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Estrogen Signaling and Portopulmonary Hypertension: The Pulmonary Vascular Complications of Liver Disease Study (PVCLD2)

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Abstract

Objectives: Portopulmonary hypertension (POPH) was previously associated with a single nucleotide polymorphism (SNP) rs7175922 in aromatase *(CYP19A1)*. We sought to determine if genetic variants and metabolites in the estrogen signaling pathway are associated with POPH.

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Author contributions:

All authors collected data, provided critical revisions to the study manuscript and approved the final version submitted for publication. MJK, MBF, SMK contributed to the study design. NA, RF, MJP, ND, RA, MRM and SMK analyzed the data. NA, MJK, KAF, KLK, GAH, RAD, SB, TMB, KER, EDA, ARH, JKO, GL, MFD, RA, MRM, MBF and SMK contributed to the interpretation of the results and the drafting of the manuscript.

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A listing of additional members of the Pulmonary Vascular Complications of Liver Disease Study Group can be found in the Appendix located before the References.

Methods: We performed a multicenter case-control study. POPH patients had mean pulmonary artery pressure > 25 mmHg, pulmonary vascular resistance > 240 dynes•s•cm^{-5}, and pulmonary artery wedge pressure ≤ 15 mmHg without another cause of pulmonary hypertension. Controls had advanced liver disease, right ventricular (RV) systolic pressure < 40 mmHg and normal RV function by echocardiography. We genotyped three SNPs in *CYP19A1* and *CYP1B1* using TaqMan and imputed SNPs in *ESR1* using genome-wide markers. Estrogen metabolites were measured in blood and urine samples.

Main Results: There were 37 patients with POPH and 290 controls. The mean age was 57 years and 36% were female. The risk allele rs7175922 in *CYP19A1* was significantly associated with higher levels of estradiol (p = 0.02) and an increased risk of POPH (OR 2.36, 95% CI 1.12–4.91, p = 0.02) whereas other SNPs were not. Higher urinary 2-hydroxyestrogen/16-α-hydroxyestrone (2-OHE/16α-OHE1) (OR per 1 ln increase = 2.04, 95%CI 1.16–3.57, p = 0.01), lower plasma levels of dehydroepiandrosterone-sulfate (DHEA-S) (OR per 1 ln decrease = 2.38, 95%CI 1.56–3.85, p < 0.001) and higher plasma levels of 16-α-hydroxyestradiol (16α-OHE2) (OR per 1 ln increase = 2.16, 95%CI 1.61–2.98, p < 0.001) were associated with POPH.

Conclusions: Genetic variation in aromatase and changes in estrogen metabolites were associated with POPH.

Introduction

Pulmonary arterial hypertension (PAH) is characterized by elevated pulmonary artery pressure and pulmonary vascular resistance, right heart failure, exercise limitation, and an increased risk of death. PAH associated with portal hypertension is termed portopulmonary hypertension (POPH). POPH is the fourth most common form of PAH in the Pulmonary Hypertension Association Registry, the REVEAL Registry, and other registries, comprising approximately 5–10% of patients with PAH (1–3). Patients with POPH have an increased risk of death compared to idiopathic PAH, even with specific PAH treatment (3–6). In many cases, POPH greatly complicates or precludes liver transplantation, impacting significantly on the lives of patients with cirrhosis and portal hypertension (7–9).

Altered estrogen metabolism has been implicated in the pathogenesis of idiopathic and heritable PAH as well as POPH. We have shown that genetic variants in the gene that encodes aromatase (*CYP19A1*) (which produces estradiol, estrone, and 16α – hydroxyestrone (16αOHE1)) and estrogen receptor (*ESR1*) were possibly associated with the presence of POPH in patients with advanced liver disease in a multicenter hypothesis-generating study (PVCLD) (10). Specifically, genetic variation in rs7175922 and rs1902584 in *CYP19A1* was associated with an increased risk of POPH. Increasing numbers of the risk allele of rs7175922 were also associated with monotonically higher levels of circulating estradiol, suggesting function or linkage disequilibrium with another locus which was functional.

CYP1B1 metabolizes estrogen and estrone and wildtype *CYP1B1* genotype *Asn453Ser* (*N453S*) was associated with PAH in women with bone morphogenetic receptor type II (*BMPR2)* mutations, but not in men (11). Moreover, the wildtype *CYP1B1* genotype was also associated with lower ratio of urinary 2-hydroxyestrogen (2-OHE)/16α-OHE1, which

has also been associated with increased breast cancer risk in pre-menopausal women and increased prostate cancer risk in men (12, 13). Other sex hormones play a role in the pathogenesis of PAH. For example, dehydroepiandrosterone-sulfate (DHEA-S) has been shown to attenuate pulmonary hypertension in experimental models and have a protective effect on right ventricular function (14–16).

We therefore hypothesized that variation in *CYP19A1*, *CYP1B1*, and *ESR1* would be associated with the presence of POPH in patients with advanced liver disease undergoing evaluation for liver transplant. We also hypothesized that lower urine 2-OHE/16α-OHE1 ratio and estrogen metabolite levels in blood would be associated with POPH. Some of these data have been previously published in abstract form (17).

Methods (See Online Data Supplement for details.)

Study cohort and study sample

The Pulmonary Vascular Complications of Liver Disease 2 (PVCLD2) Study enrolled a cohort of patients evaluated for liver transplantation or pulmonary hypertension at eight centers in the United States between 2013 and 2017. Similar to the PVCLD cohort, the PVCLD2 study recruited a distinct cohort of patients using the same inclusion and exclusion criteria. The only inclusion criterion was the presence of chronic portal hypertension with or without intrinsic liver disease. We excluded patients with evidence of active infection, recent (< two weeks) gastrointestinal bleeding, or who had undergone liver or lung transplantation. The institutional review boards at each of the participating centers approved this study, and informed consent was obtained.

The study sample included patients undergoing initial liver transplantation evaluation who were evaluated with transthoracic echocardiography (performed routinely) during the study period. “Prevalent” patients with POPH who met the case definition (see below) were also included. We excluded patients with pulmonary function testing showing a significant obstructive ventilatory defect defined as forced expiratory volume in one second (FEV1)/ forced vital capacity (FVC) < 0.70 with FEV1 % predicted < 50% or a significant restrictive ventilatory defect, defined as FVC % predicted < 60% using standard reference equations (18, 19) (See Online Data Supplement for details). We also excluded patients with HIV infection or the presence of more than moderate aortic or mitral valvular disease or significant left ventricular systolic dysfunction as assessed by transthoracic echocardiography (left ventricular ejection fraction < 50%).

Case and control definitions

Cases with POPH met the following criteria at initial evaluation: 1) mean pulmonary artery (PA) pressure > 25 mm Hg, pulmonary artery wedge pressure (PAWP) (or left ventricular end-diastolic pressure (LVEDP)) ≤ 15 mm Hg, and pulmonary vascular resistance > 240 dyne-s•cm^{-5} measured by right heart catheterization prior to study enrollment without another etiology of pulmonary hypertension. “Prevalent” cases who had previously undergone evaluation and were subsequently being treated were included. Liver disease controls had portal hypertension with or without intrinsic liver disease and met the following

echocardiographic criteria at entry into the cohort: 1) right ventricular (RV) systolic pressure < 40 mm Hg (if estimable) and 2) absence of right ventricular dysfunction.

Clinical variables and blood sampling

Data were prospectively collected from subjects during a scheduled study day and the medical record. A history and physical examination, transthoracic echocardiography, spirometry, and six-minute walk testing were performed using research protocols and interpreted centrally. The Model for End-stage Liver Disease (MELD) score was calculated (20). Phlebotomy was performed after an overnight fast (except water). Plasma and buffy coat layers were stored at −80 °C.

Candidate genes and single nucleotide polymorphism (SNP) selection

We performed TaqMan SNP Genotyping Assays for rs7175922 and rs1902584 in *CYP19A1* and rs1800440 in *CYP1B1*. Whole-genome data were also available from the Multi-Ethnic Genotyping Array (MEGA, Global version D2, Illumina, San Diego, CA). All genotyping was performed by HudsonAlpha Institute for Biotechnology (Huntsville, AL) (See Online Data Supplement for details). We imputed *ESR1* SNPs (rs1913474, rs1801132, rs3020317, rs985694, rs932477, rs7757956, and rs3020368) using MACH software with 500kb regions typed genotypes and 1000 Genomes EUR population as the reference (21).

Measurement of estradiol, estrogen metabolites, DHEA-S

Plasma estradiol and DHEA-S as well as urinary estrogen metabolites were measured using immunoassay (See Online Data Supplement for details). Estradiol and its metabolites were also measured using LC-MS/MS methods (See Online Data Supplement for details).

Statistical analysis

Continuous data were summarized using mean ± standard deviation or median [interquartile range], as appropriate. Categorical variables were summarized using n (%). Unpaired Student's t-tests, Wilcoxon rank sum tests, chi-squared tests, and Fisher's exact tests were used, as appropriate. Correlation analysis between estradiol measurements was performed using Spearman rank correlation coefficients.

Hardy-Weinberg equilibrium (HWE) was assessed for genetic alleles using Fisher's exact tests in controls. Principal components analysis was conducted on genome-wide markers to derive the components representing population stratification. The association of genotype (independent variable) with case/control status (dependent variable) was assessed using additive (for *CYP19A1* and other *ESR1* SNPs) and recessive (for *CYP1B1*) multivariate logistic regression models, adjusted for age, sex, and the top 4 principal components. The association between each hormone (independent variable) and case-control status (dependent variable) was assessed by logistic regression models. We also performed mediation analyses to explore whether estradiol mediated the association of *CYP19A1* SNPs with POPH using nonparametric bootstrapping estimation methods using the *mediation* package in R (22). We ran sensitivity analyses limiting the cohort to post-menopausal women and men, and limiting the cohort to patients without hepatocellular carcinoma (HCC).

All analyses were performed using R (version 3.6.1) and PLINKv1.9 (23).

Results

Study subject characteristics

There were 454 patients in the PVCLD2 cohort. Thirty-seven patients with POPH and 290 controls with liver disease were included in the study sample (Figure 1). The mean age of the subjects was 57 years, and 117 (36%) were female. One hundred and twenty-one (87%) were white and thirty-three (10%) were African American. Fifty-six (20%) of the white subjects were of Hispanic ethnicity (17% of the study sample).

Age, sex, race/ethnicity, and body mass index were similar between the groups (Table 1). The majority of women in our cohort were post-menopausal (100% among POPH and 75% among controls). Hepatitis C infection was less commonly the cause of the liver disease in patients with POPH when compared to liver disease controls ($p = 0.08$), and primary biliary cholangitis was more common ($p = 0.03$). There was a significant difference in prevalence of hepatocellular carcinoma between cases and controls (3% vs 34%, $p < 0.001$). Patients with POPH more commonly complained of dyspnea and possibly syncope and lower extremity edema and had significantly worse WHO functional class, but six-minute walk distance was not significantly different. The Model for End-stage Liver Disease was similar between POPH cases and liver disease controls.

Patients with POPH more commonly had echocardiographic evidence of pulmonary hypertension (by design, in terms of RV dysfunction for controls) (Table 1). Patients with POPH had lower tricuspid annular plane systolic excursion, higher right ventricular systolic pressure, larger right atrial area, and lower RV factional change (in those in whom it was interpretable). Notching in the right ventricular Doppler envelope was seen in one quarter of the POPH patients. The baseline hemodynamics were characteristic of POPH.

Genetic and hormonal association

The A allele in rs7175922 in the *CYP19A1* gene was associated with an increased odds of POPH (OR 2.36, 95% CI 1.12–4.91, $p = 0.02$) (Table 2) and increase in circulating estradiol levels in a monotonic fashion (Figure 2). We observed a similar association when limiting the cohort to patients without HCC (OR 2.48, 95% CI 1.09–5.61, $p = 0.03$). We also observed similar associations when results were stratified by sex (not shown). The association of the A allele with POPH case status was not mediated by estradiol levels (average causal mediation effect 1.2%, 95% CI 4.5–100%). The SNP rs1902584 in *CYP19A1* and SNPs in *ESR1* and *CYP1B1* were not statistically significantly associated with the risk of POPH (Table 2).

There were significant differences in plasma sex hormone levels between POPH cases and liver disease controls (Table 3, Figure 3). Patients with POPH had lower DHEA-S compared to liver disease controls (Figure 3A). A 1 natural logarithm (ln) lower circulating DHEA-S level was associated with a 2.38-fold increase in odds of POPH even after multivariable adjustment ($p < 0.001$) (Table 3). Patients with POPH also had higher levels of 16α-hydroxyestradiol (estriol) (16α-OHE2), which persisted despite adjustment for age, sex,

race/ethnicity, body mass index, and liver disease etiology. The urinary ratio of 2-OHE/16α-OHE1 was also associated with POPH in both unadjusted (not shown) and adjusted analyses with lower ratio being associated with a higher odds of POPH (Table 3). These results were similar when the cohort was limited to post-menopausal women and men (data not shown).

While levels of estradiol measured by immunoassay and LC-MS/MS were highly correlated (r 0.74, $p < 0.001$), estradiol levels measured by immunoassay were approximately 3-fold higher than estradiol levels measured by LC-MS/MS (Figure 3). Lower levels of circulating estradiol (by immunoassay) were associated with increased odds of POPH; although this association was not observed between circulating estradiol levels measured by LC-MS/MS and POPH.

Discussion

In this prospective multicenter study, we confirmed that the rs7175922 SNP in *CYP19A1* was associated with elevated circulating estrogen levels and the presence of POPH in patients with advanced liver disease. Patients with POPH had higher 16α-OHE2 levels, lower levels of urinary 2-OHE/16α-OHE1, and lower levels of DHEA-S independent of other covariates. There were no associations between *ESR1* SNPs or *CYP1B1* SNP with the risk of POPH. Estradiol levels assessed by immunoassay were lower in POPH, while estradiol levels assessed by LC/MS-MS were not associated with case status. To our knowledge, this is the first multicenter epidemiologic study of plasma and urine sex hormones and metabolites in POPH, and the first to show differences in 16α-OHE2 between patients with PAH and suitable control patients.

Female sex and sex hormones, particularly estrogen and its metabolites, have been implicated in the pathogenesis of PAH. Estrogen binds to the promoter region of the *BMPR2* gene and regulates its expression (24). Polymorphisms in *CYP1B1* which metabolizes estrogen were associated with penetrance of PAH in women with *BMPR2* mutations, but not men (11). This same study showed lower urinary 2-OHE/16α-OHE1 in women with familial PAH and *BMPR2* mutations compared to unaffected *BMPR2* mutation carriers (11). Expression of *CYP1B1* has also been shown to be increased in experimental models and human PAH (25).

More recent experimental studies of preferential metabolism of estrogens to 16α-OHE1 have shown increased penetrance and severity of PH in murine models (26, 27). 16α-hydroxyestrogens have a strong affinity for estrogen receptors and promote proliferative and proinflammatory processes (28–31). 16α-OHE1 leads to the development of PH in animals via upregulation of miR-29 and has been associated with abnormal markers of insulin resistance (25, 32). 16α-OHE1 has also been shown to increase oxidative-stress related proliferation in pulmonary arterial smooth muscle cells from PAH patients (33). In our cohort, POPH patients had lower urinary 2-OHE/16α-OHE1 ratios and higher circulating 16α-OHE2 (estriol) levels, which is produced by 17β-hydroxysteroid dehydrogenase type 2 from 16α-OHE1 in the liver or the placenta (34). In non-pregnant women, estriol levels are usually very low or unmeasurable. To our knowledge, this is the first study to show increases in circulating 16α-hydroxyestrogens in patients with PAH compared to controls. Inhibition

of 16α-OHE1 production using aromatase inhibitors or 16α-OHE2 with 17β-hydroxysteroid dehydrogenase type 2 inhibitors may be worthy of future study.

The administration of anastrozole or fulvestrant prevented and treated PH in experimental models and led to reversal of the impaired BMPR2 signaling and reversal of the metabolic defects including insulin resistance (26, 35). A small pilot study of anastrozole in postmenopausal women and men with PAH showed that anastrozole decreased levels of estradiol and increased 6-minute walk distance (36). A larger phase-II multicenter randomized clinical trial of anastrozole in PAH is currently underway (ClinicalTrials.gov Identifier: NCT03229499).

In this sample of carefully-phenotyped POPH patients and liver disease controls, we validated the association of the rs7175922 SNP in the aromatase gene with the risk of POPH and its functional role with a dose-dependent increase in estradiol measured by immunoassay as in our prior study. However, there were also some differences. We did not find significant associations between *ESR1* and POPH as in the previous study potentially due to differences in the patient and disease characteristics or Type I error. Moreover, in the current study, female sex was not associated with the risk of POPH, as in our prior study. The current study sample was more racially and ethnically diverse than the previous cohort and had a different distribution of etiologies of liver disease and significantly greater hepatocellular carcinoma in controls. Our findings were similar when limiting the cohort to patients without HCC but there may be additional racial, ethnic and liver disease specific differences in sexual dimorphism in POPH.

DHEA-S, a precursor to testosterone and estrogen, has been linked to the pathogenesis of PAH. Lower DHEA-S levels have been associated with PAH in men and post-menopausal women, and lower levels of circulating DHEA-S tracked with greater clinical severity and higher risk of death (37, 38). Prior studies did not include patients with POPH or advanced liver disease. DHEA-S regulates endothelial nitric oxide and endothelin-1 pathways (39, 40). Supplementation of DHEA has also been shown to restore normal endothelial function in men and post-menopausal women and currently there is a proof-of-concept randomized double-blind placebo-controlled crossover trial of DHEA treatment in men and women with PAH (clinicaltrials.gov Identifier: NCT03648385). Our study shows that lower DHEA-S levels may be a novel mechanism associated with the development of POPH in advanced liver disease.

Estradiol was measured using two techniques: immunoassay and LC-MS/MS. We found a strong correlation between the aromatase SNP rs7175922 and estradiol levels measured by immunoassay (as in our prior study) which was weaker when measured by LC-MS/MS ($p = 0.16$). While levels were highly correlated between the two techniques, LC-MS/MS measurements were significantly lower than those measured by immunoassay and in some cases undetectable. This is consistent with previous literature as LC-MS/MS provides a precise assessment of 17β-estradiol whereas immunoassay has some cross-reactivity with estrone and to a lesser degree with other estrogenic hormones (41–43), which may actually more meaningfully capture the full extent of aromatase activity. As our prior study used the immunoassay for validation (and aromatase also generates several of these estrogenic

compounds), we used the immunoassay data to support the finding of the genetic variant in *CYP19A1*; estrogen levels did not appear to mediate the association. This may imply that local estrogen production, levels over time, or estrogen metabolites such as 16α-OHE2 may explain the aromatase association with POPH.

There were several limitations to this study. First, the number of POPH cases was small; however, this is one of the largest epidemiological cohorts of POPH with detailed phenotyping and genotyping. Cases were recruited across 8 centers over 5 years and represent POPH with exclusion of other contributors to PH. Other studies of POPH have not excluded patients with restrictive or obstructive ventilatory defects or left-sided heart and valvular disease. While controls were patients presenting for liver transplant evaluation, they were similar to other patients with advanced liver disease in terms of sex and racial/ethnic diversity. Patients who did not have evidence of pulmonary hypertension by echocardiography did not undergo right heart catheterization, hence some patients with POPH could have been missed and included amongst the controls. If so, this would have biased our results to the null. Not all subjects provided a urine sample so urinary estrogen metabolites were only available on a subset of the cohort, yet we were able to detect strong associations with POPH.

Our results support the hypothesis that estrogen and its metabolites play a crucial role in the pathogenesis of POPH and that the risk of POPH is influenced by variations in the aromatase gene. Future studies should focus on assessing the therapeutic benefits of altering the estrogen pathway as a potential new treatment approach for patients with POPH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

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Appendix:

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List of Abbreviations

2-OHE	2-hydroxyestrogen
16α-OHE1	16-α-hydroxyestrone
BMPR2	bone morphogenetic protein receptor type II
DHEA-S	Dehydroepiandrosterone-sulfate
ESR1	Estrogen receptor 1
FEV1	Forced expiratory volume in one second
FVC	Forced vital capacity
LVEDP	Left ventricular end-diastolic pressure
MELD	Model for End-stage Liver Disease
PA	Pulmonary artery
PAH	Pulmonary arterial hypertension
PAWP	Pulmonary artery wedge pressure
POPH	Portopulmonary hypertension
PVCLD2	Pulmonary Vascular Complications of Liver Disease 2
SNP	Single nucleotide polymorphism
RV	Right ventricular

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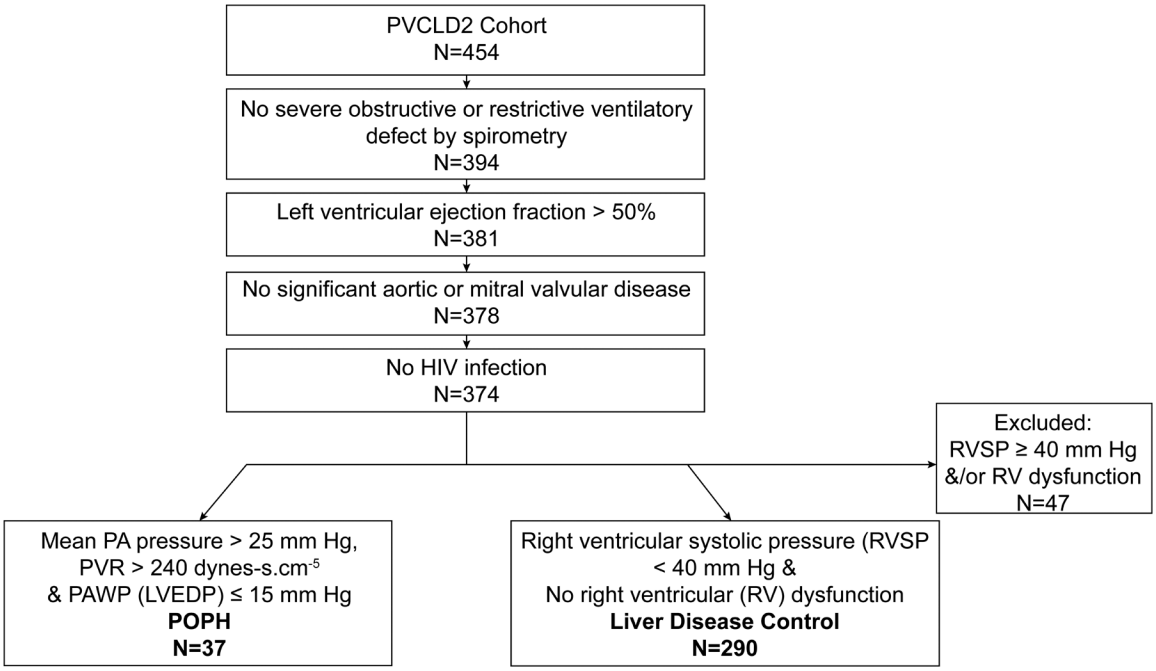

Figure 1.
Flowchart of study inclusion

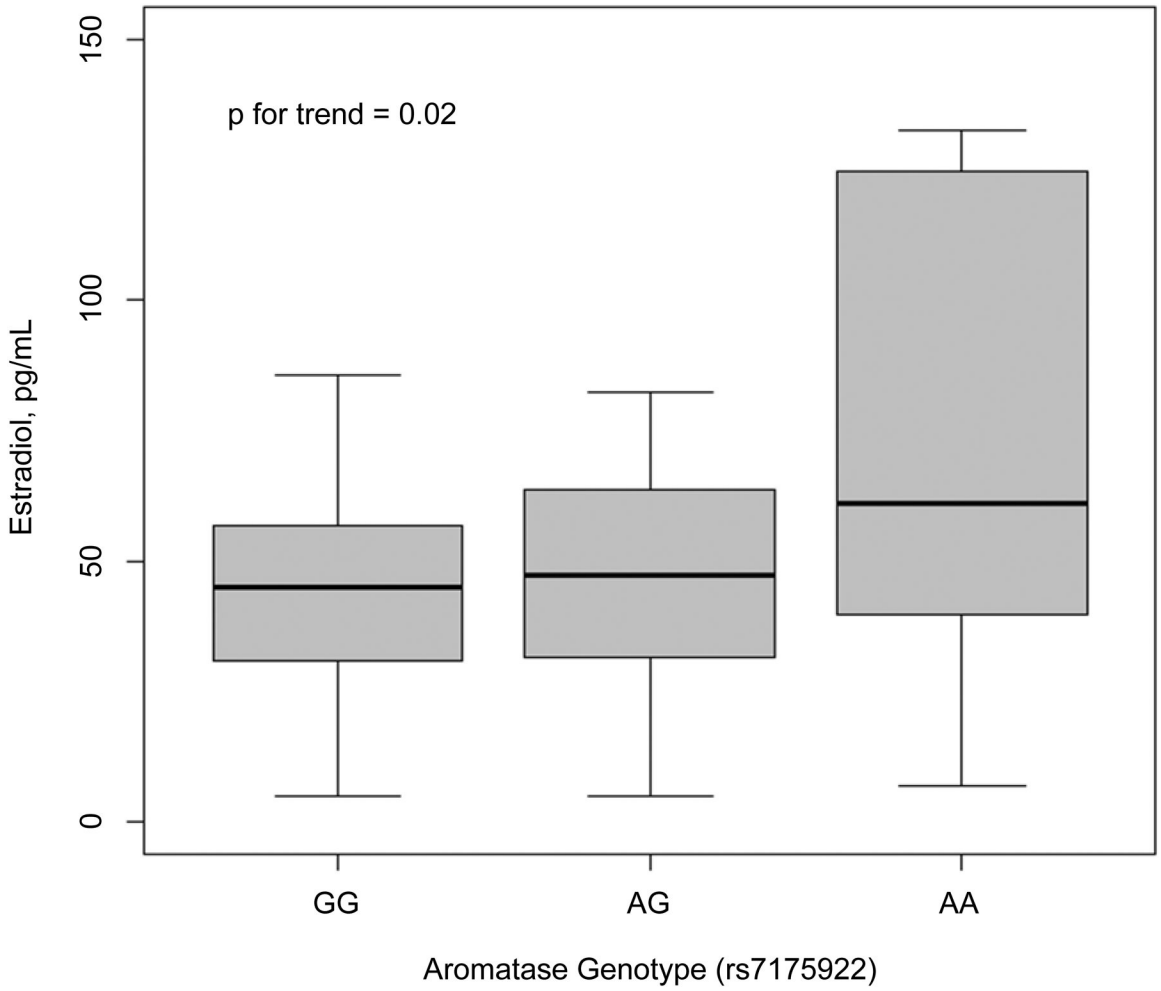

Figure 2.

Estradiol levels and aromatase genotype (Test for trend, p = 0.02, N = 204). Median, interquartile range (box), and adjacent values (whiskers) are shown. Aromatase genotype distribution: GG (N = 148), AG (N = 46), AA (N = 10)

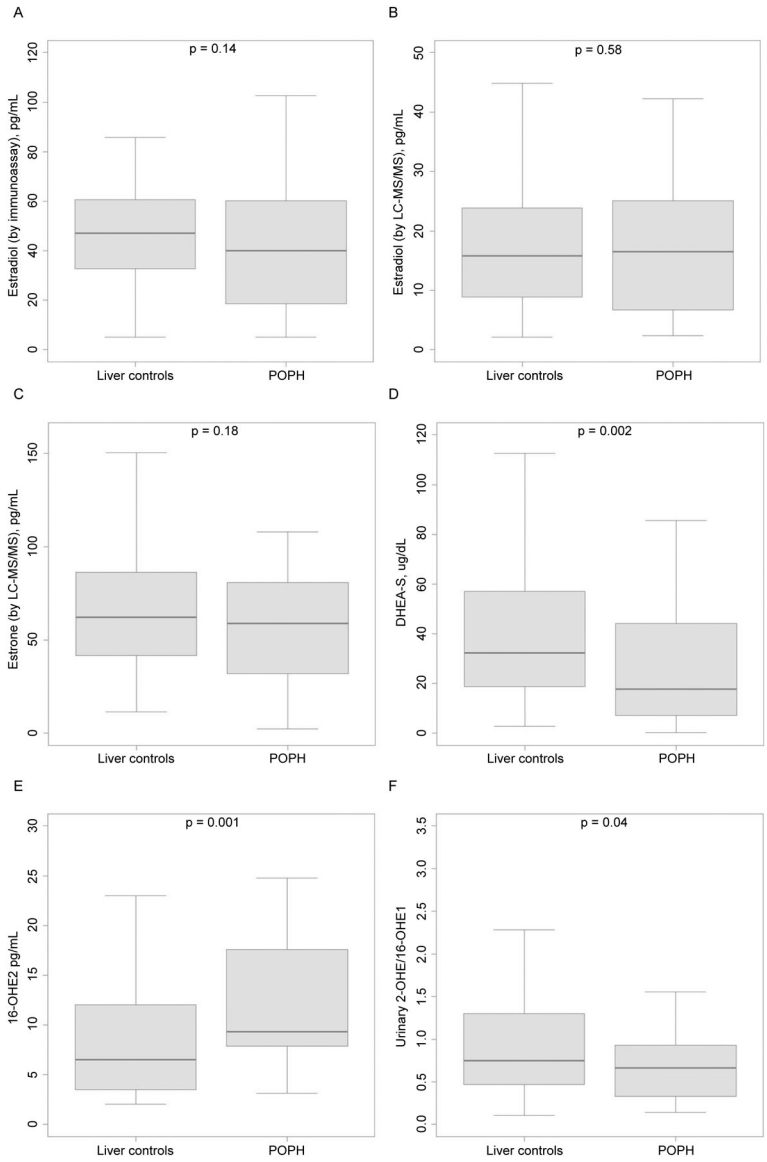

Figure 3.

Sex hormone levels by POPH case status. A) Estradiol measured by immunoassay; B) Estradiol measured by LC-MS/MS; C) Estrone; D) DHEA-S; E) Estriol; F) Urinary 2-OHE/16α-OHE1

Table 1.

Demographic and clinical data for cases and controls.

	Liver disease controls (n=290)	POPH (n=37)	*P* value
Age, years	57 ± 9	57 ± 8	0.90
Male sex, %	65	59	0.58
Race/Ethnicity, %			0.66
Non-Hispanic White	69	78	
Non-Hispanic African American	10	8	
Hispanic white	18	11	
Other	3	3	
Body mass index, kg/m^2	30.3 ± 6.8	30.6 ± 6.9	0.81
Liver disease etiology, %*			
Alcoholic hepatitis	38	46	0.37
Autoimmune hepatitis	4	8	0.20
Hepatitis B	2	--	1.00
Hepatitis C	43	27	0.08
Non-alcoholic fatty liver disease	22	16	0.53
Primary biliary cholangitis	6	16	0.03
Primary sclerosing cholangitis	4	--	0.37
Co-morbidities, %			
COPD	8	5	0.75
Diabetes mellitus	37	30	0.47
Hypertension	49	43	0.60
Hepatocellular carcinoma	34	3	<0.001
Signs and symptoms, %			
Dyspnea	32	84	<0.001
Orthopnea	4	5	0.66
Syncope	2	8	0.07
Edema	47	62	0.08
Clubbing, (n=326)	7	8	0.74
WHO functional class, %			<0.001
I	33	8	
II	48	46	
III	18	46	
IV	1	--	
6-minute walk distance, m (n=286)	400 ± 99	371 ± 114	0.16
SF-36 scores			
Physical component score	38 ± 10	37 ± 8	0.45
Mental component score	47 ± 10	47 ± 11	0.65
Spirometry			
FEV1, % predicted	86 ± 14	84 ± 11	0.25
FVC, % predicted	86 ± 14	89 ± 12	0.30

	Liver disease controls (n=290)	POPH (n=37)	*P* value
FEV1/FVC ratio	77 ± 7	73 ± 7	<0.001
Laboratory findings			
MELD score (n=312)	14 [10–18]	15 [11–18]	0.39
GFR by MDRD, ml/min/m^2 (n=326)	77 [60–98]	68 [58–98]	0.43
Hemoglobin, g/dL	12.4 [10.8–13.6]	13.1 [11.4–14.4]	0.05
Platelets, $\times 10^9$/L	89 [65–128]	84 [53–112]	0.37
Echocardiogram findings			
TAPSE, mm (n=250)	27 ± 6	24 ± 6	0.01
RV systolic pressure, mmHg (n=221)	28 [24–32]	60 [49–71]	<0.001
Right atrial area, cm^2 (n=281)	16.9 ± 4.1	19.7 ± 4.9	0.003
Right ventricular function, %			<0.001
Normal function	100	51	
Mild dysfunction	--	27	
Moderate dysfunction	--	14	
Severe dysfunction	--	8	
RV fractional area change, % (n=150)	51 [45–57]	41 [33–47]	<0.001
Notching present, %	1	27	<0.001
Hemodynamics			
Right atrial pressure, mm Hg	--	9 ± 5	
Mean pulmonary artery pressure, mm Hg	--	46 ± 11	
Pulmonary artery wedge pressure, mm Hg	--	10 ± 3	
Cardiac output, L/min	--	5.90 [4.40–6.90]	
Cardiac index, L/min/m^2	--	3.00 [2.30–3.40]	
Pulmonary vascular resistance, dynes-s-cm^{-5}	--	449 [299–730]	

Abbreviations: RV: Right ventricular; TAPSE: Tricuspid annular plane systolic excursion

Data presented as mean ± SD or median [interquartile range].

Table 2.

Additive multivariate logistic regression models for SNPs and the risk of POPH (adjusted for age, sex and genomic control)

		SNP			Risk Allele Frequency			
Chr	**Gene**	**Identification**	**Location**	**Risk Allele**	**Cases**	**Controls**	**OR (95% CI)**	***P* value**
15	Aromatase (*CYP19A1*)	rs7175922	5’	A	0.25	0.16	2.36 (1.12–4.91)	0.02
		rs1902584	Intron 1	T	0.12	0.08	1.65 (0.68–3.63)	0.23
6	Estrogen receptor 1 (*ESR1*)	rs1913474 *	Intron 3	A	0.23	0.25	0.87 (0.42–1.79)	0.71
		rs1801132	P324P	C	0.22	0.25	0.89 (0.47–1.69)	0.72
		rs3020317 *	Intron 4	C	0.15	0.20	0.66 (0.26–1.65)	0.37
		rs985694	Intron 4	A	0.11	0.18	0.58 (0.25–1.37)	0.22
		rs932477 *	Intron 4	A	0.06	0.10	0.55 (0.16–1.98)	0.36
		rs7757956 *	Intron 4	A	0.15	0.14	1.09 (0.45–2.61)	0.85
		rs3020368 *	Intron 5	T	0.13	0.09	1.62 (0.63–4.15)	0.31
2	*CYP1B1*	rs1800440		T	0.15	0.15	1.09 (0.56–2.36)	0.80

* Imputed for European Ancestry (n=211)

Abbreviations: Chr: Chromosome; SNP, single nucleotide polymorphism; OR, odds ratio

Table 3:

Association of DHEA-S, estrogen and its metabolites with POPH

	N	OR of POPH (95% CI)	*P* value
DHEA-S, per 1 ln decrease	209	2.38 (1.56–3.84)	<0.001
Estradiol (immune-assay), per 1 ln decrease	209	2.08 (1.11–3.84)	0.02
Estradiol (LC-MS/MS), per 1 ln decrease	268	1.21 (0.80–1.79)	0.34
16α-hydroxyestradiol, per 1 ln increase	268	2.13 (1.59–2.92)	<0.001
Urinary 2-OHE/16α-OHE1, per 1 ln decrease	199	2.04 (1.16–3.57)	0.01

Adjusted for age, sex, race (Non-Hispanic white vs other), body mass index, alcoholic liver disease and Hepatitis C.