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human reproduction **ORIGINAL ARTICLE** ***Reproductive epidemiology***

The prevalence and phenotypic characteristics of spontaneous premature ovarian failure: a general population registry-based study

K. Haller-Kikkatalo[1,2,3,4,*], R. Uibo[1,2], A. Kurg[5], and A. Salumets[1,2,3]

[1]Institute of Biomedicine and Translational Medicine, University of Tartu, Ravila 19, Tartu 50411, Estonia [2]Competence Centre on Health Technologies, Tiigi 61b, Tartu 50410, Estonia [3]Department of Obstetrics and Gynecology, University of Tartu, L. Puusepa 8, Tartu 51014, Estonia [4]Women's Clinic, Tartu University Hospital, L. Puusepa 8, Tartu 51014, Estonia [5]Institute of Molecular and Cell Biology, University of Tartu, Riia 23, Tartu 51010, Estonia

*Correspondence address. Department of Immunology, Institute of Biomedicine and Translational Medicine, University of Tartu, Ravila 19, Tartu 50411, Estonia. Tel: +372-50-2002; E-mail: kadri.haller-kikkatalo@ut.ee

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STUDY QUESTION: What is the measured prevalence and phenotype of spontaneous premature ovarian failure (POF) in the general population?

SUMMARY ANSWER: Spontaneous POF occurs in ~1% of the general population with unique phenotype of post-menopausal ageing distinct from surgically induced premature menopause.

WHAT IS KNOWN ALREADY: POF is multifactorial ovarian quiescence before the age of 40. The clinical features of POF are diverse and the population prevalence of POF is still not known.

STUDY DESIGN, SIZE, DURATION: This population-depictive registry-based case-cohort study included 34 041 women from the Estonian Genome Center registered between 2003 and 2013.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Spontaneous POF was selected retrospectively by excluding other causes for premature menopause under the age of 40 ($N = 310$) and women with surgically induced premature menopause participated as a reference group ($N = 242$).

MAIN RESULTS AND THE ROLE OF CHANCE: The prevalence of spontaneous POF was 0.91% (0.81–1.02%) among women of the general population in Estonia. In women with POF, menarche occurred a few months later than in the reference group and a significantly higher number of live births during their reproductive life was recorded. Women with POF also consumed less alcohol and had smaller waist-to-hip ratios than those in the reference group, although both groups of women were similar in body mass index a decade after menopause. The prevalence of concomitant diseases was similar between two groups of women by their fifties, but the pattern of onset of these diseases was different. Surgically induced premature menopause associated with faster development of osteoporosis, hypertension, and connective tissue diseases, but slower development of allergies, compared with spontaneous POF. The age of menopause was determined by irregular menstrual cycles, but not by the length of regular menstrual cycles, the age of menarche, the number of pregnancies or live births, smoking or alcohol consumption, or the use of oral contraceptives for some time during the reproductive period.

LIMITATIONS, REASONS FOR CAUTION: POF is rarely stated in medical records and cannot be diagnosed retrospectively by standard procedures. Therefore the data on all cases of women with primary amenorrhea or premature menopause before the age of 40 were requested from the registry and spontaneous POF was predicted retrospectively by excluding other extraovarian causes for premature menopause. Since the current study is retrospective registry-based data analysis, no genetic evaluation concerning possible candidate genes and no blood analysis concerning immunologic disorders could be performed to describe etiopathogenesis of POF.

WIDER IMPLICATION OF THE FINDINGS: Spontaneous POF most likely comprises several diseases with different etiopathologies and there may be a unique phenotype of post-menopausal ageing distinct from that in surgically induced premature menopause. Irregular menstrual cycles may be a prospective risk for developing spontaneous POF. Compared with spontaneous POF, surgically induced premature menopause

associates with faster development of age-related diseases. The data point to new ideas and hypotheses for further studies on etiopathologies and treatment options for spontaneous POF.

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Key words: fecundity / concomitant diseases / phenotype / premature ovarian failure / population prevalence

Introduction

Premature or primary ovarian failure (POF) is one of the hypergonadotrophic hypogonadisms defined as premature amenorrhea with elevated gonadotrophin levels observed under the age of 40. The designation of POF has been under discussion for long time, but it is still preferred among many alternatives, including primary ovarian insufficiency (POI), dysfunction or insult (Shelling, 2010). There are still differences of opinion concerning the use of proper name. More authors are starting to prefer the term POI to POF or use them alternatively (Bakhsh *et al.*, 2014; Miyazaki *et al.*, 2015). There is also a growing confidence in, and the need for a term describing, a clinical situation between regular ovarian cycles and the sustained amenorrhea of POF, leading to the implication of POI. In this context, the authors define POI as ovarian function that is disturbed but not completely stopped (Cox and Liu, 2014). Some authors use POI to define sustained amenorrhea and the term diminished ovarian reserve to stand for the clinical stage where menstrual cycles are still regular (Silva *et al.*, 2014). Also, POI has been used to discriminate spontaneous ovarian cause for premature menopause from POF which sometimes reflects both spontaneous and iatrogenic premature menopause (Shah and Nagarajan, 2014). Here we agree with Shelling (Shelling, 2010) that none of the alternative names is perfect and since there is no consensus on the name of the diagnosis and since POF is still most widely used, it is also the preference herein. Further, to minimize misinterpretation, we have chosen the term spontaneous POF to retrospectively define ovarian quiescence before age 40 with no known obvious extraovarian cause for premature menopause and we contrast this with (surgically) induced iatrogenic premature menopause and premature menopause induced by extraovarian illness (systemic disease or treatment).

In addition to many names, the condition complied as POF is highly heterogeneous and can be associated with autoimmune (Monnier-Barbarino *et al.*, 2005) or genetic factors (Pouresmaeili and Fazeli, 2014), as well as iatrogenic factors such as unilateral oophorectomy, ovarian resection or cystectomy reducing ovarian reserve (Busacca *et al.*, 2006), chemo-therapy or systemic diseases (Group, 2002). The common gynecological diagnostic methods, including assessment of ovarian and uterine parameters by ultrasound technique, bone mineral density or serum estradiol and follicle stimulating hormone levels do not distinguish different etiologies of POF (Conway *et al.*, 1996). In fact, even today the clinical diagnosis of POF does not require the determination of etiology of premature menopause and it might not be possible to investigate this retrospectively. The wide heterogeneity of POF syndrome makes it almost impossible to study the prevalence of it in a population unless there is a retrospective, registry-based survey to select cases according to the age of menopause. Population-based registries have widely been used to study different diseases (Pupillo *et al.*, 2014; van Nielen *et al.*, 2014) but not POF. So far, most authors have been predicting the prevalence of POF by screening some of the risk factors in certain subgroups of patients. For example, the prevalence of autoimmunity has been reported to range from ~4% (Reimand *et al.*, 2000) up to 30 (Conway *et al.*, 1996) or even 50% (Monnier-Barbarino *et al.*, 2005) in cases of POF. The overall presumption regarding the prevalence of POF is that occurs in 1–2% of the general population (Coulam *et al.*, 1986; Group, 2002).

Imminent POF is one of the causes for female infertility and poor outcome of infertility treatments by assisted reproductive techniques (de Boer *et al.*, 2003). Furthermore, POF affects a woman's well-being far beyond the well-recognized reproductive concerns. Concomitant with POF, there is a decline in ovarian hormones, which is responsible for multisystem effects including increased bone resorption, osteoporosis, sexual distress and disturbed sleep (Pal *et al.*, 2008). Additionally, early oophorectomy increases the risk of ischemic heart disease (Lokkegaard *et al.*, 2006), and vice versa, heart disease risk determinants during premenopausal years, including increased body mass index (BMI) and lifestyle (Guthrie *et al.*, 2004) and especially smoking (Adamopoulos *et al.*, 2002), also fasten the transition to menopause (Kok *et al.*, 2006). Systemic diseases or the treatment of it prior to menopause, as shown in case of cyclophosphamide therapy in systemic lupus erythematosus may induce POF (Tincani, 2001). At the same time, there have been discussions about whether premature menopause, either surgically induced or natural, is associated (Rocca *et al.*, 2007) or not (Vearncombe and Pachana, 2009) with increased risk of mental impairment. All of this makes POF a considerable health, socio-economic and mental burden for an affected person and her family and for public health in general.

As there are no data concerning the actual prevalence of POF available to date, in particularly for the Estonian population, we aimed to assess the prevalence of spontaneous POF in our population and exclude cases induced by either iatrogenic manipulations or systemic diseases. For this purpose, we used data from the Estonian Genome Center which registers confirmed anamnestic clinical and phenotypic data of people from general population. Population-based registry is the only way to study rather than predict the prevalence of POF in a general population. Secondly, we focused on characterizing the POF population from the perspective of fecundity and general health anamnesis. This study did not aim to characterize spontaneous POF patients according to the etiology of POF for three reasons. Firstly, the main perspective of current research was to assess the prevalence of spontaneous POF regardless of etiology. Secondly, as this study was a retrospective survey of registry-based data, any laboratory testing would be unmanageably large in scale. An enormous number of different etiologic factors and pathogenic mechanisms described to lie behind POF would need to be tested and these factors are reviewed comprehensively elsewhere (Persani *et al.*, 2010; Ebrahimi and Akbari Asbagh, 2011). Thirdly some etiologic

factors, including autoimmune mechanisms, may not be detectable retrospectively after a period of time giving advantage to the more preserved risks including genetic alterations in the etiopathogenesis of POF.

Methods

Study design and prevalence

The study used registry data collected by the Estonian Genome Center of University of Tartu (EGCUT) between June 2003 and November 2013. The EGCUT was founded in 2000 (under the name of Estonian Genome Project Foundation until 2007) to establish a large-scale population-based Biobank of population-based genetic and health data for research and development in human genetics and implementation of the results from genetic studies for the promotion of public health. The cohort size is currently 51 535 genetic donors (≥18 years of age), which closely reflects the age, sex and geographical distribution of the Estonian population. All subjects have been recruited randomly and personal data (place of birth, place(s) of living, nationality, etc.), genealogical data (family history of medical conditions spanning four generations), educational and occupational history, lifestyle data (physical activity, dietary habits, smoking, alcohol consumption, women's health and quality of life) have been registered and anthropometric measurements, such as blood pressure and resting heart rate, were measured. Recruitment into the registry of Biobank was completed in 2013 and there are hundreds of ongoing scientific projects where the EGCUT either is a collaborator or has been delivering data for the research. Previously, data from EGCUT registry have provided evidence to associate genetic loci with early menopause (Perry *et al.*, 2013) or DNA repair processes with ovarian ageing of natural menopause (Stolk *et al.*, 2012; Perry *et al.*, 2014).

In contrast with previous studies on early and natural menopause currently, we aimed to study the data from Biobank by focusing primarily on the subgroup of women with premature menopause (before the age of 40) and on the calculation of the prevalence of POF and description of the phenotypic data and health parameters. The Ethics Review Committee on Human Research of the University of Tartu and Scientific committee of EGCUT approved the study.

The selection criteria of women with premature menopause are illustrated by Fig. 1. Anamnestic and phenotypic data were extracted for women who either suffered from primary amenorrhea ($N = 15$) or secondary amenorrhea before the age of 40 ($N = 665$). The additional anamnestic data were studied and two study groups of participants were formed: (i) the group with ovarian cause for spontaneous POF ($N = 310$), where no iatrogenic manipulation, comorbidities affecting menstrual cycle nor genetic or developmental disorders were reported by the participants or recorded by family doctors; and (ii) the reference group of surgical premature menopause ($N = 242$) was considered where there was a record of hysterectomy or bilateral oophorectomy before the time when menstruation had stopped. The remaining population of women from the registry with premature menopause caused by other extraovarian disorders/iatrogenic manipulation secondarily affecting the function of ovaries ($N = 128$) were excluded from the study. The exclusion criteria included the presence of an expressed systemic disease or comorbidity outside the ovaries but influencing ovarian function (including type 2 diabetes (T2D) jointly with overweight with BMI ≥ 25 kg/m^2, obesity with BMI ≥ 30 kg/m^2, cancer treatments, HIV infection, hyperprolactinaemia, renal failure), iatrogenic manipulation (including ovarian resection or unilateral oophorectomy affecting ovarian reserve, use of oral contraceptives or other medications causing amenorrhea), developmental disorder of uterus, chromosomal disease (Turner syndrome, Dandy–Walker syndrome) affecting menstrual cycle, and insufficient

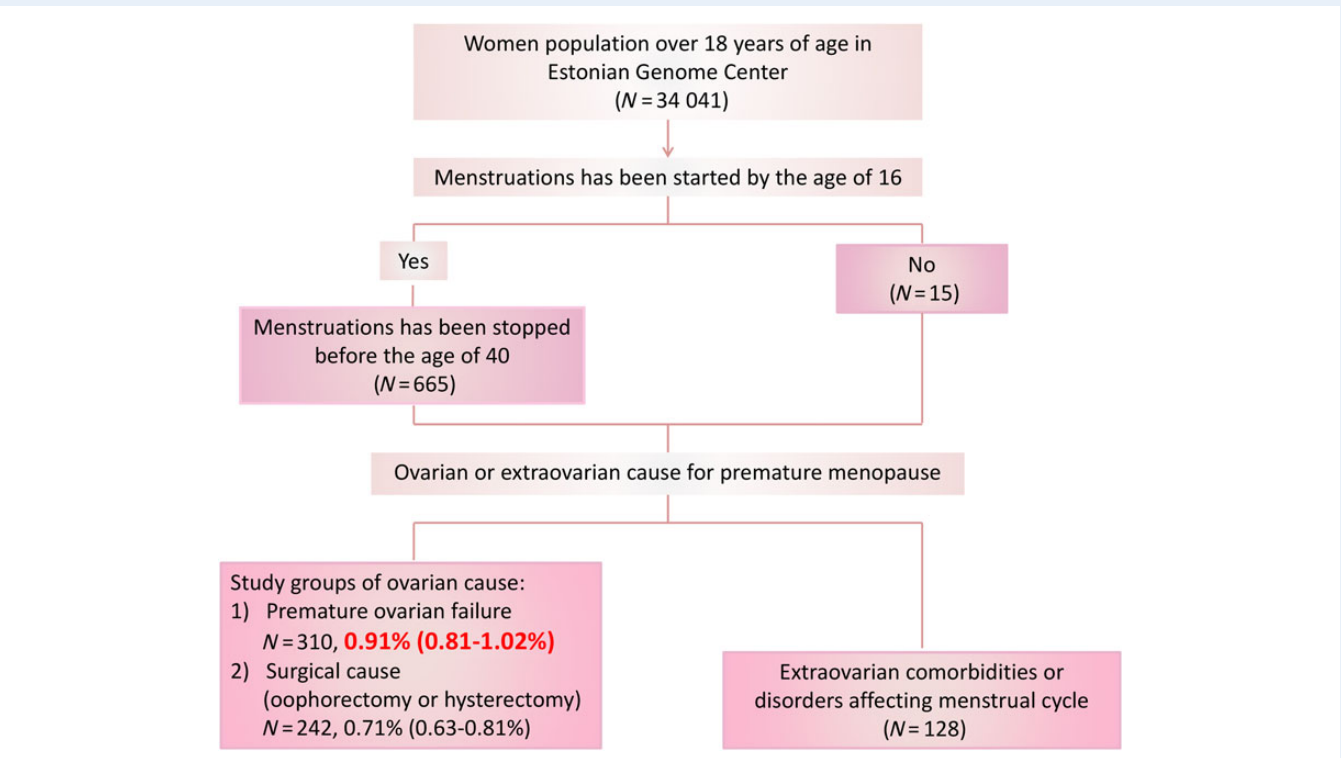

Figure 1 The study design and prevalence of premature menopause of ovarian cause among Estonian women population. The prevalence is given as a percentage with the 95% confidential interval of the percentage.

data for grouping under either of the two study groups. The prevalence of spontaneous POF in Estonian women population was 0.91% (0.81–1.02%). The prevalence of all premature menopause ($N = 680/34\,041$) was 2.00% (1.85–2.15%).

Statistical analysis

The R3.1.0 a Language and Environment (Free Software Foundation, Boston, MA, USA) was used for statistical analysis. Crude and multifactorial linear and logistic regression analyses were performed to assess associations between phenotypic data and POF where women with induced i.e. surgical premature menopause were used as reference, as well as between different phenotypic data inside the group of POF. The confounding effect of age difference between women with POF and women of the reference group was considered by adjusting regression models in comparisons of study groups with regards to diseases or clinical characteristics related to the person's age. Models describing clinical characteristics related to menopause were adjusted by the duration of amenorrhea, if not stated otherwise. Adjusted regression coefficients (ad *r*) from linear regression and adjusted odds ratios (adOR) from logistic regression were calculated; *P*-values <0.05 were considered significant and *P*-values >0.05 and <0.1 were considered as tendencies in statistical comparisons.

Results

Description of study groups and associations with POF

The overall age of participants at the recruitment to the study was 54.35 ± 13.43 years (mean ± standard deviation (SD)), but the women with POF were ∼5 years older than women in reference group (Table I). Both study groups had been in menopause and had used hormonal replacement therapy (HRT) during similar period of time. Also, the smoking habits were similar, but there were fewer people with regular alcohol consumption among women with POF. Menarche in women of POF appeared to have happened about 3 months later ($r = 0.23$ years, $P < 0.1$) than in woman from the reference group, but both groups reached menopause at same age. Also, there was no significant difference in the duration of reproductive age (years between menarche and menopause), the distribution of different lengths of menstrual cycle, the percentage of women ever being pregnant and the age of first pregnancy. Despite this, women with POF tended to have more pregnancies and had significantly more live births during their reproductive life when the age of the first pregnancy was considered in statistical analysis. Both study groups were similar in BMI at the time of study, but women with POF were likely leaner at their waist or more pear-shaped rather than apple-shaped in body structure, since their waist-to-hip ratio measure at the time of study was smaller after adjusting for the duration for HRT.

There were differences between study groups also in the onset of concomitant diseases (Table II and Fig. 2). Compared with women with surgical menopause, women with POF were diagnosed with allergies ∼10 years earlier, but with connective tissue diseases (CTD) ∼4.5 years later, with hypertension, 4 years later and with osteoporosis 7 years later; however, by the age of 54 (the average age at the time of study) the prevalence of these diseases did not differ between study groups. Also, when we counted women who were diagnosed with CTD before and after the menopause, significantly more women were found in the POF group to be diseased after the menopause compared with women from reference group (83.86 and 68.7%, respectively). The difference remained significant also when smoking (either former or current smoker compared with non-smokers) or alcohol consumption (either former or current user compared with non-users) statuses, and number of pregnancies or live births were taken into account (data not shown). At the same time, the prevalence of other concomitant diseases (allergies, thyroid diseases, T2D, metabolic symptoms, hypertension, breast cancer and osteoporosis) either diagnosed before or after menopause was not different between study groups (Table II), even after considering other risk factors like age at study, duration of amenorrhea, smoking and alcohol consumption statuses, and the number of pregnancies or live births (data not shown). POF may be associated with higher a prevalence of type 1 diabetes (T1D), since there were four cases of T1D among POF women and none were revealed among women in reference group. The critical post-menopausal period where majority of concomitant diseases were diagnosed in women with premature surgical menopause seemed to be narrowed down to the 5th to 15th post-menopausal year, while women with POF were diagnosed with these diseases evenly throughout the entire postmenopause (Fig. 2).

Determinant of the time of menopause in spontaneous POF

Among women with POF, the age at menopause in women with secondary amenorrhea in the group of POF ranged from 12 to 40 years ($N = 302$, mean ± SD was 37.2 ± 4.42 years) while eight women had primary amenorrhea. Women with irregular menstrual cycles (at their age between 25 and 35 years) developed POF ∼2 years earlier than women with regular menstrual cycle (ad $r = -2.04$ years, $P = 0.006$). There was no association between the POF starting age and the lengths of menstrual cycle if the cycles were regular. Also, no associations were revealed between the age of POF and the age of menarche, number of pregnancies, number of live births, statuses of smoking and alcohol consumption, and whether the person had been used oral contraceptives for some time during the reproductive life.

The associations between the age of menopause and the diseases developed after menopause in POF were assessed with linear regression models adjusted by the duration of amenorrhea. Women who had CTD and hypertension were found to reach POF later than women without these diseases (ad $r = 1.1$ years, $P = 0.033$ and ad $r = 1.7$ years, $P = 0.003$, respectively). Expectedly, osteoporosis developed later if POF developed later (ad $r = 0.28$, $P = 0.007$). At the same time women who were diagnosed with T1D tended to develop POF earlier (ad $r = -3.9$ years, $P = 0.080$). There were no associations between the age of menopause and the presence of allergies, T2D, breast cancer, and thyroid diseases (data not shown).

Discussion

The study assessed the prevalence of POF patients in Estonian population and characterized their reproductive and general health in comparison to women with surgically induced premature menopause. Most importantly, the prevalence of spontaneous POF in Estonian population was revealed to be ∼1% among women over 18 years of age. In addition, we observed four main phenotypic phenomena of POF. The first was that the menarche in women of spontaneous POF may have occurred few months later than for women from reference group, but despite this,

Table I Characteristics of spontaneous POF compared with women with surgical premature menopause (reference group).

	POF ($N = 310$)	Reference group ($N = 242$)	Statistical model	Result
Age at study (years)	57.65 ± 12.82	52.66 ± 12.26	Lin	$r = 4.99$, $P < 0.00001$**
Duration of amenorrhea (years)	20.71 ± 11.62	16.49 ± 12.26	LinAge	ad $r = -0.45$, $P = 0.218$
Smoking status:				
Never smoker	212 (68.4%, 62.8–73.5)	157 (64.9%, 54.5–70.8)	LogAge	reference
Former smoker	28 (9.0%, 6.2–12.9)	25 (10.3%, 6.9–15.0)		adOR 0.90, $P = 0.716$
Current smoker	70 (22.6%, 18.1–27.7)	60 (24.8%, 19.6–30.8)		adOR 1.12, $P = 0.595$
Age at regular smoking (years)	21.68 ± 5.98	21.58 ± 5.97	LinAge	ad $r = 1.05$, $P = 0.805$
Duration of regular smoking (years)	18.40 ± 9.91	13.60 ± 9.41	LinAge	ad $r = 1.54$, $P = 0.453$
Alcohol consumption status:				
Never user	64 (20.6%, 16.4–25.7)	33 (13.6%, 9.7–18.8)	LogAge	reference
Former	20 (6.5%, 4.1–9.9)	12 (5.0%, 2.7–8.7)		adOR 0.74, $P = 0.468$
Current	226 (72.9%, 67.5–77.7)	197 (81.4%, 75.8–86.0)		adOR 0.69, $P = 0.125$
Frequency of alcohol consumption:				
Do not use	70 (22.6%, 18.1–27.7)	33 (13.6%, 9.7–18.8)	LogAge	reference
2–3 times a week	14 (4.5%, 2.6–7.6)	21 (8.7%, 5.6–13.1)		adOR 0.38, $P = 0.021$**
2–4 times a month	64 (20.6%, 16.4–25.7)	68 (28.1%, 22.6–34.3)		adOR 0.56, $P = 0.038$**
≥4 times a week	2 (0.6%, 0.1–2.6)	4 (1.7%, 0.5–4.5)		adOR 0.36, $P = 0.252$
Once a month	64 (20.6%, 16.4–25.7)	44 (18.2%, 13.6–23.8)		adOR 0.76, $P = 0.353$
Few times a year	82 (26.5%, 21.7–31.8)	65 (26.9%, 21.5–33.0)		adOR 0.58, $P = 0.046$**
Less than once a year	14 (4.5%, 2.6–7.6)	7 (2.9%, 1.3–6.1)		adOR 1.06, $P = 0.917$
Age of menarche (years)	13.76 ± 1.65	13.39 ± 1.51	LinAge	ad $r = 0.23$, $P = 0.093$*
Length of menstruation cycle at age 25–35:				
25–29 days	174 (60.0%, 50.4–61.7)	133 (57.3%, 48.5–61.3)	LogAge	reference
≤20 days	8 (2.8%, 1.2–5.2)	7 (3.0%, 1.3–6.1)		adOR 0.92, $P = 0.884$
21–24 days	58 (20.0%, 14.6–23.6)	47 (20.3%, 14.7–25.1)		adOR 0.84, $P = 0.468$
30–35 days	12 (4.1%, 2.1–6.8)	19 (7.9%, 4.9–12.2)		adOR 0.57, $P = 0.156$
>35 days	1 (0.3%, 0.02–2.1)	0 (0%, 0–1.9)		–
Irregular	25 (8.6%, 5.4–11.8)	16 (6.6%, 4.0–10.7)		adOR 1.32, $P = 0.438$
Do not know	12 (4.1%, 2.1–6.8)	10 (4.1%, 2.1–7.7)		adOR 0.65, $P = 0.344$
Duration of fertile period (years)	23.01 ± 6.24	22.83 ± 4.65	LinAge	ad $r = -0.35$, $P = 0.463$
Has been pregnant	274 (88.4%, 84.2–91.6)	222 (91.7%, 87.3–94.8)	LinAge	adOR 0.74, $P = 0.340$
Age at first pregnancy (years)	22.44 ± 3.58	21.57 ± 3.67	LinAge	ad $r = 0.39$, $P = 0.240$
No. of pregnancies	3.56 ± 2.01	3.45 ± 2.14	LinAge	ad $r = 0.14$, $P = 0.450$
			LinPreg	ad $r = 0.31$, $P = 0.071$*
No. of live births	2.02 ± 1.13	1.91 ± 1.21	LinAge	ad $r = 0.14$, $P = 0.203$
			LinPreg	ad $r = 0.21$, $P = 0.037$**
Has used oral contraceptive	24 (7.7%, 5.3–11.7)	31 (12.8%, 9.0–17.8)	LogAge	adOR 0.94, $P = 0.845$
Age at menopause (years)	36.20 ± 7.35	36.17 ± 4.30	LinAge	ad $r = -0.60$, $P = 0.256$
Duration of HRT (years)	0.35 ± 1.84	0.40 ± 1.92	LinAge	ad $r = -0.02$, $P = 0.869$
Body mass index (kg/m^2)	28.86 ± 6.62	28.44 ± 5.87	LinAm	ad $r = 0.42$, $P = 0.447$
Waist-to-hip ratio	0.84 ± 0.08	0.85 ± 0.08	LinAm	ad $r = -0.13$, $P = 0.059$*
			LinHRT	ad $r = -0.14$, $P = 0.045$**

Numeric data are provided in means ± standard deviation; non-parametric data are provided as count, percentage and 95% CI of percentage.
adOR, adjusted odds ratio; ad *r*, regression coefficient from adjusted regression model; CI, confidential interval; CTD, connective tissue diseases; Lin, crude linear regression model; LinAge, linear regression adjusted by age at the study; LinAm, linear regression model adjusted by the duration of amenorrhea; LinHRT, linear regression model adjusted by the duration of hormonal replacement therapy; LinPreg, linear regression model adjusted by the age of 1st pregnancy; LogAge, logistic regression model adjusted by age at the study; T1D, type 1 diabetes; T2D, type 2 diabetes.
**Statistically significant difference ($P < 0.05$).
*Statistical tendency to be different ($P < 0.1$).

Table II Comparison of the age at diagnosis and the prevalence of women with concomitant diseases between study groups.

Concomitant disease	POF	Reference group	Statistical model	Result
Allergies[A]				
Age at diagnoses (years)	39.77 ± 20.99	46.32 ± 17.27	LinAge	ad $r = -9.74$, $P = 0.034$**
Total, *N* (%, 95% CI)	31 (10.0%, 7.0–14.0)	25 (10.3%, 6.9–15.0)	LogAge	adOR 0.94, $P = 0.819$
Premenopausal	13 (41.9%, 25.1–60.7)	7 (28.0%, 12.9–49.6)	LogSm	reference
Post-menopausal	18 (58.1%, 39.3–74.9)	18 (72.0%, 50.4–87.1)		adOR = 0.53, $P = 0.292$
T1D				
Age at diagnoses (years)	46.50 ± 19.91	–	–	–
Total, *N* (%, 95% CI)	4 (1.3%, 7.0–14.0)	0 (0%, 0–1.9)	–	–
Premenopausal	0 (0%, 0–60.4)	0	–	–
Post-menopausal	4 (100%, 39.6–100)	0		–
Thyroid disease[B]				
Age at diagnoses (years)	48.33 ± 16.30	43.75 ± 15.21	LinAge	ad $r = 1.25$, $P = 0.714$
Total, *N* (%, 95% CI)	32 (10.3%, 7.3–14.4)	24 (9.9%, 6.6–14.6)	LogAge	adOR 1.04, $P = 0.888$
Premenopausal	11 (34.4%, 19.2–53.2)	8 (33.3%, 16.4–55.3)	LogSm	reference
Post-menopausal	21 (65.6%, 46.8–80.8)	16 (66.7%, 45.0–83.6)		adOR = 0.98, $P = 0.972$
CTD[C]				
Age at diagnoses (years)	53.71 ± 15.82	43.40 ± 18.25	LinAge	ad $r = 4.53$, $P = 0.017$**
Total, *N* (%, 95% CI)	117 (37.7%, 32.4–43.4)	67 (27.7%, 22.2–33.9)	LogAge	adOR 1.30, $P = 0.174$
Premenopausal	19 (16.2%, 10.3–24.5)	21 (31.3%, 20.9–44.0)	LogSm	reference
Post-menopausal	98 (83.8%, 75.5–89.7)	46 (68.7%, 56.0–79.1)		adOR = 2.31, $P = 0.022$**
Breast cancer				
Age at diagnoses (years)	54.12 ± 16.79	47.00 ± 14.14	LinAm	ad $r = 8.99$, $P = 0.519$
Total, *N* (%, 95% CI)	8 (2.6%, 1.2–5.2)	2 (0.8%, 0.1–3.3)	LogAm	adOR 2.33, $P = 0.297$
Premenopausal	1 (12.5%, 0.7–53.3)	0 (0%, 0–80.2)	LogSm	reference
Post-menopausal	7 (87.5%, 46.7–99.3)	2 (100%, 19.8–100)		adOR = 0, $P = 0.998$
Hypertension				
Age at diagnoses (years)	55.4 ± 12.26	49.11 ± 14.70	LinAm	ad $r = 3.81$, $P = 0.009$**
Total, *N* (%, 95% CI)	121 (39.0%, 33.6–44.7)	90 (37.2%, 31.1–43.6)	LogAm	adOR 0.79, $P = 0.229$
Premenopausal	18 (14.9%, 9.3–22.8)	17 (18.9%, 11.7–28.8)	LogSm	reference
Post-menopausal	103 (85.1%, 77.2–90.7)	73 (81.1%, 71.2–88.3)		adOR = 1.31, $P = 0.465$
Metabolic symptoms[D]				
Age at diagnoses (years)	56.21 ± 11.85	47.93 ± 16.0	LinAm	ad $r = 3.65$, $P = 0.143$
Total, *N* (%, 95% CI)	49 (15.8%, 12.0–20.5)	30 (12.4%, 8.6–17.4)	LogAm	adOR 1.18, $P = 0.514$
Premenopausal	5 (10.2%, 3.8–23.0)	7 (23.3%, 10.6–42.7)	LogBMI	reference
Post-menopausal	44 (89.8%, 77.0–96.2)	23 (76.7%, 57.3–89.4)		adOR = 2.57, $P = 0.142$
T2D				
Age at diagnoses (years)	56.62 ± 10.60	54.50 ± 15.97	LinAm	ad $r = 0.16$, $P = 0.955$
Total, *N* (%, 95% CI)	28 (9.0%, 6.2–12.9)	12 (5.0%, 2.7–8.7)	LogAm	adOR 1.56, $P = 0.225$
Premenopausal	3 (10.7%, 2.8–29.4)	3 (25.0%, 6.7–57.2)	LogBMI	reference
Post-menopausal	25 (89.3%, 70.6–97.2)	9 (75.0%, 42.8–93.3)		adOR = 2.77, $P = 0.282$
Osteoporosis				
Age at diagnoses (years)	58.32 ± 11.0	50.29 ± 14.83	LinAm	ad $r = 7.26$, $P = 0.024$**
Total, *N* (%, 95% CI)	23 (7.4%, 4.9–11.1)	17 (7.0%, 4.3–11.2)	LogAm	adOR 0.91, $P = 0.774$

Continued

Table II *Continued*

Concomitant disease	POF	Reference group	Statistical model	Result
Premenopausal	2 (8.7%, 1.5–29.5)	2 (11.8%, 2.1–37.7)	LogSm	reference
Post-menopausal	21 (91.3%, 70.5–98.5)	15 (88.2%, 62.2–97.9)		adOR = 1.18, $P = 0.890$

Numeric data are provided in means ± standard deviation; non-parametric data are provided as count, percentage and 95% CI of percentage.
adOR, adjusted odds ratio; ad *r*, regression coefficient from adjusted regression model; CI, confidential interval; CTD, connective tissue diseases; LinAge, linear regression adjusted by age at the study; LinAm, linear regression model adjusted by the duration of amenorrhea; LogAge, logistic regression model adjusted by age at the study; LogAm, logistic regression model adjusted by the duration of amenorrhea; LogBMI, logistic regression model adjusted by body mass index at the study; LogSm, logistic regression model adjusted by smoking status (either never, former or current smoker); POF, premature ovarian failure; T1D, type 1 diabetes; T2D, type 2 diabetes.
[A]Allergies included allergic rhinitis, allergic asthma, mixed asthma, allergic contact dermatitis, atopic dermatitis, food allergy, and lactose intolerance.
[B]Thyroid diseases included diffuse and multinodular goiter, hypo- and hyperthyroidisms, autoimmune thyroiditis, unspecified thyroiditis.
[C]CTD included ankylosing spondylitis, osteoarthritis, podagra, psoriasis, rheumatism, rheumatoid arthritis, lupus erythematosus, unspecified polyarthritis.
[D]Metabolic symptoms included adiposity, dyslipidemia, and isolated and familiar hypercholesterolemia.
**Statistically significant difference ($P < 0.05$).

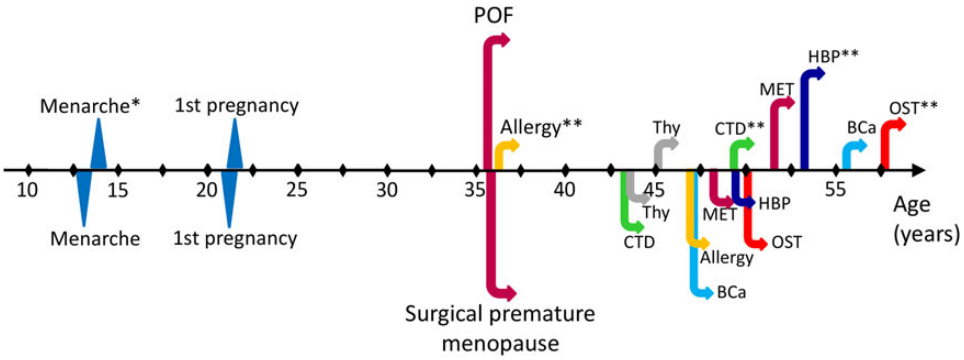

Figure 2 The pattern of clinical characteristics of women with POF (top) compared with women with surgical menopause (bottom) on a timescale. Starting ages are calculated from statistical models provided in Table II. BCa, breast cancer; HBP, hypertension; CTD, connective tissue disease; MET, features of metabolic syndrome; OST, osteoporosis; POF, premature ovarian failure; Thy, thyroid disease. **statistically significant difference in starting age ($P < 0.05$), *statistical tendency to be different ($P < 0.1$).

women with POF seem to have good fecundity ending up with significantly more live births during their reproductive life. The second phenotype was that women with spontaneous POF consumed less alcohol and were more likely to be pear-shaped in their body structure with smaller waist-to-hip ratio than women with surgically induced premature menopause. However, at the same time, both groups of women were similar in BMI a decade after menopause. The third observation concerned the prevalence and time of concomitant diseases. The critical postmenopausal period for the majority of concomitant diseases appeared to be ~5–15 years after menopause, approximately, in women with surgical menopause, while women with spontaneous POF were diagnosed with these diseases throughout the entire postmenopause period. Therefore the prevalence of concomitant diseases was similar between two groups of women by their fifties, but the pattern of onset of these diseases was different. The fourth phenomenon was that irregular menstrual cycles, and likely also T1D, but not the lengths of regular menstrual cycle, age of menarche, number of pregnancies or live births, smoking or alcohol consumption, or use of oral contraceptives for a period of time, determined an earlier starting age of menopause in spontaneous POF.

The first aim of this study was to assess the prevalence of spontaneous POF among the Estonian population. For these purposes we were privileged to have data from Estonian Genome Center which registers confirmed phenotypic and anamnestic clinical data of people from the general population of Estonia. The Estonian Genome Center database included 34 041 women systemically recruited from and representative of the whole population of grownups. The shortcoming of the data available was that POF is rarely stated in medical records and cannot be diagnosed retrospectively by its cold standard procedures meaning no hormone profiles nor etiopathogenic descriptions of POF patients were available. Therefore we applied a study design (Fig. 1) where all cases of women with primary amenorrhea and premature menopause before the age of 40 were studied and spontaneous POF was predicted retrospectively by excluding other extraovarian causes for premature menopause. Chromosomal diseases, developmental malformations of the genital tract and severe illnesses were also excluded from our target group. Similarly, women who passed into premature menopause due to surgical hysterectomy or bilateral oophorectomy were not included in the spontaneous POF group, but used as the reference group. To best of our knowledge, this is an exclusive approach to

study, rather than predict, the prevalence of POF in a general population. Also, there are no similar studies found in literature where population-based registries have been used for similar purposes. As the result, we calculated the prevalence of POF to be ~1% among women of 18 years of age and older, which is in concordant with the estimations of several studies using different approaches (Coulam et al., 1986; Group, 2002). In this study, cases of premature menopause induced either surgically or by other extraovarian illness or treatment were not considered among the POF group in contrast to the recommendations of some researchers (Busacca et al., 2006); if that were the case, the prevalence of all premature menopause ($N = 680/34\,041$) would be 2%. This is concordant with the previous presumption of the prevalence of POF being 1–2% in the general population (Coulam et al., 1986; Group, 2002).

In concordance with the definition of POF which points to decreased lengths of reproductive age (or complete absent of the reproductive period in case of primary amenorrhea), we found that women with spontaneous POF tended to have their menarche ~3 months later than those in the reference group. However, despite the same duration of fertile years, women with POF had had more pregnancies and gave birth to more babies (mean no of babies in reference group added adjusted regression coefficient from linear regression analyses revealed the average of 2.12 babies for one woman with spontaneous POF), when the age at first pregnancy was considered in the statistical comparison. These data indicate that there is no reduced fertility among woman with spontaneous POF compared with women with surgically induced premature menopause, unless there is primary amenorrhea. The current study does not compare these fertility parameters to the general population, still according to the data provided by National Institute for Health Development (NIHD, 2014), the cumulative birth coefficient in women between 18 and 39 years from entire Estonian population ranged from 1.36 (in 2003) and 1.54 (in 2013), which may indicate that patients with POF had more live births than women of the same age in the general population. Further, one could form a hypothesis that more childbirths during life could pull menopause into earlier years as if the reserve of reproduction had been consumed. However, this would be highly speculative and would definitely need research. These results apply for period prior to POF and do not argue against the previous findings that once the ovarian function has failed (when POF has been diagnosed) or POF is about to be expressed clinically, ovarian function and fertility cannot be restored even by assisted reproduction (Awwad et al., 2013; Mittal et al., 2014), although use of donated oocytes would be an option for these women (Silva et al., 2014). Thus, infertility still can be a major problem for certain women (de Boer et al., 2003; Mittal et al., 2014) with oncoming POF if pregnancy is to be desired too close to the clinical manifestation of POF. Irregularity of menstrual cycle has been suggested to be the first symptom to appear in developing POF (Pouresmaeili and Fazeli, 2014). In concordance with the literature, here we showed that POF manifested earlier in those patients whose menstrual cycles had been irregular compared with women who had regular 25–29 day cycles before POF. The regularity and length was recorded according to the situation between the ages of 25 and 35.

Here we show that women with POF were diagnosed with various allergies significantly earlier in their lives than women from reference group. Also, all cases of T1D were found in the POF group, where it tended to be a risk factor for earlier POF. As the number of T1D cases in POF was very low, the association did not reach to statistical significance. All together, these results may indicate that allergies and T1D may share common risk factors with POF and that the majority of POF cases, particularly those with relatively early onset of POF, might be associated with deviations in the immune system regulation. This in turn agrees with data found in literature (Monnier-Barbarino et al., 2005; Silva et al., 2014) that at least some cases of POF might be due to immune-mediated disease. However, since the women with POF had been in menopause ~20 years by the time of study, any assessment of immune-mediated ovarian impairment would have not been informative.

Overweight and increase in subcutaneous adipose tissue is achieved faster after iatrogenic menopause and the most remarkable changes occur during the first 3–4 years after menopause (Lovejoy et al., 2008). Here we show that when one assesses the body structure decades after menopause, at the average of 16–20 years later as in this study, BMI was not significantly different between women with POF and women with premature surgical menopause. Also, the prevalence of metabolic symptoms and the time of diagnosis were not different before and after menopause or between study groups. However, women with spontaneous POF were leaner at the waist irrespective of the duration of amenorrhea and the use of HRT. The use of HRT is associated with weight loss or less weight gain after menopause (Espeland et al., 1997) and was therefore considered in the statistical analysis. It is not known whether the difference was because of the more pear-shaped body structure of the spontaneous POF women or because the reference group gained weight faster just after menopause (Lovejoy et al., 2008) but the manifestation of osteoporosis and other clinical features related to menopause such as CTD and hypertension occurred significantly later in women with POF compared with women with surgically induced menopause. There was also a positive correlation between the starting age of POF and the age of diagnosing CTD, hypertension and osteoporosis. This is rather intriguing since many researchers have claimed that women with POF will more likely face diseases such as cardiovascular diseases at their postmenopause years (Shah and Nagarajan, 2014). Since POF indeed means earlier menopause, it is rather expected that there is more time to develop diseases associated with postmenopause. Decreased levels of estrogen following ovarian quiescence is unfavorable, permitting the development of hypertension and ventricular remodeling after menopause (Zhao et al., 2014). The current study has two features to stress; firstly we assessed the associations with the time of menopause in spontaneous POF and secondly, we compared these women to those with surgically induced premature menopause, which makes any comparison to the literature rather misleading in this case. However, there is a chance that heart disease risk factors at premenopause determine the age of menopause age and not the other way round (Kok et al., 2006). Here we suggest, along with the results from literature discussed above, that POF is not just a heterogeneous disease, but that it consists of a number of subdiseases with certain risk factors that likely determines the time of manifestation of POF and pattern of concomitant diseases. In accordance, breast cancer in women with spontaneous POF was diagnosed ~9 years later than in women with surgically induced menopause, but due to too few cases, the difference was not proved to be statistically significant. The same associations were seen when analysis was done only among women with spontaneous POF. Commonly premature menopause has been associated with cancer treatment but not the other way round. However, there are some suggestions that the age of natural and

premature menopause is heritable and is closely associated with breast cancer via transforming growth factor β receptor II and miR-518 gene polymorphisms (Ma *et al.*, 2014).

In addition to different patterns of concomitant diseases occurring after menopause between the study groups, it was surprising to detect that the critical post-menopausal period for majority of concomitant diseases to appear was ~5–15 years after menopause in women with surgical menopause; while women with spontaneous POF were diagnosed with these diseases throughout the entire period of postmenopause. But by the time of study, when most of the participants were in their fifties, the prevalence of concomitant diseases was similar between two groups of women. This result indicates that post-menopausal diseases in women with surgical menopause might primarily be caused by the hormonal changes following ovarian removal and changes in adipose tissue metabolism thereafter (Lovejoy *et al.*, 2008); while the post-menopausal years in women with spontaneous POF continue to be influenced by the same risk factors which may also cause POF. Thus the authors believe that spontaneous POF likely represents a unique phenotype of post-menopausal ageing distinct from that in surgically induced premature menopause. For instance, a critical age windows for neuroprotection have been suggested for age-dependent effects of oophorectomy or naturally occurring menopause on benign concomitant diseases (Matthews, 2013) and cognitive impairment and dementia (Rocca *et al.*, 2007; Vearncombe and Pachana, 2009) in women at postmenopause.

The current study was not able to detect associations between the starting age of menopause in POF and the lengths of regular menstrual cycle, age of menarche, number of pregnancies or live births, smoking or alcohol consumption, or use of oral contraceptives for some time in the life, as reviewed previously (Harlow and Signorello, 2000). The reason for this could be first, again, that most of the research on POF has used a certain subgroup of patients where either genetic factors or immune mechanisms or other particular etiologic factors might be prevalent. Our study design excludes as much as possible any bias towards preference of any particular etiologic subgroup of POF. Secondly, since the factors like smoking and alcohol consumption are usually self-reported, the way of reporting alone can be confused by many indistinguishable factors and cause bias.

In conclusion, this is the first study which uses data from a registry representative of the whole Estonian population to assess the prevalence of women with an ovarian cause of premature menopause. The prevalence of spontaneous POF of 1% among women population is comparable to other studies reported or predicted in literature. Intriguingly, although the reproductive age is short according to the definition of POF, our results suggest that these women possess no less fecundity during their fertility years. Also, the pattern of menopause-related health problems differs between women with spontaneous POF and women with surgically induced menopause. Most distinctly these data stress that irregular menstrual cycles and early development of diseases affecting immune system, like allergies and T1D, may possess prospective risks for developing spontaneous POF, particularly early onset POF. But nevertheless, surgically induced premature menopause, which we used as a comparison with spontaneous POF, was associated with much more rapid development of menopause- or age-related diseases, including osteoporosis, hypertension and connective tissue diseases. All together, these results reiterate that POF most likely comprises several diseases with different etiopathologies and we believe that spontaneous POF may represent a unique phenotype of post-menopausal ageing distinct from that of surgically induced premature menopause. Importantly, since POF was defined only by the age of menopause and by excluding other extra-ovarian causes for premature menopause, the accuracy of defining POF was dependent on the availability of anamnestic data, and ovarian causes, by elevated FSH levels, could not have been verified retrospectively. Therefore, these results have certain limits and the data are primarily suggestions. However, the authors emphasize again that this population-depictive registry-based epidemiologic study is the only way to actually measure the POF in a population. Nevertheless, the wider implications of these findings are the new ideas and hypothesis for further studies on etiopathologies and treatment options of POF.

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Authors' roles

Study was conceived by A.S., K.H.-K. designed the groups and data request from registry was performed by K.H.-K, A.K. and A.S. The search for literature, statistical evaluation and preparation of first draft and final manuscript was performed by K.H.-K, and the revision of the paper was performed by R.U., A.K. and A.S. All authors approved the final manuscript.

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Conflict of interest

None declared.

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