

# Liver phenotypes in PCOS: Analysis of exogenous and inherited risk factors for liver injury in two European cohorts

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**Handling Editor:** Luca Valenti

## Abstract

**Background & Aims:** Fatty liver disease (FLD) is common in women with polycystic ovary syndrome (PCOS). Here, we use non-invasive tests to quantify liver injury in women with PCOS and analyse whether FLD-associated genetic variants contribute to liver phenotypes in PCOS.

**Methods:** Prospectively, we recruited women with PCOS and controls at two university centres in Germany and Poland. Alcohol abuse was regarded as an exclusion criterion. Genotyping of variants associated with FLD was performed using TaqMan

**Abbreviations:** ALT, alanine aminotransferase; APOE, apolipoprotein E; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; DHEAS, dehydroepiandrosterone sulphate; FIB-4, fibrosis-4; FLI, fatty liver index; FLD, fatty liver disease; FSH, follicle-stimulating hormone; GGT,  $\gamma$ -glutamyl transferase; GPAM, glycerol-3-phosphate acyltransferase-mitochondrial; HbA1c, haemoglobin A1c; HSD17B13, hydroxysteroid 17 $\beta$ -dehydrogenase 13; HSI, hepatic steatosis index; HWE, Hardy-Weinberg equilibrium; IR, insulin resistance; kPa, kilopascal; LH, luteinizing hormone; LSM, liver stiffness measurement; MAF, minor allele frequency; MBOAT7, membrane-bound O-acyltransferase domain containing 7; MTARC1, mitochondrial amidoxime-reducing component 1; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; PCOS, polycystic ovary syndrome; PNPLA3, patatin-like phospholipase domain-containing protein 3; pSWE, point shear wave elastography; SHBG, sex hormone binding globulin; TE, transient elastography; TM6SF2, transmembrane 6 superfamily member 2.

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assays. Liver stiffness measurements (LSM), controlled attenuation parameters (CAP) and non-invasive HSI, FLI, FIB-4 scores were determined to assess hepatic steatosis and fibrosis.

**Results:** A total of 42 German (age range 18–53 years) and 143 Polish (age range 18–40 years) women with PCOS, as well as 245 German and 289 Polish controls were recruited. In contrast to Polish patients, Germans were older, presented with more severe metabolic profiles and had significantly higher LSM (median 5.9 kPa vs. 3.8 kPa). In the German cohort, carriers of the *PNPLA3* p.I148M risk variant had an increased LSM ( $p = .01$ ). In the Polish cohort, the minor *MTARC1* allele was linked with significantly lower serum aminotransferases activities, whereas the *HSD17B13* polymorphism was associated with lower concentrations of 17-OH progesterone, total testosterone, and androstenedione (all  $p < .05$ ).

**Conclusions:** FLD is common in women with PCOS. Its extent is modulated by both genetic and metabolic risk factors. Genotyping of variants associated with FLD might help to stratify the risk of liver disease progression in women suffering from PCOS.

#### KEYWORDS

fatty liver, fibrosis, polycystic ovary, steatosis

## 1 | INTRODUCTION

Fatty liver disease (FLD), which is characterized by increased deposition of lipids in the liver, belongs to the most common conditions worldwide.<sup>1</sup> Fatty liver can represent a standalone disease but it can also be a sign of chronic liver disease.<sup>2</sup> Although in most patients FLD is benign, in some cases, it may lead to advanced hepatic fibrosis, cirrhosis and deterioration of liver function.<sup>3</sup> Fatty liver can be detected at abdominal ultrasonography; however, this method is observer-dependent and does not allow measurements of liver scarring. A more reliable quantification of hepatic steatosis and fibrosis is achievable with transient elastography (TE) and ultrasonography-based point shear wave elastography (pSWE). These methods allow non-invasive measurement of liver fibrosis (based on the liver stiffness measurements—LSM) and hepatic lipid contents (based on the controlled attenuation parameter—CAP), without the need of performing liver biopsies.<sup>4,5</sup>

Numerous studies have shown that in women suffering from polycystic ovary syndrome (PCOS), the prevalence of FLD is higher than in the general population, ranging broadly from 15% to 77%.<sup>6</sup> Kumarendran et al.<sup>7</sup> analysed a total of 63 120 PCOS cases and 121 064 matched controls from the United Kingdom primary care database and showed a substantially (hazard ratio 2.23) increased risk of FLD among women with PCOS after adjusting for risk factors such as body mass index (BMI) or hyperglycemia.<sup>7</sup> The pathogenesis of fatty liver in women with PCOS is not yet fully understood, but it has been postulated that both exogenous and genetic risk factors play a role in this condition.<sup>8</sup> Hyperandrogenaemia, insulin resistance (IR), obesity and obesity-associated chronic low-grade inflammation represent established risk factors for fatty liver in

#### Key points

- Women with PCOS are known to develop fatty liver more frequently than the general population.
- Exogenous risk factors and fatty liver-associated polymorphisms modulate the extent of liver injury in women with PCOS.
- Whereas the *PNPLA3* p.I148M polymorphism increases liver injury, the *MTARC1* p.A165T variant might have protective effects on the liver phenotype in PCOS.
- *HSD17B13* rs72613567 variant modulates sex hormone levels in PCOS.

PCOS.<sup>6</sup> Interestingly, IR might lead to enhanced androgen production in ovarian theca cells.<sup>9</sup> Hyperandrogenism alone promotes apoptosis, alters hepatic lipid metabolism, and induces autophagy directly contributing to the development and progression of FLD.<sup>6,10</sup>

Several genetic variants increasing the risk of FLD have been detected in recent years.<sup>11,12</sup> The adiponutrin (*PNPLA3*) polymorphism p.I148M is the major genetic variant that confers the risk of severe FLD.<sup>13,14</sup> A recent study showed that the *PNPLA3* p.I148M polymorphism and insulin resistance are associated with the increased risk of developing fatty liver in PCOS.<sup>15</sup> Two other variants, namely *TM6SF2* (rs58542926) and *MBOAT7* (rs641738), also increase liver injury in patients with fatty livers.<sup>11</sup> Recently, an exome-wide association study<sup>16</sup> has identified two novel genetic variants in mitochondrial glycerol-3-phosphate acyltransferase (*GPAM* rs2792751)

and apolipoprotein E (APOE rs429358) as risk factors for hepatic fat accumulation and chronic liver injury.

To date, two protective variants have been recognized. The mitochondrial amidoxime-reducing component 1 (MTARC1) p.A165T variant was shown to reduce liver-related mortality,<sup>17</sup> diminish liver injury in patients with autoimmune hepatitis,<sup>18</sup> and the severity of NAFLD.<sup>19</sup> The second protective variant is 17 $\beta$ -hydroxysteroid dehydrogenase 13 (HSD17B13) polymorphism rs72613567 that inhibits the progression of fibrosis of etiologically variable chronic liver diseases, including NAFLD.<sup>20</sup>

Here, we analyse the clinical, metabolic and endocrine profiles of women with PCOS, and investigate whether common FLD-related genetic variants contribute to liver phenotypes in two independent Central European cohorts.

## 2 | MATERIALS AND METHODS

### 2.1 | Cohort characteristics

We prospectively recruited two independent cohorts of adult women patients with PCOS in Germany ( $n = 42$ , Saarland University Medical Center, Homburg) and Poland ( $n = 143$ , Medical University of Silesia, Katowice) as well as German ( $n = 245$ ) and Polish ( $n = 289$ ) female controls. The diagnosis of PCOS was established according to Rotterdam criteria.<sup>21</sup> Exclusion criteria included the diagnosis of any acute or chronic liver disease other than FLD, hyperprolactinaemia, hypercortisolaemia or impaired thyroid function. Alcohol abuse was determined by medical history and interview survey at baseline, and patients abusing alcohol were excluded from the study. Hormonal contraception, glucocorticosteroids or treatment with anti-androgen drugs within three months before inclusion also represented exclusion criteria. The study protocols (EK/271/11, PCN/0022/KB1145/19) were approved by the local ethics committees (Ethik-Kommission der Ärztekammer des Saarlandes; Medical University of Silesia in Katowice) according to the ethical guidelines of the Declaration of Helsinki (latest revision 2013). Informed consent was obtained from each patient included in the study.

All patients underwent careful clinical examination. Blood samples were drawn from fasted subjects and collected for analyses including liver function tests, serum lipids and glucose. Serum hormone levels were determined by electrochemiluminescence immunoassay (ECLIA Roche Diagnostics and IMMULITE 2000 Siemens Healthcare Diagnostics).

### 2.2 | Non-invasive assessment of liver steatosis and fibrosis

In the German cohort, transient elastography (TE, Fibroscan) was used to measure liver fibrosis (liver stiffness measurements; LSM) and hepatic steatosis (controlled attenuation parameters; CAP).<sup>5</sup> In

the Polish cohort, ultrasonography-based point shear wave elastography (pSWE) was used to measure hepatic fibrosis.<sup>22</sup> LSM  $\geq 9.2$  kPa defined diagnosis of significant liver fibrosis (stage  $\geq$  F2),<sup>23</sup> while values  $\geq 13.0$  kPa were classified as cirrhosis.<sup>24</sup> CAP cut-off  $\geq 333$  dB/m was used to determine severe steatosis, corresponding with the histological grade  $\geq$  S3.<sup>5</sup> The fatty liver index (FLI) was calculated as follows:  $FLI = (e^{0.953} \times \log_e[ TG ] + 0.139 \times BMI + 0.718 \times \log_e[ GGT ] + 0.053 \times \text{waist circumference} - 15.745) / (1 + e^{0.953} \times \log_e[ TG ] + 0.139 \times BMI + 0.718 \times \log_e[ GGT ] + 0.053 \times \text{waist circumference} - 15.745) \times 100$ , and the cut-off value of 60 and above indicated the presence of fatty liver.<sup>25</sup> Hepatic steatosis index (HSI) was determined as follows:  $HSI = 8 \times ALT/AST + BMI$  (+2 if type 2 diabetes yes, +2 if female), and values  $\geq 36$  indicate NAFLD.<sup>26</sup> The fibrosis-4 (FIB-4) index for hepatic fibrosis was calculated according to Sterling et al.<sup>27</sup>

### 2.3 | Genotyping

Genotyping of PNPLA3 (rs738409), MTARC1 (rs2642438), TM6SF2 (rs58542926), MBOAT7 (rs641738), HSD17B13 (rs72613567), GPAM (rs2792751), and APOE (rs429358) polymorphisms was performed using allelic discrimination assays, as described previously.<sup>18</sup>

### 2.4 | Statistical analyses

Association between the genotyped variants and quantitative phenotypes was analysed using ANOVA or Kruskal–Wallis tests depending on data distribution. Kolmogorov–Smirnov test was used to determine if variables followed a normal distribution. Clinical variables were analysed using  $\chi^2$ , Student's  $t$  or Mann–Whitney  $U$  tests, as appropriate. Genotype frequencies were compared between cases and controls in contingency tables, using an online tool (<https://ihg.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl>), which was also used to analyse the consistency of genotypes with Hardy–Weinberg equilibrium (HWE) by exact tests. The influence of genotypes, age, BMI and endocrine markers on hepatic steatosis and fibrosis were analysed in uni- and multivariate models by regression analysis. SPSS (version 26.0) and GraphPad Prism (version 8.0) were used for statistical analyses.  $p$ -values  $< .05$  were regarded as statistically significant.

## 3 | RESULTS

### 3.1 | Patient characteristics

A total of 42 German and 143 Polish patients with PCOS were recruited prospectively. Clinical characteristics are summarised in Table 1. The mean age of German women was  $30 \pm 9$  years. Mean BMI was  $30.7 \text{ kg/m}^2$ , 12 individuals (28.6%) were overweight, and

TABLE 1 Baseline characteristics of the study cohorts.

Variables	German cohort	Polish cohort	<i>p</i>
Number of PCOS cases	42	143	
Age (years)	30.0 ± 9	26.5 ± 5	.01
BMI (kg/m <sup>2</sup> )	30.7 ± 9.6	24.5 ± 6.7	<.01
CAP (dB/m)	255 (100–393)	NA	
Patients with CAP 333 ≥ dB/m	10	NA	
LSM (kPa)	5.9 (2.9–17.0)	3.8 (2.5–10.1)	<.01
Patients with LSM ≥ 9.2 kPa	5 (11.9%)	1 (0.7%)	<.01
Patients with LSM ≥ 13.0 kPa	2 (4.8%)	0	<.01
ALT (IU/L)	39 ± 39	15 ± 9	<.01
AST (IU/L)	29 ± 19	20 ± 7	<.01
GGT (IU/L)	33 ± 32	13 ± 21	<.01
Triglycerides (mg/dL)	128 ± 68	79 ± 50	<.01
Cholesterol (mg/dL)	186 ± 39	183 ± 32	.64
HbA1c (%)	5.4 ± 0.9	NA	
Hepatic steatosis index, HIS (points)	37.9 ± 12.0	32.7 ± 7.7	<.01
Patients with HSI ≥ 36 points	25 (59.5%)	54 (37.8%)	.01
Fatty liver index, FLI (points)	38.4 ± 39.8	13.9 ± 31.0	<.01
Patients with FLI ≥ 60 points	19 (45.2%)	30 (21.4%)	<.01
FIB-4 (points)	0.5 ± 0.2	0.5 ± 0.2	.44

Note: Values are given as means ± SD except for CAP and LSM, which are presented as medians and ranges.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; CAP, controlled attenuation parameter; FIB-4, fibrosis-4; FLI, fatty liver index; GGT, gamma-glutamyl transpeptidase; HbA1c, glycated haemoglobin; HSI, hepatic steatosis index; kPa, kilopascal; LSM, liver stiffness measurements; NA, not available; PCOS, polycystic ovary syndrome.

16 (38.1%) were obese. Six (14.3%) patients presented with type 2 diabetes. The mean age in the Polish cohort was 26.5 ± 5 years. Polish women had a mean BMI of 24.5 kg/m<sup>2</sup>, 35 (24.5%) were overweight, and 32 (22.5%) were obese. Three individuals (1.4%) had type 2 diabetes, and 25 (17.6%) showed impaired glucose tolerance in a 75 g oral glucose tolerance test. Overall, as compared to the German cohort, women in the Polish cohort were significantly younger and had better metabolic parameters, as reflected by significantly lower BMI and serum triglycerides levels (all *p* < .05; Table 1).

### 3.2 | Liver phenotypes

The median liver stiffness in the German cohort was 5.9 kPa (range 2.9–17.0 kPa), and the median CAP was 255 dB/m (range

100–393 dB/m). Overall, five patients presented with LSM ≥ 9.2 kPa (i.e., at least fibrosis stage F2).<sup>23</sup> LSM ≥ 13.0 kPa, indicating the development of cirrhosis,<sup>24</sup> was detected in two patients (4.8%). In total, 10 patients showed CAP ≥ 333 dB/m, indicating steatosis grade ≥ S3.<sup>5</sup> Polish women with PCOS had significantly (*p* < .01) lower liver stiffness (median 3.8 kPa, range 2.5–10.1 kPa; Table 1) as compared to German patients. Only one individual presented with LSM ≥ 9.2 kPa, and no one had cirrhosis. Steatosis biomarkers HSI (37.9 vs. 32.7; *p* < .01) and FLI (38.4 vs. 13.9; *p* < .01) were significantly higher in German patients as compared to the Polish cohort. HSI ≥ 36 points indicating fatty liver was detected in 59.5% and 37.8% of Germans and Poles, respectively. Based on the FLI score, fatty liver was present in 45.2% of patients in the German cohort and 21.4% of patients in the Polish cohort. There were no significant differences in FIB-4 scores between patients from both countries. As shown in Table 1, German patients had significantly higher serum activities of liver function tests, i.e., ALT, AST and GGT, as compared to patients from Poland (all *p* < .01).

### 3.3 | Endocrine profiles

We found significant differences in serum endocrine markers between German and Polish women with PCOS. They are summarized in Table 2. In brief, patients in the Polish cohort had higher androstenedione, dehydroepiandrosterone sulphate (DHEAS), as well as total and free testosterone levels as compared to German patients (all *p* < .05). On the other hand, concentration of follicle-stimulating hormone (FSH) was higher in the German cohort (both *p* < .05).

### 3.4 | Genotyping results

All seven genetic variants were genotyped in patients and controls. Each polymorphism was within HWE. Minor allele frequencies (MAF) are summarized in Figure 1. The *PNPLA3* risk variant was significantly

TABLE 2 Serum hormone levels in patients with PCOS.

Variables	German cohort	Polish cohort	<i>p</i>
Androstenedione (ng/mL)	2.8 ± 1.8	3.4 ± 1.6	.01
DHEAS (µg/dL)	201 ± 118	349 ± 131	<.01
Testosterone (pg/mL)	0.4 ± 0.4	1.5 ± 12.7	<.01
Free testosterone (pg/mL)	1.8 ± 2.7	2.0 ± 1.2	<.01
FSH (mIU/mL)	17.5 ± 82.3	5.9 ± 1.6	.02
LH (mIU/mL)	8.4 ± 6.6	8.2 ± 4.4	.68
SHBG (nmol/L)	51.5 ± 103.1	58.5 ± 35.2	.65

Note: Values are given as means ± SD.

Abbreviations: DHEAS, dehydroepiandrosterone sulphate; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone binding globulin.

more frequent among German women with PCOS as compared to controls ( $p < .01$ ). On the other hand, this polymorphism was hardly detectable in patients from Poland: It was less frequent as compared to Polish controls ( $p = .02$ ) and German women with PCOS ( $p < .01$ ). The *MBOAT7* rs641738 polymorphism was less common in German women with PCOS as compared to controls ( $p = .03$ ). In the Polish cohort, we detected a higher frequency of the *TM6SF2* polymorphism as compared to controls ( $p < .01$ ). The frequencies of the *MTARC1*, *HSD17B13*, *APOE* and *GPAM* variants did not differ between cases and controls (Figure 1).

### 3.5 | Association between liver phenotypes and genetic variants in the German cohort

As presented in Figure 2, in the German cohort, the variant *PNPLA3* was related to liver stiffness measurement: LSM differed significantly ( $p = .02$ ) between homozygous carriers of the wild-type allele (median LSM 4.9 kPa, range 3.5–7.8 kPa) and risk genotypes [IM] (median LSM 6.7 kPa, range 2.9–14.3 kPa) and [MM] (median LSM 7.7 kPa, range 4.4–17.0 kPa). LSM correlated with serum AST ( $p < .01$ ), triglyceride levels ( $p < .01$ ), HbA1c ( $p = .02$ ), and BMI ( $p < .01$ ). In the multivariate model, both the *PNPLA3* polymorphism

and BMI represented independent risk factors for liver fibrosis in women with PCOS ( $p = .01$  and  $p < .01$  respectively).

Hepatic steatosis, expressed as CAP, correlated with ALT ( $p = .03$ ), GGT ( $p = .01$ ) and HbA1c ( $p = .02$ ), but not with the *PNPLA3* genotype ( $p > .05$ ). Notably, the *PNPLA3* variant was associated with a trend for increased risk of fatty liver as indicated by HSI  $\geq 36$  (OR = 3.56,  $p = .06$ ). Carriers of at least one *MTARC1* minor allele showed a trend (OR = 0.61,  $p = .06$ ) for protection against FLD, as defined by HSI  $\geq 36$ . Although the *APOE* polymorphism did not modify the liver phenotype, carriers of the minor allele showed significantly higher serum LDL concentrations ( $p < .01$ ). Of note, carriers of the *HSD17B13* variant presented with a trend for higher levels of SHBG ( $p = .05$ ). No significant associations were observed between FLI, LSM, CAP, genotyped variants and serum concentrations of hormones including androstenedione, DHEAS, oestradiol, FSH, LH or testosterone (all  $p > .05$ ).

### 3.6 | Association between liver phenotypes and genetic variants in the Polish cohort

In the Polish cohort, the minor *MTARC1* allele was associated with lower serum activities of ALT ( $p = .01$ ; Figure 3A) and AST ( $p = .04$ ;

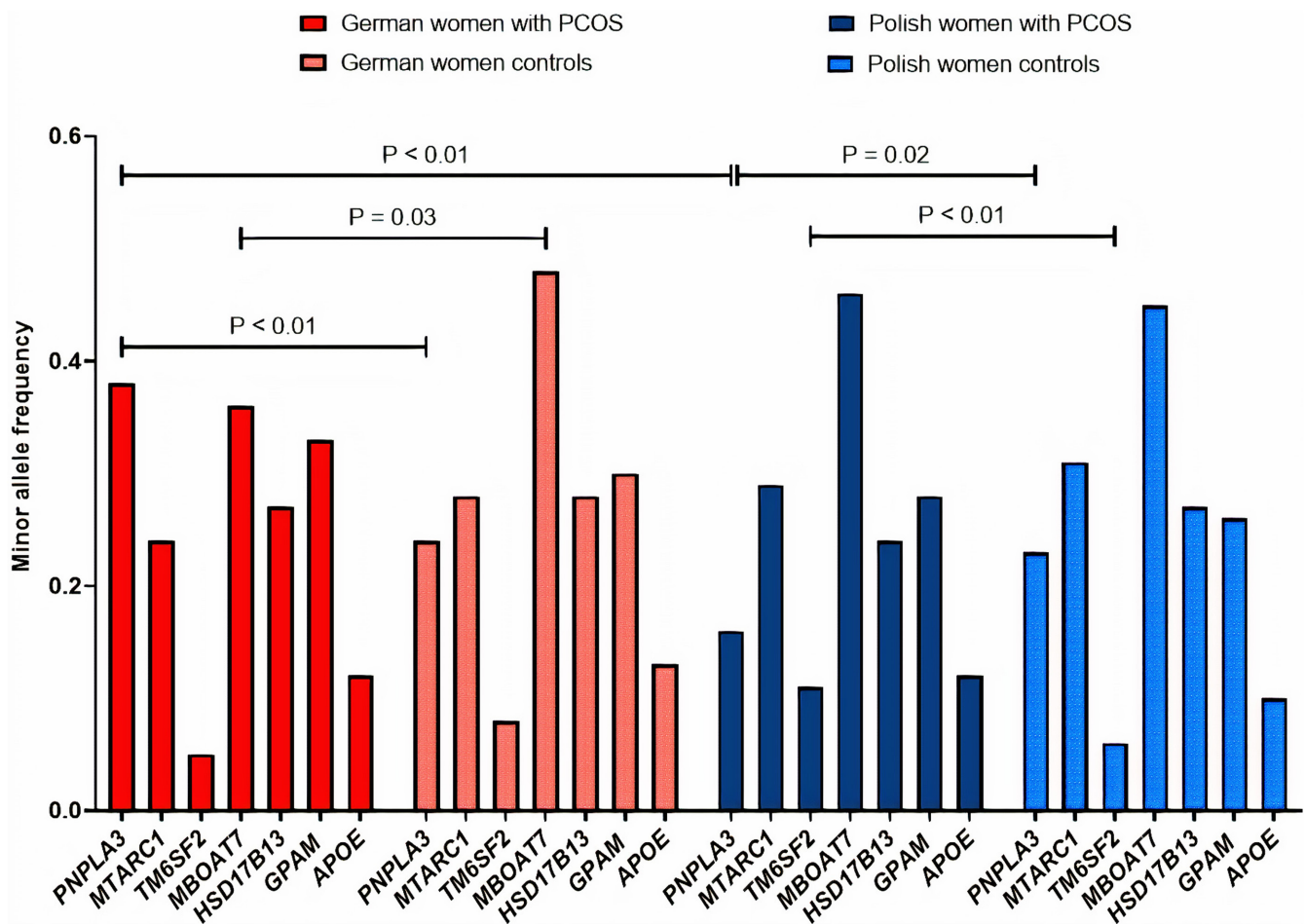
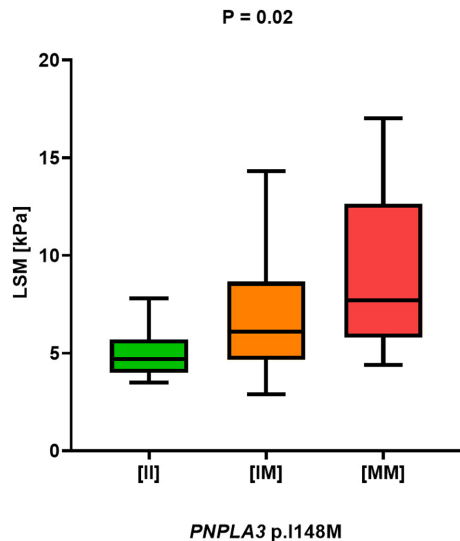


FIGURE 1 Minor allele frequencies (MAF) of all genotyped variants in Polish and German cohorts.



**FIGURE 2** Association between the *PNPLA3* p.I148M polymorphism and liver stiffness (TE) in the cohort of German women with PCOS.

Figure 3B). In line with the results in the German cohort, Polish carriers of *MTARC1* variant alleles showed a trend (OR = 0.27,  $p = .05$ ) for protection against FLD indicated by HSI  $\geq 36$ . The effect of *MTARC1* on HSI became significant after combining German and Polish patients in one cohort (OR = 0.54,  $p = .01$ ). Similar to the German cohort, Polish carriers of the *APOE* variant had higher serum levels of total and LDL cholesterol (both  $p < .01$ ). Furthermore, carriers of two protective alleles of the *HSD17B13* presented with lower concentrations of total testosterone, 17-OH progesterone, and androstenedione (all  $p < .05$ , Figure 4A-C). Of note, carriers of *MTARC1* polymorphism had higher serum SHBG levels ( $p = .04$ ). In contrast to the German cohort, the *PNPLA3* variant did not influence LSM, FLI or HSI in Polish women with PCOS. However, this variant was less frequent in the Polish cohort compared with the German one (Figure 1). We did not detect any effects of the *MBOAT7*, *TM6SF2* or *GPAM* polymorphisms on patients' clinical phenotypes.

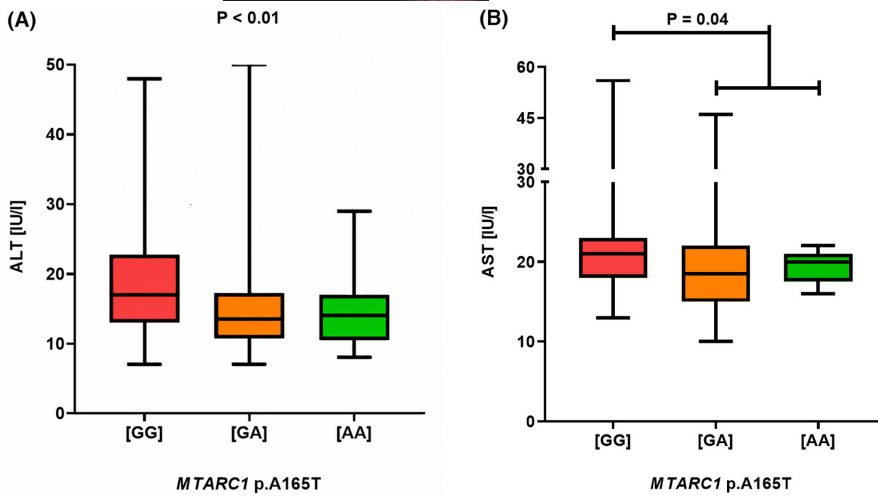
## 4 | DISCUSSION

In our study we present data concerning liver status in two independent Central European cohorts of patients with PCOS. We detected a high prevalence of fatty liver in women with PCOS, but this observation was limited to the German cohort. In the Polish cohort, the patients were significantly younger, had lower BMI and were tested less frequently positive for *PNPLA3* p.I148M, which is a recognized genetic risk factor for fatty liver. Consequently, signs of liver injury were less frequent in this cohort with only a few cases of liver fibrosis and none with cirrhosis. Concerning the genetic analyses, we showed that patients with PCOS who carry the *PNPLA3* p.I148M genotype might suffer from more severe hepatic fibrosis. On the other hand, the

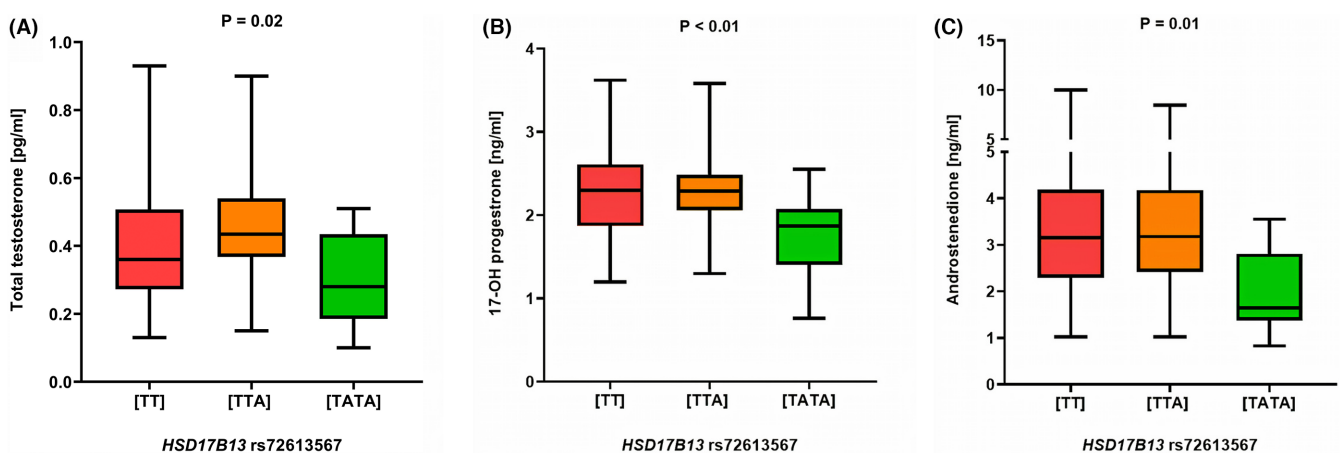
protective variant p.A165T in the *MTARC1* gene is associated with less pronounced liver injury as reflected by lower serum activities of ALT and AST, but this association was only observed in patients from Poland.

The prevalence of NAFLD in women with PCOS ranges from 15% to 77%.<sup>6</sup> Metabolic dysfunction, including dyslipidaemia and insulin resistance, is a common trait in both FLD and PCOS. A recent study from Korea reported that features of metabolic syndrome in women with PCOS correlate with liver fibrosis and steatosis.<sup>28</sup> So far, there has been limited data on the impact of the *PNPLA3* polymorphism on liver phenotypes in women with PCOS. Here, we replicated previous reports<sup>29</sup> and showed that *PNPLA3* p.I148M is independently linked with increased LSM. However, the association between the *PNPLA3* genotype and liver fibrosis was limited to German women with PCOS. This might be attributed to different population distributions of this variant or the effects of exogenous modifiers of liver injury. The p.I148M mutation in *PNPLA3* is linked with a considerable reduction of lipase activity and higher hepatic triglyceride content.<sup>11</sup> In carriers of the p.I148M polymorphism, *PNPLA3* evades ubiquitylation and proteasomal degradation, resulting in the accumulation of *PNPLA3* on the surface of hepatic lipid droplets.<sup>30</sup> Celik et al.<sup>31</sup> analysed the follicular levels of *PNPLA3* in women with and without PCOS, but did not detect significant differences between the two groups.

The *MTARC1* polymorphism p.A165T was shown to have protective effects in patients with chronic liver diseases: it decreases the risk of alcohol-related cirrhosis<sup>32</sup> and severity of NAFLD.<sup>19</sup> Recently, we have demonstrated enhanced antioxidant capacity with the protective influence on liver injury in patients with autoimmune hepatitis.<sup>18</sup> Here, we expand on these observations by showing less severe liver injury and less steatosis in PCOS women carrying the minor *MTARC1* allele. Interestingly, carriers of the *MTARC1* variant presented with higher levels of SHBG, which binds testosterone. Lower testosterone bioavailability could also be considered protective against NAFLD in PCOS. *MTARC1* is a molybdenum-containing enzyme with reductive activity on n-oxygenated compounds.<sup>33</sup> The exact functional consequences of the *MTARC1* p.A165T polymorphisms are yet to be defined.<sup>34</sup> The second known genetic variant *HSD17B13* rs72613567 is associated with protection against hepatic fat accumulation.<sup>20</sup> Recent findings have demonstrated that selective targeting of *HSD17B13* mRNA in hepatocytes might be a promising therapeutic approach in NASH.<sup>35,36</sup> Although the *HSD17B13* variant did not modify liver phenotypes in our patients, it affected testosterone and SHBG levels in the Polish and German cohorts respectively. A similar finding i.e., a lower testosterone levels, was previously described in male patients with advanced chronic liver disease.<sup>37</sup> Increased serum testosterone and decreased SHBG levels are associated with an elevated NAFLD risk.<sup>7</sup> Hence, *HSD17B13* might indeed influence distinct pathomechanisms, although larger cohorts are required to capture its effects on the PCOS-liver phenotype. In the context of ongoing clinical trials targeting *HSD17B13*, researchers should pay attention to the surveillance of sex hormones,



**FIGURE 3** Associations between the *MTARC1* p.A165T polymorphism and serum ALT (panel A) and AST (panel B) activities in Polish women with PCOS.



**FIGURE 4** Associations between the *HSD17B13* rs72613567 variant and serum levels of total testosterone (panel A), 17-OH-progesterone (panel B) and androstenedione (panel C) in Polish women with PCOS.

particularly in men. This observation is also in line with the notion that *HSD17B13* belongs to the family of  $17\beta$ -hydroxysteroid dehydrogenases involved in sex hormone metabolism.<sup>38</sup> We did not observe any major effects of *APOE* or *GPAM* variants on liver phenotypes in PCOS. *APOE* is involved in lipid circulation between tissues, whereas *GPAM* encodes an enzyme participating in triglyceride biosynthesis.<sup>16</sup> In line with the primary function, *APOE* genotypes correlated with serum cholesterol level in both cohorts.

Certain limitations might have influenced the results of our study. We included two phenotypically different cohorts of women with PCOS, which were different in many aspects (age, BMI, metabolic characterization). Overall, patients from Poland presented less advanced signs of metabolic syndrome and less severe liver injury as compared to patients from Saarland, Germany. These differences could result from the variable role of unreported alcohol consumption, dietary factors and/or physical activity, which were beyond the control in this study. In the genetic analysis, we identified very few patients who were homozygous carriers of the *PNPLA3* gene in the Polish cohort. As a result, Polish women did not present with

advanced fibrosis, and in almost all of them the liver function tests were within normal ranges.

The presence of the *PNPLA3* variant might be a strong and so far, underrated risk factor for liver injury in women with PCOS. As in other types of secondary FLD, the significance of this genetic variant might be potentiated by overweight and alcohol consumption. Hence, in women with PCOS, special attention should be given to the modifiable external risk factors for liver injury, such as obesity, alcohol consumption, insulin resistance, and diabetes mellitus. Genotyping of NAFLD-associated variants (e.g., *PNPLA3* p.148M) might help to further stratify patients with PCOS in terms of their individual risk for liver fibrosis and metabolic dysfunction.

Overall, we reckon that the presence of fatty liver in patients with PCOS is driven by multifactorial determinants including a wide range of inherited and environmental risk factors. Based on these results, one might consider the inclusion of FLD-related polymorphisms in the clinical work-up of women with PCOS. This approach, together with non-invasive instrumental and laboratory assessment of hepatic steatosis and fibrosis, might facilitate the detection of patients at risk of developing advanced liver disease. Nevertheless,

larger cohorts of women with PCOS are required to capture less evident links between tested gene variants, as well as clinical and metabolic characteristics of PCOS.

## ACKNOWLEDGEMENTS

This work has been presented in part at the Annual Meeting of the German Gastroenterological Association (DGVS) in Munich, September 2018, and published in abstract form: Papapostoli I, et al. The *PNPLA3* p.I148M polymorphism is associated with increased liver injury in patients with polycystic ovary syndrome. *Z Gastroenterol.* 2018;56:e255. Open Access funding enabled and organized by Projekt DEAL.

## FUNDING INFORMATION

The authors received no financial support for the research, authorship and/or publication of this article.

## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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**How to cite this article:** Smyk W, Papapostoli I, Żorniak M, et al. Liver phenotypes in PCOS: Analysis of exogenous and inherited risk factors for liver injury in two European cohorts. *Liver Int*. 2023;43:1080-1088. doi:[10.1111/liv.15527](https://doi.org/10.1111/liv.15527)