limited to the time necessary to obtain blood or other tissue, to verify diagnosis and perform lab studies. This is expected to be 3 years or less. Blood or tissue will be stored and tested for newly detected genes that may be identified. Your research doctor may be able to provide you with part of your information while the study is in progress if you provide a written request. Test results will be revealed to you only at the discretion of the study doctor.

What are the possible risks, side effects, and discomforts of this research study?

The possible risks of this research study are the risks associated with obtaining the blood sample. Bruising, soreness, or rarely, infection may occur as a result of the needle sticks to obtain blood from your vein.

Questionnaire: If you are unable to give information about your family's medical history, it may cause anxiety. It is okay if you are able to provide us with limited information you or your family knows.

Pedigree: Although the risk is minimal it may cause anxiety.

There is a chance of breach of confidentiality associated with release of medical and personal information. Certain risks are rare but can impact insurability, employability,

reproduction plans, or have a negative impact on family relationships or result in paternity suits or stigmatization.

If you request your results of the genetic testing, there is a small risk of learning results which may not be accurate. There is also a small risk of uncovering genetic information that you did not want to know. If you have any questions regarding these results, we will be happy to discuss them with you and provide genetic counseling.

What are possible benefits from taking part in this study?

You will receive no direct benefit from your participation in this study. However, your participation may help the investigators better understand genetic causes of infertility or POF. It is possible that your participation in this study will benefit women with POF in the future.

What treatments or procedures are available if I decide not to take part in this research study?

If you decide not to take part in this research study, you will continue to have the standard care for Premature Ovarian Failure.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?

You will be promptly notified if, during the conduct of this research study, any new information develops which may cause you to change your mind about continuing to participate.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

Neither you, nor your insurance provider, will be charged for the costs of any of the procedures performed for the purpose of this research study (i.e., the blood work). You will be charged, in the standard manner, for any procedures performed for your routine medical care.

Will I be paid if I take part in this research study?

Yes. Five dollars cash will be offered to you for participating in this research study.

Who will pay if I am injured as a result of taking part in this study?

If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

Who will know about my participation in this research study?

Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results.

Will this research study involve the use or disclosure of my identifiable medical information?

This research study will involve the recording of current and/or future identifiable medical information from your hospital and/or other (e.g., physician office) records. The information that will be recorded will be limited to information concerning the diagnostic tests

that have been performed related to Premature Ovarian Failure, as well as any pertinent medical or family history.

Who will have access to identifiable information related to my participation in this research study?

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

Authorized representatives of the UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (e.g., laboratory tests, diagnostic procedures) associated with research study

participation; (2) addressing correct payment for tests and procedures ordered by the investigators; and/or (3) for internal hospital operations (i.e. quality assurance).

We may share the research sample obtained in this study with other investigators interested in POF. The name of the participants, and family members enrolled, and all personal identifying information, such as address, social security number, and date of birth, will be removed. Therefore, the Biobank will not give out your name, or other information that identifies you, your family member or children, to the scientists who receive the samples. However, the scientists will have some data about you, such as age, sex, diagnosis, race, and outcomes of the initial study.

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?

The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to your participation in this research study for a minimum of seven years after final reporting or publication of a project.

May I have access to my medical information that results from my participation in this research study?

In accordance with the UPMC Notices of Privacy Practices document that you have been provided, you are permitted access to information (including information resulting from your participation in this research study) contained within your medical records filed with your health care provider.

Is my participation in this research study voluntary?

Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed to participate in the research study.) Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

Before agreeing to participate in this research study, or at any time during your study participation, you may discuss your care with another doctor who is not associated with this research study. You are not under any obligation to participate in any research study offered by your doctor.

May I withdraw, at a future date, my consent for participation in this research study?

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable medical record information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study.) Any identifiable research or medical information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Your decision to withdraw your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

If I agree to take part in this research study, can I be removed from the study without my consent?

The investigator or sponsor may decide to stop you from taking part in this study at any time. You could be removed from the study for reasons related only to you or because the entire study is stopped. The sponsor, investigator, Food and Drug Administration, or Institutional Review Board may stop the study at any time.

You may withdraw from the study at any time and you may request that your sample be permanently removed if it has not already been used.

****

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at

the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations that have occurred during my participation.

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____	_____	_____
Participant's Signature	Printed Name of Participant	Date

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise. I further verify that no research component of this protocol was begun until after this consent form was signed."

_____	_____
Printed Name of Person Obtaining Consent	Role in Research Study

_____	_____
Signature of Person Obtaining Consent	Date

APPENDIX B

POF QUESTIONNAIRE

Comprehensive Questionnaire
POF Patient

Title: *Genetic Basis of Female Fertility and Premature Ovarian Failure*

Please complete the following questionnaire to provide important information for our research study being conducted at the University of Pittsburgh. Your responses will provide valuable information that will help with our research study. Please be as specific as possible. All your responses will be kept strictly confidential. Thank you for participating.

I. DEMOGRAPHICS

1. Name:_____Date:_____

2. Address:_____

3. Phone_____

   Email:_____

4. Date of Birth (Month/Day/ Year): _____

II. MENSTRUAL/MEDICAL HISTORY

Please list the date of your last menstrual period:_____

At what age did your period start?_____

At what age did they stop completely?_____

Do you smoke?_____If yes, how many packs per day, and for how long_____

Please list your height and weight_____

Are/were your periods painful on a regular basis?

_____

What is your race?_____

Describe your cycle from early age:

Regular Sometimes regular Sometimes irregular Always Irregular

Please describe in detail:_____

_____

How frequent were/are your cycles?

Monthly

Every 2-3 months

Please explain:_____

III. PREMATURE OVARIAN FAILURE DIAGNOSIS

1. How old were you when you were diagnosed with premature ovarian failure?___

2. What type of provider diagnosed you? (e.g., OB/GYN, Reproductive Endocrinologist, Medical Endocrinologist, Internist)_____

3. How long had your menses stopped before you were seen by a provider and diagnosed with POF? _____

4. Did you go to several providers before the correct diagnosis was made? How many?_____

5. Do you think most providers are aware of premature ovarian failure? Are they helpful with POF information including treatment options? Please comment.

_____

_____

_____

6. Have you ever been told a reason WHY you have POF?

If yes, please explain in detail._____

_____

_____

7. Which of the following symptoms related to POF did you experience?

___Irregular cycles

___Heavy Bleeding

___Abrupt cessation of menses

___Absence of menses after stopping birth control pills

___Absence of menses after delivery of a baby

___Hot flashes

___Mood Swings

___Vaginal Dryness

___Painful Intercourse

___Sleep Disturbances

___Depression

___Night sweats

___Fatigue

___Anorexia

___Weight Loss

___Abdominal Pain

___Pelvic Pain

___Changes in skin pigmentation (vitiligo, premature grey hair, etc)

9. Have you ever had symptoms of menopause for a long period of time? (hot flashes, mood swings, vaginal dryness, or dry skin) If so, please describe for how long:

_____

_____

_____

List other significant symptoms related to POF not noted above:

_____

10. Please indicate if you have been previously diagnosed with any of the following: Please note when you were diagnosed and comment if necessary.

Endometriosis

Pelvic inflammatory disease (PID)

Sexually transmitted infections (ex. gonorrhea, Chlamydia, etc.)

Thyroid Disorder

Polycystic Ovarian Syndrome

Uterine Fibroids

Diabetes

Adrenal Disorder (Addison's disease, Cushing's)

Lupus

Autoimmune Disease

Anorexia or Bulimia

Galactosemia

Cancer with chemotherapy or radiation treatment

Grave's Disease

Hashimoto's Thyroiditis

Myasthenia Gravis

Hypoparathyroidism

Autoimmune polyglandular syndrome

Fragile X Syndrome

Turner's syndrome

Mumps

Inherited Enzyme deficiency

Inherited Syndrome-please list_____

Pituitary Disorder

Osteoporosis

11. Have you ever been exposed to chemicals or radiation through your work or residence? Explain._____

12. Have you ever had a cyst or ovary removed or other surgery on your ovary (ies)? (If so, state year and type of surgery)

_____

_____

Are both of your tubes and ovaries present?_____

13. Do you have a family history of POF? If so, who also has been diagnosed with this disorder? Please list (e.g., mother, sister, aunt, cousin, grandmother)

_____

14. Do any inherited diseases run in your family? If so, please list

_____

_____

15. Which of the following have you had done to evaluate for POF:

___Karyotype

___Thyroid Tests (TSH)

___Adrenal Function Tests

___Diabetes Evaluation (Glucose)

___Autoimmune Antibody Testing

___FSH/LH Levels

___Ovarian Biopsy

___Pelvic Ultrasound

16. What abnormalities were detected at the time of your diagnosis? Please explain in detail. _____

_____

_____

17. How often do you follow-up with your doctor since being diagnosed with POF?

Annually Every six months As needed No Follow-up after diagnosis

Other:_____

IV. PREGNANCY HISTORY

1. How many times have you been pregnant, if applicable?_____

2. How many live births have you had? Please list year(s) of delivery, if applicable_____

3. How many preterm births? Please list year(s) if applicable_____

4. List the sex of your children-Males_____ Females_____

5. How many miscarriages in the past? Please list year(s), if applicable_____

6. Did you ever have a genetic analysis on the tissue? If so, what was it? Male/Female or another abnormality?_____

7. How many elective terminations (abortions)?_____

8. Any unusual problems during pregnancy? (e.g., toxemia, diabetes, thyroid disease, liver problems, etc). Please list._____

_____

_____

V. FERTILITY CONCERNS

1. Since diagnosis of POF, have you ovulated on your own or while taking hormones?_____

1b. Does infertility run in your family? If so, please list relationship to you. Did they have to use fertility drugs or IVF to conceive?

_____

_____

_____

1c. Do you have a family history of early menopause or ovarian failure in your family? Please list relationship and age of menopause. _____

_____

2. Do you take hormonal medications now? Please list (estrogen, progesterone, birth control pills)_____

3. Have you become pregnant since being diagnosed with POF? _____

_____

4. Have you delivered a baby since your diagnosis?_____

5. Do you plan to use assisted reproductive technology (ART) or in-vitro fertilization (IVF) in attempts to conceive?_____

Which of the following have you considered?

___Hormone Therapy (Estrogen and/or Progesterone)

___Clomid

___Pergonal or Gonadotropins (injectable drugs)

___IVF

___Own Eggs (if possible)

___Donor Eggs (sister or anonymous donor)

6. How many times have you been treated with fertility drugs in the past? Please explain in detail: _____

_____

_____

_____

7. Have you ever been told that you do not stimulate well with fertility drugs?_____

8. If you underwent IVF in the past, please list when and how many eggs were retrieved, if you can recall.

_____

_____

VI. HEALTH STATUS

1. Have you had any health problems since being diagnosed with POF? Please list and explain._____

_____

_____

2. Was a DEXA scan or XRAY recommended to evaluate your bones? _____

3. Are you aware that you are at higher risk for heart disease and osteoporosis because of POF? _____

4. Did your provider discuss these potential health problems with you?_____

5. Where have you learned the most about your disorder?

____Physician provider

____Internet

____POF Support Group in local area

____Medical Library

____Research Study (please list which one)

____Other

VII. TREATMENT

1. Are you taking any type of hormone replacement therapy (HRT) or Birth Control Pill containing hormones NOW? Please list._____

2. Have you been placed on any other medications to help treat your POF? If so, please list.

Androgens (i.e., Testosterone)_____

Steroids_____

Immunosuppressant therapy_____

Others_____

3. Are you using alternative medications/therapies to treat your POF or to ovulate in hopes of conceiving? Please list_____

4. Were you aware that patients with POF can conceive spontaneously 5-10% of the time?_____

5. Were you aware that taking hormone replacement therapy does not prevent you from getting pregnant even if you have been diagnosed with POF?_____

6. Were you aware that the only proven method of successfully achieving a pregnancy with POF is oocyte donation? _____

7. Any comments that you would like to make:

_____

End of questionnaire. Thank you for your time!

APPENDIX C

IRB APPROVAL FORM

University of Pittsburgh

Institutional Review Board

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Aleksandar Rajkovic

From: Christopher Ryan, PhD , Vice Chair

Date: 12/20/2012

IRB#: REN12110250 / PRO09080427

Subject: Genetic Basis of Female Fertility and Premature Ovarian Failure

Your renewal for the above referenced research study has received expedited review and approval from the Institutional Review Board under:

45 CFR 46.110.(9)

Please note the following information:

Approval Date: 12/20/2012

Expiration Date: 12/19/2013

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month** prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

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