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Rīga Stradiņš University

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Polycystic Ovarian Syndrome in Adolescents – Intergenerational, Genetic, Quality of Life and Binge Eating Aspects

Summary of the Doctoral Thesis for obtaining a doctoral degree "Doctor of Science (*Ph.D.*)"

Sector – Clinical Medicine
Sub-Sector – Obstetrics and Gynaecology

Riga, 2022

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Abbreviations

ACTH	Adrenocorticotropic hormone
alpha (ESRα) or ESR1	Oestrogen receptor 1
BMI	Body Mass Index
CAH	Congenital adrenal hyperplasia
CCUH	Children's Clinical University Hospital
CI	Confidence interval
CYP21A2	Cytochrome P450, Family 21, Subfamily A, Member 2
DHEA	Dehydroepiandrostendione
DHEA-SO_4	Dehydroepiandrostendione sulphate
DNA	Deoxyribonucleic acid
E2	Estradiol
ESR beta (ESRβ) or ESR2	Oestrogen receptor 2
ESHRE	European Society of Human Reproduction and Embryology
FSH	Follicle-stimulating hormone
FSHR	Follicle-stimulating hormone receptor
GAGS	Global acne grading system
GnRH	Gonadotropin-releasing hormone
GnRHR	Gonadotropin-releasing hormone receptor
GWAS	Genome wide association study
HPO	Hypothalamic-pituitary-ovary
HRQOL	Health Related Quality of Life
IL-6	Interleukin-6
IR	Insulin resistance
LH	Luteinising hormone
LHCGR	Luteinising hormone / chorionic gonadotropin receptor

mFG	Modified *Ferriman–Gallwey* scale
OR	Odds Ratio
PCOM	Polycystic ovarian morphology in ultrasonography
PCOS	Polycystic Ovary Syndrome
PCOSQ	PCOS Quality of Life Survey
PCR	Polymerase chain reaction
RR	Relative risk
RSU SLMG	Rīga Stradiņš University, Scientific Laboratory of Molecular Genetics
SD	Standard deviation
SHBG	Sex hormone binding globulin
SNV	Single-nucleotide variant
T	Total testosterone
TNF-α	Tumour Necrosis Factor alpha
TSH	Thyrotropic hormone (thyroid-stimulating hormone)
USG	Ultrasonography
WHO	World Health Organisation

Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy in women, affecting 4.8 % to 18.6 % of women in reproductive age (1–3), depending on the population studied and the diagnostic criteria applied. Among adolescents, PCOS occurs at 3.39– 8.03 % (10 –19 years of age) (4). Data on the incidence of PCOS in puberty-age girls is inaccurate, as PCOS may not have fully developed yet in some adolescent patients and the symptoms of PCOS are similar to normal puberty signs (e.g. irregular menstrual cycle, acne, etc.) (5).

Specific diagnostic criteria are used to confirm the diagnosis of PCOS, which also reflect the main symptoms of PCOS. This disease is characterised by hyperandrogenism (biochemical and / or clinical), oligomenorrhea and a specific ovarian appearance in ultrasound – polycystic ovarian morphology (PCOM). Diagnostic criteria have changed over time (6–9). At present, the age of the patient is also taken into account in the application of the latest diagnostic criteria. In adolescents, the diagnosis is confirmed if the patient meets two diagnostic criteria, hyperandrogenism (clinical and / or biochemical) and oligomenorrhea or amenorrhea. PCOM is not used as a diagnostic criterion for at least eight years after the onset of menstruation. The new diagnostic criteria also distinguish patients who do not yet meet the two necessary diagnostic criteria to confirm the diagnosis, but who should be monitored in order to find out whether they develop PCOS diagnostic criteria – risk group patients (9).

PCOS increases the risk of infertility (10), cardiovascular disease, type 2 diabetes, insulin resistance (IR), metabolic syndrome (11, 12). Increased body mass occurs in 40–70 % of adolescents diagnosed with PCOS, which exacerbates the symptoms of PCOS and increases the risk of long-term health problems (13). Patients with PCOS are also more likely to experience mental disorders (depression, anxiety) (9) and lower health-related quality of life (HRQOL) than the overall female population (14). In PCOS patients, a confirmed diagnosis of

an eating disorder is more common, as is binge eating – both alone and as one of the symptoms of an eating disorder. Binge eating is an eating behaviour characterised by episodes of overeating over a short period of time and can also be one of the symptoms of an eating disorder (15).

PCOS and associated symptoms are commonly found in several family members in different generations. First-grade relatives of PCOS patients are more likely to experience some symptoms of PCOS (hirsutism, menstrual disorders) and a diagnosis of PCOS (16, 17). It is important to continue intergenerational research to better understand how a mother's disease and its symptoms affect the course of the daughter's disease, the phenotypic manifestation of PCOS.

PCOS is a multifactorial disease. Extensive genetic research has been carried out confirming the role of genes in the development and manifestation of the disease (18). Although genome wide association studies (GWAS) have revealed genetic loci associated with the development of PCOS, a replication of results in genetic association studies often fails. This is due to the use of robust statistical analysis in the case of GWAS, which may result in unnoticed genetic variations with weak but real association with the disease, as well as rare variants of genetic variations (19). For example, GWAS has established that genetic factors are responsible for less than 10 % of the heredity of PCOS. At the same time, twin and family studies indicate that genetic heredity could be up to 70 % (19–21). Since PCOS patients have a very wide phenotypic variation in both clinical and biochemical parameters, it is possible that this is a spectrum of diseases of different genetic origins (22). For these reasons, research on genetic associations should be continued in order to clarify the role of certain genetic factors in the development of PCOS and associated symptoms (19). Hypothalamic-pituitary-ovarian (HPO) axis dysfunction is the main pathogenetic mechanism of the development of the syndrome and is affected by genetic variations that determine the action of sex hormones.

In the pathogenesis of PCOS, excess weight and the tendency for the amount of visceral fat to increase (also in lean women with PCOS) are of great importance (22) and therefore the main tactic of treatment is lifestyle changes (9). In order for the lifestyle changes to have the best effect, PCOS and PCOS risk group patients should be diagnosed as early as possible. This would require the following steps. First, identification of patients who may develop PCOS because their mothers already have a PCOS diagnosis or clinical symptoms related to PCOS. Second, the detection of specific genetic markers in patients suspected of this syndrome, which could both help confirm the diagnosis and predict the clinical course of the disease (risks of developing metabolic syndrome, infertility, etc.). It is important that the onset of PCOS occurs at a significant stage of life – adolescence, which is characterised by simultaneous, intense changes in various areas (physical, mental and social). In addition, it is also a period when autonomous motivational systems and lifestyle habits are developed (23). The ability of health professionals to persuade and motivate to follow their guidance on lifestyle changes is of utmost importance. This is done best by understanding and accentuating precisely those aspects of the disease that are most worrying to the adolescent. Considering also that impaired eating behaviour is more common in patients with PCOS and may increase patients' body weight or interfere with its reduction and affect their mental health, it is extremely important to notice and prevent these signs in a timely manner in order to achieve the best results in PCOS treatment and improve quality of life (24). In general, identifying patients (by intergenerational and genetic aspects) and highlighting the most important factors affecting their HRQOL, as well as preventing binge eating (a widespread obstacle to achieving positive lifestyle changes), would make it possible to achieve the best PCOS prognosis.

Objectives

1. To investigate clinical symptoms of the mothers of adolescents with PCOS and their relationship with the clinical picture of patients with PCOS.
2. To study the genetic characteristics of adolescents with PCOS and those at risk for PCOS development by testing genetic variants in genes *FSHR, ESR2, LHCGR, GNRHR, CYP21A2,* their relationship to PCOS development and clinical picture of the disease.
3. To study binge eating habits and HRQOL of adolescents with PCOS and the interrelationship of the two factors.

Tasks

In order to achieve the goals of the Doctoral Thesis, the following tasks are set:

1. Investigate whether the phenotype of the adolescents with PCOS consulted at Children's Clinical University Hospital (CCUH) can be predicted by using PCOS-related symptoms in their mothers.
2. Determine the role of the most frequent variations of protein coding genes involved in the functioning of sex hormones (*FSHR, ESR2, LHCGR, GNRHR, CYP21A2*) in PCOS development for adolescent patients with PCOS and PCOS risk group patients consulted at CCUH.
3. Analyse the association of the most frequent variations of protein coding genes involved in the functioning of sex hormones *(FSHR, ESR2, LHCGR, GNRHR, CYP21A2*) to the PCOS clinical picture in adolescent patients with PCOS and risk group adolescent consulted at CCUH.

4. Analyse HRQOL components for adolescents with PCOS consulted at CCUH compared to healthy control group representatives of the appropriate age.
5. Study the incidence of binge eating behaviours in adolescent patients with PCOS consulted at CCUH compared to healthy control groups representatives of the appropriate age.
6. Identify factors that influence total HRQOL in PCOS patients.

Hypotheses

1. A mother's PCOS-related symptoms can be used to predict a daughter's PCOS phenotype in adolescence.
2. Variations in *FSHR*, *ESR2*, *LHCGR*, *GNRHR* and *CYP21A2* genes are related to the development of PCOS in adolescents.
3. Variations in *FSHR*, *ESR2*, *LHCGR*, *GNRHR* and *CYP21A2* genes are related to the PCOS phenotypes of adolescents.
4. For adolescents with PCOS, concerns about increased body weight and infertility are the most pronounced among all components of HRQOL.
5. Binge eating behaviour is more common in adolescents with PCOS than in healthy adolescents and has a negative effect on HRQOL.

Novelty of the study

1. A study on adolescents with PCOS has been conducted for the first time in Latvia. Such studies in the adolescent age group have been carried out abroad but only in small samples.
2. The study was conducted using the latest diagnostic criteria for the confirmation of the diagnosis of PCOS for adolescents (9).

3. The effects of symptoms associated with the clinical picture of PCOS in mothers have been studied on the PCOS phenotype in their daughters. Only small number of studies with limited sample sizes have been carried out in adolescent population abroad. Results of these studies are contradictory.
4. The role of the most common variations of protein coding genes involved in the functioning of sex hormones in the development of PCOS and associated clinical symptoms in adolescents has been studied. Only small number of studies with limited sample sizes have been carried out in adolescent population abroad. Results of these studies are contradictory.
5. The HRQOL aspects that are the most relevant and affect adolescents with PCOS the most have been identified in order to develop a targeted counselling and treatment strategy for this particular age group. Such studies have also been carried out in small numbers and small samples of adolescents abroad.
6. The incidence of binge eating and its role in the overall HRQOL in adolescents with PCOS have been studied. Such studies have also been carried out abroad in small numbers and in small samples of adolescents, with contradictory results.
7. A cohort of adolescent patients with PCOS and PCOS risk group adolescents living in Latvia has been established, and can be used in further longitudinal studies.

1 Materials and methods

1.1 Structure of the study

The study at the CCUH Outpatient Clinic was prepared for launch between 1 January and 31 December 2016. The inclusion of patients started on 1 January 2017. The research was carried out at the State-owned limited liability company CCUH and the Scientific Laboratory of Molecular Genetics (RSU SLMG) of Rīga Stradiņš University.

The study consisted of three sections:

I. inter-generational aspects. A study of the relationship between clinical signs in PCOS patients and their mothers.

II. genetic aspects. A study of genetic variations associated with HPO axis activity and with non-classical form of CAH in PCOS patients.

III. aspects of quality of life and binge eating. A study of health-related quality of life and binge eating for adolescents with PCOS.

1.2 Inclusion of participants in the study

Figure 1.1 shows the process of inclusion of participants in the study. The study included adolescents who had visited gynaecologists, obstetricians at the CCUH Outpatient Clinic in Torņakalns. Additionally, the mothers of these adolescents have been included.

At the beginning of the study, patients were included using modified Rotterdam criteria (6): clinical hirsutism (m*Ferriman–Gallwey* score above eight points) and oligomenorrhea (menstrual cycle longer than 45 days) or ultrasonographic ovarian appearance typical to PCOS (volume of the largest ovary above 10 ml, and no *corpus luteum* or inclusion cysts are visualised in any

of the ovaries). On 19 July 2018, ESHRE published international evidence-based guidelines for PCOS evaluation and treatment. These guidelines specify the diagnostic criteria for PCOS in adolescents (up to eight years after the onset of menstruation) as well as isolated patients in risk group for PCOS development (9). A review of patients already included and changes to the inclusion criteria were carried out.

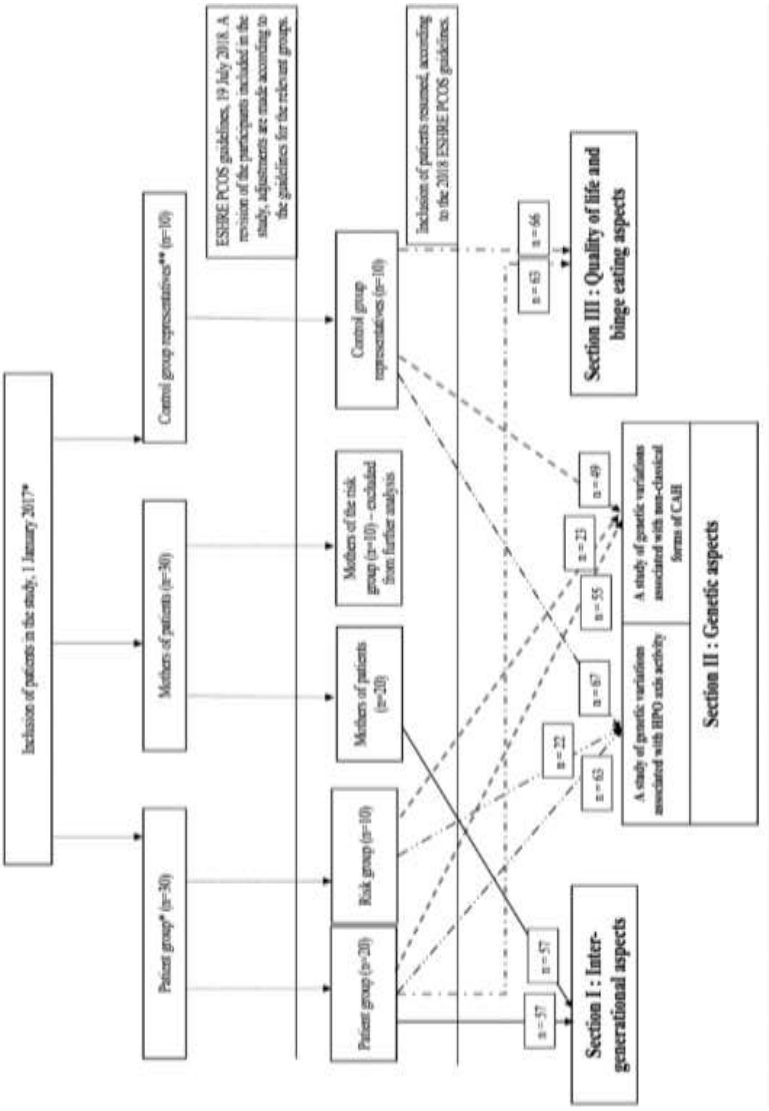

Figure 1.1 **Inclusion of patients in the study. Schematic diagram**

* Included patients with clinical hyperandrogenism (m*Ferriman–Gallwey* score > 8 points) and oligomenorrhea – menstrual cycle longer than 45 days or PCOM – volume of largest ovary above 10 ml and no *corpus luteum* or inclusion cysts are visualised in any of the ovaries. Other causes of hyperandrogenism are excluded.
** From 1 February 2017

Figure 1.1 shows the number of patients included in the study sections. A total of 63 PCOS patients, 57 mothers of PCOS patients, 23 representatives of the risk group and 67 representatives of the control group participated in the study. It was not possible to include all participants in all sections of the study for a number of reasons. First, some of the participants had not completed all questionnaires of the study (e.g. missing data on the binge eating scale). Second, in order to follow the time plan of the study, some patient data were analysed before the inclusion of all participants was completed (e.g. inter-generational study). Third, in the analysis of genetic aspects, in some cases, it was not possible to obtain genetic material of sufficient quality to analyse all the genetic variations envisaged.

1.3 Literature selection

Literature sources from the following medical databases were selected:

1. Medline (PubMed);
2. EBSCO;
3. SCOPUS;
4. The Cochrane Library.

The selection of publications was limited: only sources in English were considered. The time of publication of sources was not limited. Unsuitable publications, publications in other languages and re-listed publications were excluded from further analysis.

The keywords used were: "*PCOS*", "*PCOS adolescents OR youth OR young people*", "*PCOS genetic*", "*PCOS quality of life*", "*PCOS health related quality of life*", "*PCOS binge eating*".

In addition, information from the guidelines of relevant organisations (*European Society of Human Reproduction and Embryology, The Androgen Excess and PCOS Society*) was used.

1.4 Ethical aspects of the study

Permissions of the Central Medical Ethics Committee have been obtained for carrying out the study (Protocol No: 1/16-04-12, 3/21-02-17, 2/18-01-24). The study has been implemented in accordance with international and Latvian law, Helsinki and Taipei declarations. Prior to the start of the study, each patient became acquainted with the "Information for the Patient" and confirmed in writing their agreement to participate in the study by signing a "Confirmation of Consent". For patients in the case and risk groups, as well as in the control group up to 16 years of age, their mother or legal guardian has given additional written permission to participate in the study, as genetic tests were begun without reaching the age of majority. The representatives of the control group who were over 16 years of age and who arrived for the visit without a legal representative signed their own agreement to participate in the study. Before then, the participants were informed that the analysis of their genetic material would only begin after they have reached the age of 18 years and three months, assuming they have not withdrawn their consent by then. Such an exception for the representatives of the control group from the age of 16, who attended the visit alone, was approved by the Central Medical Ethics Committee (Authorisation Protocol No 2/18-01-24). Exceptional arrangements were requested, since healthy young women often come to a visit to a gynaecologist without a legal representative, and often do not want the legal representative to learn about the visit of the gynaecologist, so it would not be possible to involve an appropriate number of representatives of the control group without applying this exception.

1.5 Criteria for inclusion and exclusion of participants

The study included PCOS patients, their mothers, risk group patients and control group representatives who visited a gynaecologist, obstetrician at the CCUH Outpatient Clinic in Torņakalns.

PCOS patients

Inclusion criterion: confirmed diagnosis of PCOS in patients according to the diagnostic criteria of the 2018 ESHRE guidelines:

1) at least one year has passed since the start of menstruation;
2) clinical hyperandrogenism and oligomenorrhea. The criterion of clinical hyperandrogenism is hirsutism, defined as a score on the m*Ferriman–Gallwey* scale ≥ 4. Definition of oligomenorrhea: at least one to three years after *menarche*, the length of the menstrual cycle is more than 45 days and three years or more after *menarche*, the length of the menstrual cycle is longer than 35 days (9, 25).

Exclusion criteria:

1) chronic diseases of gynaecological or other organ systems, including endocrine diseases;
2) hormonal medication used within the last six months;
3) the patient or the patient's mother (legal guardian) refused to participate in the study.

PCOS patients' mothers

Inclusion criterion: PCOS patient's biological mother.

Exclusion criteria:

1) the patient's mother refuses to participate in the study;
2) the patient is adopted.

Risk group patients

Inclusion criterion: patients with clinical hyperandrogenism, other causes of hyperandrogenism have not been found, but experience regular menstruation (9).

Exclusion criteria:

1) chronic diseases of gynaecological or other organ systems, including endocrine diseases;
2) hormonal medication used within the last six months;
3) the patient or the patient's mother (legal guardian) refused to participate in the study.

Representatives of the control group

Adolescents who have visited a gynaecologist, obstetrician without any complaints about health and turned to the doctor with a desire to discuss their reproductive health or choose contraception.

Exclusion criteria:

1) chronic diseases of gynaecological or other organ systems, including endocrine diseases;
2) hormonal medication used within the last six months;
3) the patient or the patient's mother (legal guardian) refused to participate in the study.

1.6 Research tools

The following scales and instruments were used for the study:

1. Acne was evaluated using the global acne grading system (GAGS). The results were interpreted as follows: mild acne 1–18 points; average acne 19–30 points; severe acne 31–38 points; very severe acne > 38 points (26).
2. m*Ferriman–Gallwey* scale, where hirsutism is defined as a score ≥ 4 (25).

3. PCOS quality-of-life questionnaire (PCOSQ) for the evaluation of HRQOL. The questionnaire consists of 26 questions, grouped into five domains: body hair, infertility, emotions, body weight and menstrual problems. The key to the questionnaire is published (27).
4. A binge eating scale. 16 multiple-choice questions with 3–4 possible answers. Interpretation of the scores: 0–16 points – no binge eating; 17–26 points – moderate binge eating; ≥ 27 points – severe binge eating (28). The binge eating scale has been validated in the Latvian population (29).
5. A family and reproductive anamnesis was obtained from the patient's mother using a structured questionnaire specifically designed for the study. The patient's mother was asked about PCOS-related symptoms at present or in the past during childbearing age (irregular menstruation, hirsutism, acne, obesity, infertility), questions on the mother's reproductive history and patient's birth weight were included.

All research questionnaires were available in both Latvian and Russian. Translation from Latvian to Russian was performed by a certified bilingual interpreter, after which the translation was adjusted by a bilingual graduate doctor, in accordance with the guidelines adopted by the World Health Organisation (30). Prior to the start of the study, the content of the questionnaires has been piloted for 10 adolescents of the relevant age.

In addition, the following tests were performed in the study:

1. Determining of hormonal levels (total testosterone, DHEA-SO_4, androstendion, LH, FSH, E2, TSH, prolactin, 17-OH progesterone) was performed in a certified laboratory on 3rd–5th day of the menstrual cycle before 10 a.m.

2. An ultrasound examination was carried out by a single specialist with a certificate for "Ultrasonography in obstetrics and gynaecology". Examinations were carried out with a HD11 XE Philips (Amsterdam, Netherlands) or Logiq P5 General Electric (Boston, USA) ultrasound apparatus. Ultrasonographically, PCOM was defined as the volume of at least one ovary > 10 ml and ovaries without *corpus luteum* or dominant follicles or cysts (9).
3. All anthropometric measurements (body weight, height) were determined and indicators (BMI; waist-hip ratio) were calculated using standardised measuring devices. The interpretation of the results in children has been carried out in accordance with WHO guidelines with the help of the AnthroPlus programme (31). BMI of PCOS patients' mothers has been interpreted as follows: < 18.5 kg/m^2 – reduced weight; 18.5 – 24.9 kg/m^2 – normal weight; 25.0–29.9 kg/m^2 – overweight; ≥ 30 kg/m^2 – obesity (32).
4. Identification of genetic variants of DNA extracted from peripheral blood was done using standard molecular analysis methods – polymerase chain reaction (PCR), restriction fragment length polymorphism analysis, multi-stage PCR, Sanger sequencing (for more information, see 1.7 Genetic analysis).

1.7 Genetic analysis

DNA extraction for genetic analysis was carried out from venous blood collected from peripheral veins in a 5 ml EDTA tube at the CCUH procedure cabinet and delivered to RSU SLMG. DNA extraction was carried out using the commercial kit Innu Prep DNA mini kit (Analytic Jena, Germany) according to the protocol specified by the manufacturer.

Genetic variations related to genes affecting the activity of sex hormones and described in relation to PCOS in the literature were selected for genetic analysis.

The selected markers, their nomenclature and the methods used for analysis are shown in Tables 1.1 and 1.2. In further text, a more common traditional nomenclature of variations which does not comply with the latest nomenclature guidelines of the Human Genome Variation Society (HGVS) is used.

Table 1.1

Genetic variations associated with activity of the HPO axis included in the study. Methods used in genetic analysis

Gene	dbSNP/ dbVar	Tradi-tional name of change	Reference sequences	HGVS nomenclature			Minor allele	Minor allele frequency (MAF*)	Method of analysis
				Gene level	Encoding sequence level	Protein level			
ESR2	rs4986938	G1730A	NG_011535.1 NM_001040275.1	g.110453G > A	c.1406 + 1872G > A	p.=**	A	0.32	Test name – Genterf [RSU MZGL] – multi-stage PCR.
FSHR	rs6166	G2039A	NG_008146.1 NM_000145.3 NP_000136.2	g.196710G > A	c.2039G > A	p.Ser680Asn	G	0.47	Test name – Genterf [RSU MZGL] – multi-stage PCR.
FSHR	rs6165	T307A	NG_008146.1 NM_000145.4 NP_000136.2	g.195590G > A	c.919G > A	p.Ala307Thr	A	0.37	PCR and restriction fragment length polymorphism analysis (RFLP), method adapted by Unsal and colleagues (33)

Table 1.1 continued

Gene	dbSNP/ dbVar	Tradi-tional name of change	Reference sequences	HGVS nomenclature			Minor allele	Minor allele frequency (MAF*)	Method of analysis
				Gene level	Encoding sequence level	Protein level			
FSHR	rs2349415	Not applicable	NG_008146.1 NM_000145.4	g.138799A > G	c.225–533A > G	p. =	T	0.39	PCR RFLP, method developed by RSU SLMG – primers designed using Primer3 program, restriction enzyme found using *Ensyme Finder* [*https://enzymefinder.neb.com/*]

Table 1.1 continued

Gene	dbSNP/ dbVar	Tradi-tional name of change	Reference sequences	HGVS nomenclature			Minor allele	Minor allele frequency (MAF*)	Method of analysis
				Gene level	Encoding sequence level	Protein level			
LHCGR	rs2293275	A312S	NG_008193.1 NM_000233.3	g.66506A > G	c.935A > G	p.Asn312Ser	T	0.0017	PCR RFLP, method adapted from Capalbo and colleagues (34)
GNRHR	rs104893837	R262Q	NG_9293.1 NM_000406.3	g.2–4–5G > A	c.785G > A	p.Arg262Gln	A	0.32	Test name – Genterf [RSU MZGL] – multistage PCR. Sanger sequencing.

* https://www.ncbi.nlm.nih.gov/snp/, tested on 07.03.2022; ** p. = unchanged protein.

Table 1.2

Genetic variations associated with the non-classical form of CAH included in the study. Methods used in genetic analysis

Gene	Traditional name of change	dbSNP/ dbVar	Reference sequences	HGVS nomenclature			Method of analysis
				Gene level	Encoding sequence level	Protein level	
CYP21A2	−113bp	rs1246774295	NG_007941.2 NM_000500.7 NP_000491.4	g.362G > A	C. −113G > A	p.=*	Multiplex ligation analysis (MLPA) using the manufacturers protocol (MRC Holland)
	I2G allele C, A G	rs6467		g.5777A/C > G	c.293–13A/C > G	p.=*	
	del8bp	rs387906510		g.5829_5836del	c.332_339del	p.Gly110fs	
	I172N	rs6475		g.6122T > A	c.518T > A	p.Ile173Asn	
	V237E (E6 Cluster Variation)	rs12530380		g.6506T > A	c.713T > A	p.Val238Glu	
	239K (E6 Cluster Variation)	–		g.6512T > A	c.719T > A	p.Met240Lys	
	F306 + T	rs267606756		rs267606756	c.923dupT	p.Leu308Phefs	

* p.? unknown effect on protein.

A mixture with a total volume of 20 µl was prepared to carry out the PCR: 2 µl 10xPCR buffer (NH4)2SO4-MgCl2, 2 µl 25 mM MgCl2, 0.25 µl 20 mM dNTP mix, 0.05 µl 100 pmol synthetic oligonucleotides, 0.1 µl Taq polymerase (recombinant 5 U/µl). All reaction reagents produced by ThermoScientific (Waltham, USA), synthetic oligonucleotides – by Metabion (Martinsried, Germany). 1 µl (concentration of 30 ng/µl) of DNA sample was added. The prepared mixture was placed in an automatic thermocycler. In order to perform the analysis of the length of the restriction fragments (RFLP), a mixture with a total volume of 4 µl was prepared: 3 µl ddH2O, 0.8 µl buffer suitable for restriction endonuclease, 0.2 µl appropriate restrictase, 4 µl PCR product. All restrictases and suitable buffers are manufactured by Thermo Scientific (Waltham, USA). The prepared mixture is incubated for 1–16h, at a temperature of 37 °C. After restriction, the samples were tested in 6 polyacrylamide gel electrophoresis. The result was visualised with ethidium bromide and documented using photo-documentation equipment OmniProGel, manufacturer UVITEC (Cambridge, United Kingdom). Positive and negative controls were added to each evaluation to determine the quality of the response.

In order to evaluate whether the genotypes found using PCR, RFLP and GENTERF correspond with at least 10 samples (among which all 3 genotypes could be found), for each of the variations, Sanger sequencing was performed using Big Dye Terminator 3.1 from the manufacturer ThermoScientific (Waltham, USA) according to the manufacturer's protocol and using PCR primers.

Molecular analysis of the *CYP21A2* gene was performed using standard MLPA (*SALSA MLPA probemix P050-C1 CAH, MRC Holland*) according to the manufacturer's methodology. MLPA kits contain 33 different probes with 130–391 bp long amplification products. The kit of this manufacturer determines extensive *CYP21A2* gene deletions (delA2) as well as *CYP21A2* and *CYP21A1P* (pseudogenic) conversion. In addition, several allelic variants of pathogens can

be identified: –113 G > A before start codon, IVS–12A/C > G 13 bp before exon 3, del8 bp in exon 3, I172N in exon 4, V237E and M239K in exon 6, F306 + T in exon 7. *Coffalyser 8.0* (*MRC Holland*, Amsterdam, Netherlands) was used to analyse MLPA data.

2 Statistical analysis

The statistical processing of the data was carried out using IBM SPSS 22.0 programme. Genotype frequencies were tested using the Hardy-Weinberg equation.

The with normal distribution of the data was checked by the Kolmogorov–Smirnov test. The central trends of normally distributed data were characterised using mean values with standard deviations, while data not consistent with normal distribution were characterised using median and interquartile range of the median values. The differences between the groups were determined using the Mann–Whitney U test, the Student's t-test, ANOVA or Kruskal-Wallis and the Pearson's Chi-square test or Fisher's exact test, depending on the scale of the variable data. For correlation analysis, the correlation coefficients of Pearson or Spearman were used for normally distributed data and data that differed from the normal distribution, respectively. Correlation coefficients were interpreted as follows: $r \leq 0.20$ – non-significant correlation; $0.20 < r < 0.40$ – weak correlation; $0.40 \leq r \leq 0.79$ – moderately close correlation; $r \geq 0.70$ – close correlation. Multicollinearity was excluded among all relevant variables using a correlation matrix. The critical value for multicollinearity was assumed to be $r \geq 0.70$ (35). A multivariate linear regression analysis was carried out to evaluate the independent variables predicting the total HRQOL. Three models were identified as statistically significant in a stepwise regression analysis. Variables were added gradually to the regression, according to their interpretative force, adding one in each step-in addition to a statistically reliable indicator. Fisher's test showed the overall statistical significance of the model, β (non-standardised β factor) indicated by how many units the dependent variable changed when the independent variable changed by one unit. R^2 pointed to the strength of the relationship between the

model and the dependent variable. ΔR^2 showed what part of the outcome variation is explained by the independent variable.

Results were considered statistically significant if the significance level (*p*) was less than 0.05.

3 Results

3.1 Study Section I. Relationship between the clinical signs in PCOS patients and their mothers

57 PCOS patients and 57 mothers were enrolled in this section of the study. The mean age of patients was 15.9 years (SD 1.3) and mean age of mothers – 41.7 years (SD 5.9). The biochemical and clinical signs of hyperandrogenism in patients are shown in Table 3.1. As per the inclusion criteria, all patients had irregular menstruation and an mFG score ≥ 4. Increased total testosterone levels in blood were observed in 23 (42.6 %) patients (data was available for 54 patients). All but one patient had at least mild or more severe acne (according to the GAGS score). Only one third of patients (35.1 %) had a PCOS-specific ovary appearance in ultrasonography (PCOM).

Table 3.1

Biochemical and clinical signs of hyperandrogenism in patients with PCOS (n = 57)

Indicator	Value
Total testosterone level ng/ml, median (IQR)	0.48 (0.20)
Total testosterone level above 0.55 ng/ml, (n = 54*), n (%)	23 (42.6)
mFG score, median (IQR)	10.1 (5.2)
Acne grading (n = 55)**	
GAGS score (n = 55), median (IQR)	14.8 (8.7)
No acne, n (%)	1 (1.8)
Mild acne, n (%)	36 (65.5)
Moderate acne, n (%)	16 (29.1)
Severe acne, n (%)	2 (3.6)
PCOM, n (%)	20 (35.1)

* In three patients, due to technical failure, the indicator was not determined at the time of the publication. They were contacted later, the level was determined, it was within the laboratory standard for everyone. ** Due to an error, in two patients, the severity of acne was not evaluated. After this error, the design of the data collection questionnaire was changed to minimise the recurrence of such errors. These patients began using combined hormonal contraceptives and therefore no subsequent evaluation of acne was possible.

The current and previous clinical symptoms of PCOS in patients' mothers are shown in Table 3.2. A total of 19 mothers (33.3 %) reported hirsutism and menstrual irregularities, but only six (10.5 %) claimed that they still experienced these symptoms. There was a significant change in the incidence of symptoms over the course of life, in particular the incidence of hirsutism, which increased seven-fold (from 6.3 % to 43.8 %). By contrast, the incidence of acne decreased by about two thirds (51.1 % reported that they had had acne before, but only 15.6 % reported that they had acne presently). The incidence of menstrual irregularities remained relatively constant, with one-fifth of mothers reporting this symptom. Only two mothers (3.5 %) noted that they were currently diagnosed with PCOS. In addition, five (8.8 %) reported that a diagnosis of PCOS was previously confirmed, but it had been reviewed as symptoms resolved naturally. None of the mothers indicated that they were currently receiving PCOS therapy. Only four mothers (7.0 %) noted that they had difficulty getting pregnant – one took more than a year, the other three became pregnant within five years. None of the respondents reported that they had used assisted reproductive technologies.

Table 3.2

Current and past clinical symptoms of PCOS in patients' mothers (n = 57)

Indicator	Currently, n (%)	In the past, n (%)
Menstrual irregularities (n = 47)[a]	10 (21.3)	9 (19.1)
Acne (n = 45)[a]	7 (15.6)	23 (51.1)
Hirsutism (n = 48)[a]	21 (43.8)	3 (6.3)
Hyperandrogenemia (n = 49)[a]	4 (8.2)	4 (8.2)

[a] Number of responders who replied to the question.

Table 3.3 shows the body composition indicators of the participants of the study. Approximately half of the participants (50.9 % of daughters and 43.9 % of mothers) had an elevated BMI. One third of the patients (35.1 %) had

a waist-hip ratio above the critical value of 0.85. It was found that the mothers' BMI could be used to statistically reliably predict the BMI percentile of the daughter (F (1,45) = 4.35; p = 0.04). The mother's BMI explained 6.7 % of the daughter's BMI percentile. An analysis of the relationship between the mother's BMI and the daughter's waist-hip ratio showed that the mother's BMI can statistically reliably predict the daughter's waist-hip ratio (F (1,44) = 8.15; p < 0.0005) and explained 15.6 % of its variations (corrected R^2 = 13.7 %).

Table 3.3

Body composition indicators of participants (n = 57)

Indicator	PCOS patients	Mothers
BMI, kg/m^2, median (IQR)	25.7 (5.6)	26.9 (6.5)
BMI, median percentile (IQR)	73.5 (29.6)	–*
Overweight, n (%)	6 (10.5)	11 (19.3)
Obesity, n (%)	23 (40.4)	14 (24.6)
Waist-hip ratio, median (IQR)	0.84 (0.10)	–**
Waist-hip ratio > 0.85, n (%)	20 (35.1)	–**

* Not used for BMI evaluation in adults. ** Not evaluated in this study.

No statistically significant differences in mFG and GAGS scales or incidence of PCOM were observed in daughters, after their mothers were divided into groups based on the presence of current or past PCOS symptoms (acne, hirsutism, menstrual irregularities) (see Table 3.4).

Table 3.4

Relationship between current and past symptoms of PCOS in mothers and daughters' PCOS symptoms

Daughter´s indicators	Mother´s acne (n = 49)*				Mother´s hirsutism (n = 48)*				Mother´s menstrual irregularities (n = 47)*			
	Never	In the past	Currently	*p* value	Never	In the past	Currently	*p* value	Never	In the past	Currently	*p* value
P PCOM, *n* (%)	4 (8.2)	11 (22.4)	2 (4.1)	0.29	9 (18.8)	1 (2.1)	7 (14.6)	0.65	12 (25.5)	3 (6.4)	2 (4.3)	0.42
GAGS score, mean (SD)	13.2 (9.1)	16.4 (7.4)	16.4 (11.6)	0.45	16.8 (7.8)	9.3 (12.7)	13.0 (7.6)	0.25	16.3 (8.8)	14.0 (6.7)	12.9 (9.1)	0.46
mFG score, mean (SD)	11.4 (6.1)	10.7 (5.6)	10.3 (3.9)	0.96	10.4 (6.6)	8.3 (4.0)	11.0 (5.4)	0.61	10.6 (6.3)	11.3 (4.1)	9.3 (4.9)	0.47

* Number of respondents who replied to the specific question.

3.2 Study Section II: Genetic Aspects

The results obtained in this section of the study are divided into separate subsections according to the question being studied – a study of genetic variations associated with HPO axis activity; a study of genetic variations associated with non-classical form of CAH;

3.2.1 A study of genetic variations associated with HPO Axis activity in adolescents with PCOS

This subsection of the study includes 63 patients with PCOS, 22 patients in the risk group and 67 control group representatives. Table 3.5 shows clinical characteristics in PCOS patients, risk group patients and control group representatives. There were no statistically significant differences in gynaecological age (difference between age at enrolment and *menarche* age) ($p = 0.411$). In comparison to the control group, the PCOS group and the risk group patients had a greater number of adolescents with statistically significantly worse metabolic health indicators (higher BMI, higher waist-hip ratio, higher percentage of obese participants). BMI and waist-hip ratio were statistically significantly higher in PCOS and risk group patients than in the control group ($p < 0.001$ and $p = 0.001$, respectively). More details on group differences in statistically significant indicators are shown in Table 3.6. Overall, acne was more common in PCOS and risk group patients than in the control group ($p < 0.001$). Moderate acne, compared to other degrees of acne, was most common in PCOS patients ($p = 0.002$).

Table 3.5

Characteristics of participants included in the study subsection on genetic variations associated with activity of HPO Axis

Indicator	PCOS patients (n = 63)	Risk group patients (n = 22)	Control group representatives (n = 67)	*p* value
Gynaecological age, years, median (IQR)†	3.0 (2.0)	4.0 (2.0)	4.0 (1.0)	0.441
BMI, median, percentile (IQR)	89.9 (48.0)	75.4 (39.8)	55.0 (47.0)	< **0.001**
Number of subjects with BMI above 85th percentile, n (%)	31 (49.2)	6 (27.3)	9 (13.4)	< **0.001**
Waist-hip ratio, median (IQR)	0.82 (0.13)	0.80 (0.06)	0.76 (0.06)	**0.001**
mFG score, median (IQR)	9.0 (6.0)	8.0 (5.0)	1.0 (2.0)	< **0.001**
GAGS score, mean (SD)	14.5 (9.1)	10.9 (8.8)	7.0 (6.0)	< **0.001**
No acne, n (%)	1 (1.9)	2 (10.0)	6 (15.0)	Reference category
Mild acne, n (%)	35 (64.8)	13 (65.0)	31 (77.5)	0.147
Moderate acne, n (%)	16 (29.6)	4 (20.0)	3 (7.5)	**0.002**
Severe acne, n (%)	2 (3.7)	1 (5.0)	0 (0)	0.091
PCOM, n (%)	22 (34.9)	3 (13.6)	5 (7.5)	**0.001**

† Gynaecological age: the difference between age at inclusion and *menarche* age; IQR – interquartile range of the median; the statistically significant *p* values are highlighted in bold.
The differences between statistically significantly different clinical parameters are detailed in Table 3.6.

Table 3.6

Detailed analysis of statistically significantly different clinical parameters among participants of the study subsection

	BMI, median, percentile (IQR), *p*	**Number of subjects with BMI above > 85th percentile, *p***	**Waist-hip ratio, median (IQR), *p***	**mFG score, median (IQR), *p***	**GAGS score, mean (SD), *p***	**PCOM frequency, *p* value**
PCOS vs. control group representatives	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**
PCOS vs. risk group patients	0.178	**0.036**	0.450	0.186	0.116	**0.046**
Risk group patients vs. control group representatives	**0.020**	0.192	**0.008**	**< 0.001**	**0.028**	0.760

IQR – interquartile range of the median; the statistically significant *p* values are highlighted in bold.

The frequencies of carriers of the SNV genotypes studied among participants are shown in Table 3.7. There were no statistically significant differences in genotype frequency between PCOS, risk group and control groups.

Table 3.7

Frequencies of carriers of SNV genotypes studied among PCOS patients, risk group patients and control groups

SNV	Genotypes, their combinations	*FSHR* rs2349415, n (%)	*FSHR* rs6166, n (%)	*FSHR* rs6165, n (%)	*ESR2* rs4986938, n (%)	*GnRHR* rs104893837, n (%)	*LHCGR* rs2293275, n (%)
PCOS patients (n = 63)	HH	25 (39.7)	18 (28.6)	15 (23.8)	14 (22.2)	59 (93.7)	26 (41.3)
	Hh	28 (44.4)	30 (47.6)	36 (57.1)	38 (60.3)	4 (6.3)	26 (41.3)
	hh	10 (15.9)	15 (23.8)	12 (19.1)	11 (17.5)	0 (0)	11 (17.4)
R Risk group patients (n = 22)	HH	7 (31.8)	7 (31.8)	5 (22.7)	5 (22.7)	21 (95.5)	6 (27.3)
	Hh	11 (50.0)	12 (54.5)	13 (59.1)	13 (59.1)	1 (4.5)	11 (50.0)
	hh	4 (18.2)	3 (13.6)	4 (18.2)	4 (18.2)	0 (0)	5 (22.7)
Control group representatives (n = 67)	HH	20 (29.9)	24 (35.8)	21 (31.3)	10 (14.9)	64 (95.5)	22 (32.8)
	Hh	33 (49.3)	29 (43.3)	31 (46.3)	49 (73.1)	3 (4.5)	31 (46.3)
	hh	14 (20.9)	14 (20.9)	15 (22.4)	8 (11.9)	0 (0)	14 (20.9)
Frequency distribution of genotypes between groups (*p* value)		0.81	0.77	0.73	0.58	0.89	0.77

HH – carriers of the major allele in a homozygous state; Hh – allele carriers in heterozygous state; hh – carriers of the minor allele in a homozygous state.

The values for phenotypically significant clinical parameters in PCOS patients, depending on their genotype, are shown in Table 3.8. The carrier of the minor allele *ESR2* rs4986938 in a homozygous state had higher median total blood testosterone level (0.68 ng/ml (IQR 0.2)), compared to heterozygous individuals (0.38 ng/ml (IQR 0.38)) and major allele carriers at homozygous state (0.38 ng/ml (IQR 0.26)) ($p = 0.04$). Carriers of *ESR2* rs4986938 allele at heterozygous state had lower scores on the mFG scale, but the statistical confidence level was not reached in this indicator ($p = 0.056$).

Table 3.8

Phenotype - genotype association among PCOS patients (n = 63)

SNV	**Genotypes, their combi-nations**	***FSHR* rs2349415**	***FSHR* rs6166**	***FSHR* rs6165**	***ESR2* rs4986938**	***GNRHR* rs104893837**	***LHCGR* rs2293275**
mFG score, median (IQR)	HH	10.0 (6.0)	9.0 (7.5)	9.0 (7.0)	10.0 (6.0)	9.5 (6.0)	9.0 (6.0)
	Hh	9.0 (6.0)	9.5 (6.0)	9.0 (6.0)	8.0 (7.3)	10.0	10.5 (5.8)
	hh	12.5 (9.8)	14.0 (10.5)	15.0 (8.0)	11.0 (6.5)	0 (0)	14.0 (12.0)
	*p**	0.44	0.19	0.12	0.056	0.99	0.92
BMI percentile, median (IQR)	HH	82.2 (64.1)	95.6 (60.6)	95.6 (63.9)	90.4 (49.9)	90.4 (48.0)	71.6 (56.8)
	Hh	93.1 (45.0)	76.7 (46.6)	76.7 (48.0)	89.0 (53.9)	74.9	89.0 (49.4)
	hh	91.4 (26.1)	81.8 (60.4)	94.0 (41.4)	96.6 (30.8)	0 (0)	98.9 (15.9)
	*p**	0.88	0.93	0.71	0.36	0.75	0.27
Waist-hip ratio, median (IQR)	HH	0.84 (0.19)	0.83 (0.17)	0.84 (0.16)	0.81 (0.09)	0.83 (0.13)	0.81 (0.11)
	Hh	0.84 (0.11)	0.82 (0.13)	0.82 (0.14)	0.81 (0.14)	0.88	0.84 (0.17)
	hh	0.77 (0.17)	0.84 (0.10)	0.84 (0.13)	0.85 (0.05)	0 (0)	0.88 (0.09)
	*p**	0.65	0.89	0.94	0.63	0.97	0.19

Table 3.8 continued

SNV	Genotypes, their combi-nations	*FSHR* rs2349415	*FSHR* rs6166	*FSHR* rs6165	*ESR2* rs4986938	*GNRHR* rs104893837	*LHCGR* rs2293275
Total testosterone level, median (IQR)	HH	0.42 (0.37)	0.53 (0.32)	0.45 (0.49)	0.38 (0.26)	0.44 (0.40)	0.35 (0.37)
	Hh	0.48 (0.52)	0.38 (0.44)	0.40 (0.41)	0.38 (0.38)	0.33	0.59 (0.36)
	hh	0.40 (0.37)	0.44 (0.42)	0.57 (0.43)	0.68 (0.20)	0 (0)	0.67 (0.66)
	*p**	0.76	0.80	0.89	**0.04**	0.47	0.13
GAGS score, mean (SD)	IH	15.0 (10.7)	10.4 (7.8)	12.4 (8.8)	14.0 (10.9)	14.4 (8.9)	14.3 (9.0)
	Hh	13.8 (7.6)	16.5 (9.4)	15.1 (9.3)	13.3 (15.3)	12.5 (16.3)	15.6 (9.6)
	hh	13.5 (8.1)	14.0 (8.4)	13.4 (9.2)	13.4 (16.5)	0 (0)	10.6 (7.6)
	*p**	0.99	0.12	0.70	0.71	0.31	0.60
PCOM, n (%)	HH	9 (40.9)	5 (22.7)	4 (18.2)	6 (27.3)	21 (95.5)	9 (40.9)
	Hh	10 (45.5)	10 (45.5)	12 (54.5)	13 (59.1)	1 (4.5)	9 (40.9)
	hh	3 (13.6)	7 (31.8)	6 (27.3)	3 (13.6)	0 (0)	4 (18.2)
	*p**	0.849	0.371	0.492	0.791	1.000	0.814

HH – carriers of the major allele in a homozygous state; Hh– allele carriers in heterozygous state; hh – carriers of the minor allele in a homozygous state;
* *p* value indicates whether there was a statistically significant difference between carriers of different genotypes (HH vs. Hh vs. hh).

Table 3.9 shows the relationship between phenotypic manifestations of PCOS and the status of a carrier of the major allele in homozygous state (HH) and status of a carrier of a minor allele in homozygous or heterozygous state (Hh and hh) in the PCOS group. Median total testosterone levels in blood were found to be statistically significantly higher for carriers of the *LHCGR* rs2293275 minor allele (0.61 ng/ml (IQR 0.42)) than for the carriers of the major allele at homozygous state (0.35 ng/ml (IQR 0.37)) ($p = 0.044$). Carriers of the *LHCGR* rs2293275 minor allele (Hh and hh) also tended to have a higher waist-hip ratio

compared to the carriers of the major allele in the homozygous state, however, the statistical confidence level was not reached ($p = 0.08$). Carriers of the *FSHR* rs6166 minor allele also showed a tendency to have an increased incidence of acne (higher GAGS score), but it did not reach the statistical confidence level ($p = 0.084$).

Table 3.9

Relationship between phenotypic manifestations of PCOS and the carrier status of the major allele (HH) and the minor allele (Hh and hh) in PCOS group (n = 63)

SNV	Genotypes, their combi-nations	*FSHR* rs2349415	*FSHR* rs6166	*FSHR* rs6165	*ESR2* rs4986938	*GNRHR* rs104893837	*LHCGR* rs2293275
mFG score, median (IQR)	HH	10.0 (6.0)	9.0 (7.5)	9.0 (7.0)	10.0 (6.0)	10.0 (6.0)	9.0 (6.0)
	Hh and hh	9.5 (7.8)	10.0 (7.0)	10.0 (6.0)	8.0 (7.0)	10.0	11.5 (6.3)
	*p**	0.84	0.60	0.64	0.07	0.99	0.71
BMI percentile, median (IQR)	IH	82.2 (64.1)	95.6 (60.6)	95.6 (63.9)	90.4 (49.9)	90.4 (48.0)	71.6 (56.8)
	Hh and hh	92.3 (41.2)	76.7 (48.1)	83.7 (48.1)	89.4 (47.1)	74.9	94.4 (44.5)
	*p**	0.73	0.73	0.79	0.92	0.75	0.37
Waist-hip ratio, median (IQR)	HH	0.84 (0.19)	0.83 (0.17)	0.84 (0.16)	0.81 (0.09)	0.84 (0.12)	0.81 (0.11)
	Hh and hh	0.83 (0.12)	0.84 (0.13)	0.83 (0.14)	0.83 (0.12)	0.88	0.86 (0.11)
	*p**	0.36	0.90	0.98	0.45	0.97	0.08
Total testosterone level, median (IQR)	HH	0.42 (0.37)	0.53 (0.32)	0.45 (0.49)	0.38 (0.26)	0.45 (0.40)	0.35 (0.37)
	Hh and hh	0.46 (0.47)	0.38 (0.42)	0.43 (0.39)	0.47 (0.42)	0.33	0.61 (0.42)
	*p**	0.90	0.50	0.74	0.40	0.47	0.044
GAGS score, mean (SD)	HH	14.9 (10.7)	10.4 (7.8)	12.4 (8.6)	14.0 (10.9)	14.5 (9.0)	14.3 (9.0)
	Hh and hh	14.0 (7.6)	16.3 (9.1)	15.1 (9.2)	13.3 (8.7)	12.5 (16.3)	14.5 (9.4)
	*p**	0.902	0.084	0.434	0.476	0.312	0.902

Table 3.9 continued

SNV	**Genotypes, their combi-nations**	***FSHR* rs2349415**	***FSHR* rs6166**	***FSHR* rs6165**	***ESR2* rs4986938**	***GNRHR* rs104893837**	***LHCGR* rs2293275**
PCOM, n (%)	HH	9 (40.9)	5 (22.7)	4 (18.2)	6 (27.3)	21 (95.5)	9 (40.9)
	Hh and hh	13 (59.1)	17 (77.3)	18 (81.8)	16 (72.7)	1 (4.5)	13 (59.1)
	*p**	0.879	0.389	0.747	0.566	1.000	0.792

HH – carriers of the major allele in a homozygous state; HH – carriers of the allele in a heterozygous state; HH – carriers of the minor allele in a homozygous state; * *p* value indicates whether there was a statistically significant difference between the carrier of the different alleles (HH vs. Hh vs. hh) within the given indicator; the statistically significant *p* values are highlighted in bold.

3.2.2 A study of genetic variations associated with non-classical form of congenital adrenal hyperplasia in adolescents with PCOS

This subsection of the study includes 55 patients with PCOS, 23 risk group patients and 49 representatives of the control group. The characteristics of the participants are shown in Table 3.10. The gynaecological age did not differ significantly among participants ($p = 0.411$). A more detailed comparison between the groups regarding the statistically relevant clinical variables is shown in Table 3.11 below. Overall, patients with PCOS had poorer metabolic health parameters (BMI percentile, waist-hip ratio, incidence of excessive weight), however, no differences were observed between PCOS patients and risk group patients.

Table 3.10

Characteristics of participants of the study subsection on the genetic variations associated with non-classical form of CAH

Indicator	PCOS patients (n = 55)	Risk group patients (n = 23)	Representatives of the control group (n = 49)	*p* value
Gynaecological age, median (IQR)	3.0 (2.0)	4.0 (2.0)	4.0 (1.0)	0.441
mFG score, median (IQR)	9.0 (6.0)	7.5 (6.3)	2.0 (2.0)	< **0.001**
PCOM, n (%)	19 (34.5)	4 (17.4)	2 (4.1)	**0.001**
BMI, median, percentile (IQR)	89.4 (46.1)	75.6 (38.3)	45.1 (45.8)	< **0.001**
Waist-hip ratio, median (IQR)	0.81 (0.1)	0.81 (0.1)	0.76 (0.1)	**0.001**
GAGS score, mean (SD)	16.0 (17.5)	8.0 (9.0)	6.0 (11.0)	< **0.001**
Testosterone level in blood (ng/mL), median (IQR)	0.4 (0.4)	0.3 (0.3)	NN*	0.547
DHEA-SO_4 level in blood (μg/mL), median (IQR)	221.5 (177.2)	248.0 (190.7)	NN	0.232
Androstendione level in blood (ng/mL), median (IQR)	3.1 (2.7)	2.7 (1.8)	NN	0.287
17-OH progesterone level in blood (ng/mL), median (IQR)	1.1 (0.7)	1.3 (1.0)	NN	0.972

* NN – not specified; statistically significant *p* values are highlighted in bold. Statistically significant differences in clinical parameters are shown in Table 3.11 in more detail.

Table 3.11

Detailed analysis of statistically significant differences in clinical parameters between the sub-section groups of the study

	BMI, median, percentile (IQR), *p*	**Number of participants with BMI above 85th percentile, *p***	**Waist-hip ratio, median (IQR), *p***	**mFG score, median (IQR), *p***	**GAGS score, mean (SD), *p***	**PCOM frequency, *p value***
PCOS vs. control group representatives	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**
PCOS vs. risk group patients	0.272	0.072	0.599	0.225	**0.045**	0.132
Risk group patients vs. control group representatives	0.007	0.030	0.003	< 0.001	**0.115**	0.084

IQR – interquartile range of the median; statistically significant *p* values are highlighted in bold.

Altered variants of the *CYP21A2* gene were observed in three patients in the PCOS group, two in the risk group and three in the control group (see Table 3.12). These changes were not observed in a homozygous state, in any of the subjects in the study. Two of the control group representatives were compound heterozygote carriers of two variations (IVS2–12A > G and −113G > A). The frequency of allele carriers was not statistically different between groups, however, variation of I172N was observed only in patients in the PCOS group. Table 3.13 shows the clinical and biochemical characteristics of the eight participants of the study, that had altered genetic variants. In general, no common clinical and biochemical signs were observed among these participants.

Table 3.12

Incidence of *CYP21A2* gene abnormalities in subjects

Allelic variant of *CYP21A2*	**PCOS patients (n = 55)**	**Risk group patients (n = 23)**	**Control group (n = 49)**	***p* value**
IVS2-12A > G, n	2	1	1	0.831
−113G > A, n	0	1	0	0.181
I172N, n	1	0	0	1.000
IVS2–12A > G + −113G > A, n	0	0	2	0.319

Table 3.13

Characteristics of carriers of modified *CYP21A2* gene variants

Indicator / participant of the study	**No 1**	**No 2**	**No 3**	**No 4**	**No 5**	**No 6**	**No 7**	**No 8**
Group	PCOS Group	PCOS Group	PCOS Group	Risk group patient	Risk group patient	Control group	Control group	Control group
Altered variants of *CYP21A2**	IVS2–12A > G	IVS2–12A > G	I172N	−113G > A	IVS2–12A > G	IVS2–12A > G	IVS2–12A > G + −113G > A	IVS2–12A > G + −113G > A
Age, years	18	17	14	18	16	16	17	16
BMI, percentile	31.7	18.8	31.1	24.6	29.7	22.8	24.5	26.7
Waist-hip ratio	0.93	0.81	0.99	0.82	0.85	0.84	0.69	0.86
mFG score	28	9	8	5	26	2	0	0
Menarche age, years	12	13	12	12	12	13	14	14
Menstrual cycle, days	30–90	20–50	90–360	28	28	30	22	21
GAGS score	18	24	31	24	9	13	0	12

Table 3.13 continued

Indicator / participant of the study	No 1	No 2	No 3	No 4	No 5	No 6	No 7	No 8
Testosterone levels in blood, ng/mL	0.59	0.63	0.78	0.64	0.33	NN**	NN	NN
DHEA-SO_4 level in blood, μg/mL	208.0	90.6	180.0	298.0	313.0	NN	NN	NN
Androstendione level in blood, ng/mL	4.10	4.48	5.77	5.88	3.16	NN	NN	NN
17-OH progesterone level in blood, ng/mL	1.77	1.45	1.48	1.05	0.99	NN	NN	NN
LH/FSH ratio	1.36	1.62	2.58	0.67	0.35	NN	NN	NN
Fasting glucose, mmol/L	6.11	4.70	4.94	4.65	5.07	NN	NN	NN

* Allele carriers in heterozygous state; ** NN – not specified.

The relationship between the genotype of study participants and their clinical parameters is shown in Table 3.14. The GAGS score of the carrier of I172N in the PCOS group (one out of 55) was statistically significantly higher than in PCOS patients without this variant ($p = 0.038$). In compound heterozygous subjects (IVS2–12A > G and −113G > A) the BMI was statistically significantly higher in the control group (two out of 49) than in control group without the genotype ($p = 0.036$). Risk group patients who are carriers of the IVS2–12A > G allele, were observed to have higher mFG scores, but this trend did not reach the level of statistical significance ($p = 0.091$).

Table 3.14

Relationship between allelic variants and clinical characteristics

Group: PCOS patients (*n* = 55)			
Allelic variant: I172N			
Indicator	**Carriers of variant (*n* = 1, 1.8 %)**	**Unchanged genotype (*n* = 54, 98.2 %)**	***p* value**
mFG scale, median (IQR)	8	9.0 (6.0)	0.741
PCOM, *n* (%)	0 (0)	19 (35.2)	0.627
BMI percentile, median (IQR)	99.5	89.2 (47.3)	0.353
Waist-hip ratio, median (IQR)	0.99	0.81 (0.14)	0.192
GAGS scale, median (IQR)	31	15.5 (16.8)	**0.038**
Testosterone (ng/mL), median (IQR)	0.78	0.40 (0.39)	0.235
Allelic variant: IVS2–12A > G			
Indicator	**Carriers of variant (*n* = 2, 3.6 %)**	**Unchanged genotype (*n* = 53, 96.4 %)**	***p* value**
mFG scale, median (IQR)	18.5	9.0 (6.0)	0.274
BMI percentile, median (IQR)	59.4	89.4 (45.3)	0.659
Waist-hip ratio, median (IQR)	0.87	0.81 (0.14)	0.462
GAGS scale, median (IQR)	21.0	15.0 (17.0)	0.395
Testosterone (ng/mL), median (IQR)	0.61	0.39 (0.40)	0.480
Allelic variant: IVS2–12A > G			
Indicator	**Carriers of variant (*n* = 1, 4.3 %)**	**Unchanged genotype (*n* = 22, 95.7 %)**	***p* value**
mFG scale, median (IQR)	26	7.0 (6.0)	0.091
BMI percentile, median (IQR)	98.3	75.4 (39.2)	0.348
Waist-hip ratio, median (IQR)	0.85	0.80 (0.06)	0.273
GAGS scale, median (IQR)	9	8.0 (9.5)	0.857
Testosterone (ng/mL), median (IQR)	0.33	0.37 (0.35)	1.000
Allelic variant: −113G > A			
Indicator	**Carriers of variant (*n* = 1, 4.3 %)**	**Unchanged genotype (*n* = 22, 95.7 %)**	***p* value**
mFG scale, median (IQR)	5	8.0 (6.0)	0.545
BMI percentile, median (IQR)	82.0	75.4 (40.3)	0.783
Waist-hip ratio, median (IQR)	0.82	0.80 (0.07)	0.910
GAGS scale, median (IQR)	24	8.0 (7.8)	0.190
Testosterone (ng/mL), median (IQR)	0.64	0.32 (0.28)	0.471

Table 3.14 continued

Group: Control group (*n* = 49)			
Allelic variant: IVS2–12A > G			
Indicator	**Carriers of variant (*n* = 1, 2.0 %)**	**Unchanged genotype (*n* = 48, 98.0 %)**	***p* value**
GAGS scale, median (IQR)	13	6.0 (10.3)	0.318
BMI percentile, median (IQR)	74.0	45.6 (46.5)	0.429
Wasit-hip index, median (IQR)	0.84	0.75 (0.06)	0.168
Allelic variant: −113G > A + IVS2–12A > G			
Indicator	**Carriers of variant (*n* = 2, 4.1 %)**	**Unchanged genotype (*n* = 47, 95.9 %)**	***p* value**
GAGS scale, median (IQR)	3.5	6.0 (11.5)	0.382
BMI percentile, median (IQR)	89.3	45.1 (45.8)	**0.036**
Waist-hip ratio, median (IQR)	0.77	0.76 (0.06)	0.957

The statistically significant *p* values are highlighted in bold.

3.3 Study Section III: study on health-related quality of life and binge eating for adolescents with PCOS

63 adolescents with PCOS and 66 representatives of the control group were included in this subsection. The median age was 16.0 years (IQR 2.0) for patients with PCOS and 17.0 years (IQR 1.0) for control group representatives. The difference was not statistically significant ($p = 0.067$). The gynaecological age for both groups was 4 years ($p = 0.240$). Clinical signs of hyperandrogenism, PCOM and body composition for all subjects are summarised in Table 3.15 PCOS patients had statistically significantly higher hirsutism levels ($p < 0.001$) and severity of acne ($p < 0.001$). PCOS patients also had a higher BMI ($p < 0.001$) and waist-hip ratio ($p < 0.001$). Half of PCOS patients (49.2 %) were overweight or obese, and slightly more than one third (34.9 %) had a waist-hip ratio higher than the critical value of 0.85.

Table 3.15

Characteristics of participants of the study subsection on HRQOL and binge eating in adolescents with PCOS

Indicator	PCOS group (n = 63)	Control group (n = 66)	*p* value
mFG score, median (IQR)	10.0 (6.0)	1.0 (2.3)	< **0.001**
PCOM, n (%)	22 (34.9)	4 (6.1)	< **0.001**
BMI, median percentile (IQR)	89.9 (46.7)	46.9 (46.3)	< **0.001**
Overweight, n (%)	6 (9.5)	4 (6.1)	0.090
Obesity, n (%)	25 (39.7)	3 (4.5)	< **0.001**
Waist-hip ratio, median (IQR)	0.83 (0.1)	0.75 (0.8)	< **0.001**
Waist-hip ratio above 0.85, n (%)	22 (34.9)	5 (7.6)	< **0.001**
Severity of acne	**(n = 60)**	**(n = 64)**	***p* value**
GAGS score, median (IQR)	15.5 (16.0)	6.0 (10.0)	< **0.001**
No acne, n (%)	2 (3.3)	7 (10.9)	–
Mild acne, n (%)	38 (63.3)	54 (84.4)	0.313
Moderate acne, n (%)	18 (30.0)	3 (4.7)	**0.002**
Severe acne, n (%)	2 (3.3)	0	0.109

Statistically significant *p* values are highlighted in bold.

The results of PCOSQ and the binge eating scale are shown in Table 3.16. In all subscales except menstrual disorders, the results were statistically significantly lower in the PCOS group than in the control group. Although the infertility subscale was statistically significantly lower in the PCOS group compared to the control group, both were still high (above six points on the seven-point scale). This indicates that this subscale is not so relevant for this sample of adolescents. There was no difference between the PCOS and control groups in the median binge eating score (p = 0.727), and the distribution of participants according to the levels of severity of binge eating did not differ.

Table 3.16

PCOSQ and binge eating scale results for PCOS group and control group

Indicator	Members of the PCOS group	Representatives of the control group	*p* value
PCOSQ results	**(n = 60*)**	**(n = 66)**	
PCOSQ total score, median (IQR)	4.9 (1.5)	5.8 (0.9)	< **0.001**
Subscale: emotions, median (IQR)	5.1 (1.3)	6.3 (0.5)	< **0.001**
Subscale: body hair, median (IQR)	4.0 (2.7)	6.4 (1.4)	< **0.001**
Subscale: weight concerns, median (IQR)	4.3 (3.7)	5.6 (2.1)	**0.013**
Subscale: infertility concerns, median (IQR)	6.3 (1.6)	6.8 (0.5)	< **0.001**
Subscale: menstrual problems, median (IQR)	4.5 (1.3)	4.8 (2.1)	**0.350**
Binge eating scale results	**(n = 61*)**	**(n = 64*)**	***p* value**
Total score, median (IQR)	12 (14.5)	12 (17.0)	0.727
No or minimal binge eating, n (%)	38 (62.3)	41 (64.1)	0.455
Mild to moderate binge eating, n (%)	15 (24.6)	11 (17.2)	
Severe binge eating, n (%)	8 (13.1)	12 (18.8)	

* Patients who failed to answer to one or more question were not included in further analysis. Statistically significant *p* values are highlighted in bold.

The rate of sexually active adolescents was statistically significantly higher in the control group (58.7 % (37/63) than in the PCOS group (22.6 % (14/62) ($p < 0.001$). However, the age of beginning sexual activity was not statistically significant between the groups, the median age was 15.5 years (IQR 2) in the PCOS group and 16 years (IQR 1) in the control group ($p = 0.433$). In the PCOS group, the total number of PCOSQ points did not differ between participants who had entered into sexual relations and those who did not.

The relationship between PCOSQ subscales and the relevant objective patient parameters was assessed. A moderately strong, significant negative correlation between the mFG score and the body hair subscale of the PCOSQ scale was found in the whole sample of the study ($r_s = -0.627$; $p < 0.001$). In a separate analysis of the two subgroups of the study sample, the correlation

was maintained in the PCOS group ($r_s = -0.664$; $p < 0.001$) but it lost its statistical significance in the control group ($r_s = 0.007$; $p = 0.959$).

Another significant correlation of moderate strength was found between BMI percentile results and the weight domain in the whole study sample ($r_s = -0.633$; $p < 0.001$), the PCOS group ($r_s = -0.561$; $p < 0.001$) and the control group ($r_s = -0.524$; $p < 0.001$). Furthermore, a weak but significant correlation was found between BES scores and the weight domain in the whole study sample ($r_s = -0.288$, $p = 0.001$), the PCOS group ($r_s = -0.342$; $p = 0.007$) and the control group ($r_s = -0.287$; $p = 0.025$).

A multiple linear regression analysis was carried out to evaluate the independent variables predicting the total HRQOL (see Table 3.17). Three models were identified as statistically significant in the stepwise regression analysis. The total three variables (diagnosis of PCOS by itself, total score on the binge eating scale, the BMI percentile) accounted for 40.5 % of total HRQOL ($R^2 = 0.405$, $F(3,89) = 21.9$, $p = 0.013$). For more details see Table 3.17.

Table 3.17

HRQOL multiple linear regression analysis in PCOS patients and control group representatives

Independent variable	β	95 % CI	p	F	R^2	ΔR^2	*p*
Step 1							
Diagnosis / No diagnosis of PCOS	−1.162	−1.530; −0.794	< 0.001	39.4	0.294	0.302	< 0.001
Step 2							
Diagnosis / No diagnosis of PCOS	−1.117	−1.466; −0.769	< 0.001	27.9	0.369	0.080	0.001
Result in binge eating scale	−0.031	−0.049; −0.013	< 0.001				

Table 3.17 continued

Independent variable	β	95 % CI	F	R^2	ΔR^2		*p*
Step 3							
Diagnosis / No diagnosis of PCOS	−1.002	−1.352; −0.651	< 0.001	21.9	0.405	0.042	0.013
Result in binge eating scale	−0.27	−0.044; −0.009	0.004				
BMI percentile	−0.007	−0.013; −0.002	0.013				

β – non-standardised β coefficient; 95 % CI – 95 % confidence interval; F – Fisher test; R^2 – R square; ΔR^2 square change.

4 Discussion

4.1 Relationship between the clinical signs in PCOS patients and their mothers

The results of the study show a number of poor metabolic health indicators in the sample of the study. The frequency of an increased BMI in the study sample coincides with data from similar studies (36). The frequent occurrence of increased weight among PCOS patients and excess weight as a significant sign of the syndrome is also indicated by the fact that the incidence of an increased BMI in the sample of this study was almost five times higher than in the population of 15-year-old girls in Latvia (37). The discovery that an elevated waist-hip ratio (central obesity indicator), which is a risk factor for cardiovascular disease, type 2 diabetes development and metabolic syndrome, is present in a third of our sample of PCOS patients, is worrying. At the same time, in our sample, this rate is lower than reported in a study conducted by Leibel and colleagues in the US, which found central obesity in 72 % of adolescents with a PCOS diagnosis (38). This may be partly explained by an increased incidence of elevated BMI in the general girl population. In the USA, the rate is 21 % (39), and in Latvia – 10 % (37). The high incidence of obesity among adolescents with PCOS suggests that healthcare professionals should also screen overweight patients for signs of PCOS (e.g. hirsutism, menstrual irregularity). In order to evaluate central obesity, special attention should also be paid to the waist-hip ratio. It would also be important to educate patients and their families about possible future metabolic diseases and to ensure regular evaluation of signs of metabolic syndrome.

In this study, the relationship between the mothers' BMI and the daughters' BMI and waist-hip ratio is consistent with the observations in other studies, both in the general population (40, 41) and in adults with PCOS (42, 43). Regarding adolescents with PCOS, unfortunately, only a small number of studies

have been conducted. The research by Leibel et al. (38) did not measure the correlation of these variables between mothers and daughters; however, those authors did find that BMI and waist–hip ratio were higher in adolescents with PCOS and their mothers. It has also been found that daughters whose mothers have PCOS have a higher BMI and waist-hip ratio (17, 44). It is impossible to exclude that the relationship between the BMI of mothers and daughters' BMI is explained not only by the diagnosis of PCOS, but also by the fact that the parameters of the composition of the body tend to be similar in different generations of the family. This is determined by both genetic, epigenetic and common environmental factors (e.g. dietary habits) (45). In general, the recurrence of symptoms of obesity in the family demonstrates the need to involve the whole family in the lifestyle modification programme, especially given that it is the most effective intervention in patients with PCOS (9).

The incidence of PCOS (12.3 %) in mothers in our study was lower than reported by Kahsar-Miller and colleagues at 24 % (16). However, it is not significantly different from figures reported by other researchers – 13.2 % in a study by Cheang and colleagues (46) and 14 % in a study by Leibel and colleagues (38). However, our study had a higher incidence of self-reported oligomenorrhea (40 %) and hirsutism (50 %), which is higher than in other studies (16, 38, 46, 47). The results of these studies are summarised in Table 4.1.

Given that there are no data available on the prevalence of PCOS in Latvia, it is difficult to determine whether the prevalence of PCOS among PCOS patients' mothers in our country is actually higher. However, the 12.3 % we found in our study is relatively high given that in the world as a whole, PCOS accounts for 4.6 % – 16.6 % of women in the general population (3, 48).

Table 4.1

Incidence of PCOS diagnosis and PCOS related symptoms in mothers of PCOS patients

Author, (PCOS patients / relatives / controls, n) Indicator	Kahsar-Miller and colleagues (195/78/119)	Cheang and colleagues (182/182)	Leibel and colleagues (36/35/21)	Torvinen and colleagues (43/183/412)	This study (57/57)
Confirmed diagnosis of PCOS, %	24[a]	13.2[b]	14[c]	NZ	12,3
Oligomenorrhea, %	17[d]	13.2	NZ	31.4	40.4
Hirsutism, %		23.6	NZ	18.6	50.1

[a] Defined as ovulation dysfunction coupled with hirsutism and / or hyperandrogenemia;
[b] defined as the treatment of irregular menstruations or infertility or difficulties with conception and clinical signs of hyperandrogenemia;
[c] defined as the diagnosis of irregular menstruations and PCOM or self-reported PCOS;
[d] reflects both hirsutism and oligomenorrhea together;
NZ – not known.

Differences in the incidence of clinical symptoms may be explained by ethnic differences as well as different diagnostic criteria used both in the PCOS determination and in the definition of the main clinical symptoms – oligomenorrhea and hirsutism. At the same time, when data are reported by respondents themselves (as in this study), the results may also be affected by the individual's different understanding of the meaning of excessive hair growth. An understanding of the menstrual cycle may also be inaccurate, as revealed in a study conducted in Latvia on parents' knowledge of sexual and reproductive health, especially women's menstrual cycle (49). Study participants and their mothers were encouraged to clarify questions if they were not clear to them.

In this study, there was no direct link between the symptoms of mothers with PCOS and the symptoms of her adolescent daughter. Therefore, according to our data, it is not possible to predict future symptoms of the daughter based on their mother's PCOS symptoms. At the same time, depending on the severity of

the mother's symptoms, this discovery can be both a consolation (if mother's symptoms are very pronounced) and a warning (if the mother's symptoms are mildly expressed) that it is of utmost importance to try and mitigate potential PCOS aspects, by following a healthy diet and lifestyle habits. It should also be borne in mind that it may take several years for PCOS symptoms to manifest completely, therefore monitoring of adolescent health and PCOS symptoms is very important.

The disadvantage of the study is that all data except the BMI are self-reported by mothers. This means that some information could be forgotten or reported incorrectly by mothers. In order to reduce this risk, the questionnaire was set up using the simplest possible terms, it was piloted in the appropriate population and was available both in Latvian and Russian, thus reducing the language barrier. In addition, the patient's visit to the clinic and the drawing of laboratory tests were adapted to the follicular phase of her menstrual cycle, so it was practically impossible to match the visit with the mother's menstrual cycle and also to perform all the same examinations. There is also a lack of healthy adolescents and their mothers, for the control group. The reason for the lack of control group is that healthy adolescents mostly visit a gynaecologist on their own, to address issues like contraception, testing of sexually transmitted infections, etc. This means that they usually do not want to inform mothers about the visit and invite them to take part in the study.

Despite the above shortcomings, the results of the study will help to understand this complex disease and can be used to improve the care of PCOS patients.

4.2 Genetic aspects

PCOS is a complex, multifactorial disease because of the interaction between environmental (especially family life), genetic and antenatal programming factors in its development(50). The results of genetic research in the PCOS group in global literature are also different and, at times, contradictory.

Genome-wide association studies (GWAS) have made a significant contribution to understanding the genetic origins of PCOS. However, they only explain about 10 % of the genetic aspects of PCOS (19). GWAS studies have a significant drawback – they often do not indicate a specific gene, but rather a genetic loci near which there are various genes that may be involved in PCOS pathogenesis. At the same time, these loci may also be regulators of other genes present in the remote areas of the genome. Therefore, GWAS does not provide reliable answers for the causality of the disease, only for the statistical probability that DNA loci changes are more common in these patients. GWAS also shows the association of only common genetic variants with the disease, the rarest variants may not reach the level of statistical significance in the large sample of participants included in the GWAS (50).

It is therefore essential to carry out studies of genetic associations, such as this one, in which it is possible to identify the genetic variants that cause the disease, or influence the risk of its development, or are present in genes in pathophysiological pathways of the disease. Such studies also allow targeted research of certain loci of specific genes, thus also identifying rare genetic variants associated with the disease. Research on genetic associations is essential for as many ethnic groups as possible, since the frequency of genetic variations and, consequently, the impact on the development of the disease may vary (51). Although the results of this study cannot be interpreted clearly, they can significantly contribute to the overall understanding of the disease and be included in a meta-analysis of studies of genetic associations.

Unfortunately, research into genetic associations also has significant shortcomings, which are particularly relevant for diseases such as PCOS with multifactorial origins. Even when identifying a particular genetic variant that is statistically significantly related to the disease or its symptom, it usually has little impact on the end outcome (confirmation of diagnosis) or the severity of the symptom. This requires an interaction analysis of several genetic variants, analysing several genetic variants at the same time, but it is difficult to include a large number of participants in the study in order to achieve a sufficient number of carriers of all genotype combinations in each subgroup. The classification of phenotypic manifestations (e.g. the threshold above which a patient is thought to have hirsutism or increased testosterone levels) is also important in the PCOS studies. A specific genetic marker may contribute to the expression of these indicators, but it alone does not lead to such a pronounced increase in the clinical score that it is already interpreted as increased (19).

It should also be noted that the minor allele frequency (MAF) of the SNVs included in the study, in *ESR2, LHCGR* and *FSHR* genes was close to 50 %, so the minor allele may vary between ethnic groups. The incidence of the analysed alleles is not known specifically in the Latvian population, which also makes it difficult to interpret the results.

4.2.1 A study of genetic variations associated with HPO Axis activity in adolescents with PCOS

There were no statistically significant differences in the frequency of genotypes of SNVs analysed in this study (*GNRHR* (rs104893837), *ESR2* (rs4986938), *LHCGR* (rs2293275) and *FSHR* (rs6166, rs6165, rs2349415) between PCOS patients, risk group patients and control group representatives. In the PCOS group, the homozygous carriers of the minor allele of *ESR2* rs4986938 had a statistically significantly higher total testosterone level than heterozygous

and homozygous allele carriers of this allele ($p = 0.04$). Total testosterone levels in blood were higher in PCOS patients who were carriers of the minor allele *LHCGR* rs2293275 SNV than those who were homozygous carriers of the common allele ($p = 0.044$). The genotype-phenotype relationship in other analysed SNVs (*GNRHR* (rs104893837) and *FSHR* (rs6166, rs6165, rs2349415) did not reach the statistical confidence level.

The *ESR2* gene is located in the 14q23.2 – q23.3 chromosome locus. It encodes the oestrogen receptor 2 – the steroid hormone receptor located in the nucleus of the cell. *ESR2* rs4986938 is present in the non-coding sequence of the gene. The *ESR2*-encoded product is involved in ovarian function, and studies have been conducted in PCOS patients that show differences in the expression levels of this receptor in ovarian cells both in patients and control group representatives (52, 53). Several research teams have analysed the relationship of this SNV with the development of PCOS in different ethnic populations (54–60).

Several research teams have also analysed the relationship of ESR2 rs4986938 to the severity of PCOS symptoms in different populations. The results obtained are contradictory. Most researchers have not found a relationship between the development of this SNV and PCOS symptoms (56–59). Douma and his colleagues found that the level of hyperandrogenism was lower in Tunisian patients, who were less likely to carry the allele, but this relationship disappeared with the Bonferroni correction (60). The pathogenetic mechanism of how variations in the *ESR2* gene could increase testosterone levels in blood is unclear. However, the *ESR1* gene study showed that lower expression of this receptor in PCOS patients could increase the conversion of androgen precursors to testosterone (61).

PCOS patients, carriers of the minor allele of *LHCGR* rs2293275, had statistically significantly higher total testosterone levels in blood than patients who did not have this allele. This indicator was not affected by the participant's

BMI, but the relationship disappeared when the waist-hip ratio was measured. The *LHCGR* gene is in 2p16.3 locus. It encodes the receptor that binds both LH and human chorionic gonadotropin. The gene is expressed in different cell types in the ovaries, including theca cells and granulosa cells. *LHCGR* transmits LH-mediated signals that are critical to the process of ovulation (62). Consequently, normal follicle maturation and ovulation are impaired in a situation where LH levels increase, as in PCOS. At the same time, aromatase expression increases, which promotes androgenic production (63). Data from genetic association studies published so far on the role of rs2293275 in the development of PCOS is contradictory (34, 65–70). A meta-analysis has also been carried out in which the homozygous minor allele carriers with European origins had 4.1 times higher risk of PCOS development (64).

The relationship of this SNV to the phenotypic manifestations of PCOS has also been analysed by other authors. A study by Thathapudi and colleagues in South India found that the carriers of the most common allele at homozygous state (GG) are associated with PCOS patients' BMI, waist-hip ratio, IR, LH levels and LH/FSH ratios. In contrast, in our study, carriers of A allele (minor allele) had higher testosterone levels in blood than in patients with no A allele in genotype. The study of Thathapudi did not report on testosterone levels in the participant's blood (67).

The study of patients with PCOS conducted in Egypt by El-Shal et al. revealed a relationship between *LHCGR* rs2293275 and a number of anthropometric and biochemical properties, including increased free androgen index and hirsutism scores (66). In a study of PCOS in European women (Netherlands), lower basal levels of follicular stimulating hormones were reported in patients who had neither *LHCGR* rs2293275 SNV nor 18insLQ SNV; however, no other clinical association was identified (58). In another study with European participants (study conducted in Sardinia), an association of this SNV with the clinical picture of PCOS was not identified (34).

For *FSHR* rs6166, rs6165 and rs2349415, no statistically significant differences in the frequency of genotypes or alleles were identified between PCOS patients, risk group patients and control group representatives. *FSHR* is a G-protein receptor located in the membrane of granulose ovarian cells. Several of the SNVs in this gene are described in relation to the development of PCOS. SNVs rs6166 and rs6165, which cause amino acid replacement in *FSHR*, are the most commonly studied. The results of genetic association studies regarding FSHR rs6166 and FSHR rs6165 role in PCOS development (55, 65, 69, 70–81) were summarised in three meta-analyses. Two of them found that carriers of the rs6166 AA genotype (the most common alleles in the homozygous state) have a lower risk of developing PCOS than carriers of the AG and GG genotypes (74, 82). In the meta-analysis of Chen and colleagues, no link between *FSHR* rs6166 and an increased risk of PCOS was found, including when taking into account a distribution of women on the basis of ethnicity – Caucasians and Asians (83). None of these meta-analyses found rs6165 to be related to the development of PCOS. The effects of these SNVs on increased levels of FSH in PCOS have been identified in separate studies, however other results on the effects of rs6166 and rs6165 on the phenotypic manifestations of PCOS are inconsistent and most do not reveal a relationship between the occurrence of these SNVs in genotype and the clinical or biochemical symptoms of the syndrome (63, 80, 82).

FSHR rs2349415 is found in the intron of the *FSHR* gene. Its relationship with the development of PCOS was found in GWAS (84) and family association (85) studies in the Chinese population, as well as confirmed by a meta-analysis carried out in the European population (86). Analysis of the relationship between rs2349415 and the clinical picture of PCOS showed that this SNV is related to the levels of SHBG in blood of PCOS patients (87). However, no other results on the association of this SNV to the clinical picture of PCOS have been published so far.

In this study no relationship was found between the development of *GNRHR* rs104893837 SNV and the development or associated symptoms of PCOS. During the complete sequencing of the *GNRHR* gene, this SNV was found to be the most common variation in the Latvian population (unpublished results). GnRHR transmits GnRH signals in the pituitary gland, ovaries and breast tissue. Its main role is the regulation of the menstrual cycle, stimulating the secretion of LH and FSH, as well as participating in the formation and atresia processes of *corpus luteum* (88). The main pathogenetic mechanism of PCOS is an increased pulse frequency of GnRH secretion (89). Several studies of genetic associations have revealed the relationship of SNV of the *GNRHR* gene to the development of PCOS and its symptoms (89–92).

Although this study did not reveal a statistically significant relationship between the SNVs studied and the development of PCOS or the phenotype of PCOS, higher testosterone levels in blood were observed in carriers of certain alleles (*ESR2* rs4986938, *LHCGR* rs2293275). There could be a number of explanations for this. This may be the effect of epigenetic factors (e.g. DNA methylation, histone modification, etc.). Similarly, these alleles may be relevant at individual level, but the observation is not replicated in larger groups. Family research is essential to study this aspect, and it should be continued in the future. The fact that the subjects are adolescents should also be taken into account – some of the risk group patients may develop a complete PCOS that could alter the results.

The differences between the results of this study and other similar studies may be related to the different ethnicities of participants in studies carried out elsewhere in the world. It is also important that the diagnostic criteria for PCOS have changed over time. In this study, we used the most up-to-date, refined and evidence-based diagnostic criteria that apply to the diagnosis in adolescent specifically. It is therefore likely that the results of previous studies will be reviewed and that future studies will show different results.

Risk group patients have also been included in this study. This is important both because they have symptoms affecting their health and well-being (hyperandrogenism) and because these adolescents have the potential to become PCOS patients in the next few years.

The limitation of the study is the small size of the sample. In the future, we plan on including additional participants, as well as monitor the population (particularly risk group patients) of this study in the long term.

4.2.2 A study of genetic variations associated with non-classical form of congenital adrenal hyperplasia in adolescents with PCOS

Our finding that the frequency of carriers genetic variations associated with non-classical form of CAH between PCOS patients is not higher than in the general population is consistent with most of the studies carried out in this field.

In this study, we found that the carrier of the I172N mutation in the PCOS group had a statistically significantly higher GAGS score than PCOS patients who did not have this variation (p = 0.038). Although the I172N allele has been included in several studies of *CYP21A2* gene variants in PCOS patients, only one study has reported characteristics of carriers of this particular allele. Ghanaati and colleagues reported one carrier of I172N with excess weight and oligomenorrhea as well as one carrier of compound heterozygous I172N and IVS2–12A > G variation with obesity, hyperandrogenism and secondary infertility, among PCOS patients. Unfortunately, no control group was included in this study, so there is no real clarity about the contribution of the I172N allele to the PCOS phenotype (97). In a study that included acne patients (n = 51), none of the patients had the I172N variation.

Carriers of compound heterozygous IVS2–12A > G and −113G > alleles had a statistically significantly higher BMI in the control group than in the control group without this variation (p = 0.036). This genotype was not detected

in either PCOS or risk group patients. Both of these variations are part of the non-coding *CYP21A2* gene and both are associated with severe cases of the illness (105). The IVS2–12A > G variation has been previously included in studies of a similar design, however, the exact effect of this variation on the PCOS phenotype has not been reported on. This variation in heterozygous state has also been detected in women with a diagnosis of acne, but its relationship to BMI was not mentioned in the publication of the study (106).

Our study included a 113G > A variation that has not been compared between PCOS patients and healthy control group representatives previously. It is located in the upstream region of the *CYP21A2* gene, where, in the −113 position, one nucleotide has been replaced by an upstream sequence specific to the *CYP21A2* pseudogene. Consequently, transcription of the *CYP21A2* gene is reduced by at least 20 % (107). To date, this variation (together with other promoter variations in this gene) in patients with PCOS has been described by Polat and colleagues. These variations have been detected by carrying out a full gene sequencing (104). Unfortunately, this study did not include representatives of a control group. Although we did not identify differences in the incidence of this variation between study groups, it was found in one patient in risk group and, in combination with IVS2–12A > G, in two control group representatives who, in turn, had a higher BMI than the control group representatives without this variation. The results suggest that studies on this combination of alleles and its clinical relevance should be continued. For example, the combination of *CYP21A2* gene variations with the G972R variant of the insulin receptor substrate-1 gene, which is more common in PCOS patients with elevated adrenal androgens (DHEA-SO_4 and 11-hydroxyandrostendione), has shown promising results in literature (99).

The methodology for genetic testing used in this study has been developed carefully and precisely, and molecular diagnostics of the *CYP21A2* gene were performed using a CAH mutation detection protocol. The study identified a wide range of *CYP21A2* pathogenic variants, and the test kit includes variant –113G > A, which has not been studied in PCOS patients in comparison to the control group. In most studies, all findings of genetic variants are presented together, but, in this study, the relationship of each variation with clinical signs of the disease is considered separately, providing additional insight into the impact on the clinical picture of these variations. It should be noted that, even though the protocol and method used in the study make it possible to identify a wide range of genetic variants, full sequencing of *CYP21A2* gene in the future would be useful to detect minor changes in the gene that cannot be detected using this method.

4.3 Health-related quality of life and study of binge eating for adolescents with PCOS

The finding of this study that adolescents with PCOS have a lower total HRQOL (4.9 (IQR 1.5) in the PCOS group versus 5.8 (IQR 0.9) points in the control group, in the PCOSQ questionnaire) coincides with a recent systemic review of studies analysing the HRQOL of adolescents with PCOS diagnosis (14). A total of 11 studies included 513 adolescents with PCOS and mostly showed lower quality of life compared to healthy adolescents. The main parameter influencing the quality of life of adolescents is body weight (23, 108–119). Overall, the research results are difficult to compare for a number of reasons. First, only a small part of the studies used the Rotterdam criteria to confirm the diagnosis of PCOS, and none of the studies used the latest evidence-based ESHRE criteria of 2018 (9). Second, studies have used a variety of instruments to assess the quality of life (see Table 4.5). Only two small studies

in adolescent age group have used the PCOS-specific tool, PCOSQ (111, 113). None of these studies involved a control group and the total score at the beginning of the study was not reported in the HRQOL questionnaire, thus it is not possible to directly compare the results obtained in these studies with those found in our study.

In this study, PCOS patients had the lowest scores in the body hair and weight subscales. The menstrual subscale also showed relatively low scores in both PCOS patients (4.5 (IQR 1.3) and control group (4.8 (IQR 1.3)), without reflecting statistically significant differences between the two groups ($p = 0.35$). This could be explained by the fact that menstruation is a new event in the life of adolescents, which can cause worries. The infertility subscale score was high in both study population groups, which is due to the fact that infertility is not such a significant issue at the age of adolescence in Latvia.

A negative body image, as indicated by the low score in the body hair subscale and the weight subscale, is typical for adolescent generations in Latvia also according to data from other researchers. A HBSC study in 2018 found that in the general adolescent population, 43 % of 15-year-old girls thought they were overweight, but only 13 % were indeed overweight (120).

Different results of PCOSQ subscales were found in samples of different countries. For example, young women in India noted infertility as the most important subscale, only then followed by body hair (113). Meanwhile, the weight subscale, followed by a subscale of menstrual problems, were the two most important subscales for adolescents in the US (111). This indicates cultural differences that should be taken into account when advising the patient. There is also evidence that it is important to use an illness-specific tool in clinical practice to assess the unique needs of each patient. A multivariate regression analysis carried out in this study, the BMI was found to be a significant variable that reduces the total HRQOL, which also coincides with the low score obtained in the PCOSQ subscale. BMI has been extensively studied as a key factor

influencing the overall HRQOL among PCOS patients (121). The relevance of this issue for adolescents with PCOS has only been addressed in a few studies (110, 111). By evaluating the results of their previous studies taking into account the BMI, Trent and colleagues found that differences in quality of life between PCOS patients and control group representatives disappear. At the same time, Harris-Glocker and colleagues found that the weight subscale had the lowest results in PCOS patients and that the quality of life significantly improved with weight loss.

There was no evidence of a higher incidence of binge eating in the PCOS group than in the control group. At the same time, binge eating was negatively predictive of the overall HRQOL score. This relationship was maintained even when the binge eating scale score in a multivariate regression analysis was controlled by the proportion of variation explained in the PCOS diagnosis. These results show the effect of disordered eating behaviour on HRQOL in adolescents. In other studies, disordered eating, particularly binge eating, was found to be more common in adults with PCOS than in their healthy peers (122). There is little data on this issue in the adolescent population. Mizgier and colleagues reported that problematic eating is 5 times more common in overweight PCOS patients 14–17 years of age compared to PCOS patients of normal weight and the same age. It should be noted that a healthy control group was not included in this study (123). In another study of binge eating in young women aged 15–24 years, there was no difference between PCOS patients and healthy patients, but only lean participants were included in the study (124). In contrast, other investigators also found a higher incidence of binge eating in PCOS patients with normal body weight (125). Although we did not detect a higher incidence of binge eating in PCOS patients, this was common among subjects in both study groups (37.7 % in the PCOS group and 35.9 % in the control group) and had a significant impact on total HRQOL. This demonstrates the importance of early detection of binge eating in patients with PCOS, as well as careful

assessment of all girls in this age group, with particular attention to patients with excess weight, weight fluctuations, decreased weight and patients with mental health problems reported by their relatives or by themselves.

An interesting finding in our study group is that the control group had a statistically significantly higher number of sexually active participants than the PCOS group. The age of onset of sexual life was not statistically significantly different between the two groups. A similar discovery was reported by Trent and US colleagues who found that control group representatives were 2.8 times more likely to be sexually active than PCOS patients (109). These data reaffirm the findings of other studies that adult PCOS patients have impaired sexual function and have difficulties with building relationships (126). At the same time sexuality in adolescents with PCOS still needs to be studied in depth.

The results of this study confirm that PCOSQ is a useful tool to be applied in both research and clinical practice, as it helps to understand problematic aspects of PCOS in the specific patient population as a whole and on a patient-by-patient basis. This understanding is essential as PCOS covers a wide range of aspects of life, and an in-depth understanding of each patient's values and important aspects of the illness helps to build collaboration with them and to apply individual treatment and observation tactics. This is particularly important in adolescence, as it is a time when self-motivation is strengthened and lifestyle habits are integrated into everyday life (23). Improving key health-related components would help young women to raise HRQOL and increase their sense of control over their health – one of the main goals of health education.

It is important for healthcare professionals to be aware that PCOS patients may have undiagnosed eating disorders and disordered eating behaviour. Overweight adolescents use inappropriate weight management tactics such as vomiting or taking laxatives more often than their peers with no excess weight (127). It should therefore be kept in mind that insistent advice on weight reduction can actually harm the patient's mental or even physical health,

especially if the patient is already prone to problematic eating behaviour. If even the slightest suspicion of disordered eating behaviour appears, a multidisciplinary team with mental health professionals should be involved in the treatment of the patient.

4.4 Strengths and weaknesses of the study

The study has several strengths. The study looks at a wide range of questions about adolescent PCOS patients, with the aim of studying aspects that would help to diagnose this syndrome as early as possible (the higher incidence of PCOS in female patients; genetic variations that determine the development of the syndrome) as well as the individualisation of therapy of patients (identifying the most important aspects of the disease that reduce HRQOL, interfere with disordered eating behaviour). Aspects that could help predict the different phenotypes of patients in the future were also studied (genetic variations that determine the clinical and biochemical characteristics of PCOS; prediction of the phenotype of daughters based on symptoms of their mothers, related to PCOS). Early confirmation of diagnosis and prediction of clinical course of the syndrome is essential for patients, given that lifestyle changes are most effective in reducing existing symptoms and improving future prognosis. This is particularly important because, at a time when metabolic health complications have already developed, the possibility of using certain types of medication (e.g. combined hormonal contraceptives to reduce hirsutism) may be limited.

Other studies on these issues have mostly involved this target group – adolescents with PCOS – in small numbers and small samples. They have been carried out in different countries and many questions are ethnically specific (genetic aspects) and socio-culturally different (the most important sub-sections in the HRQOL questionnaire). Therefore, each newly created study sample

provides a contribution to the overall understanding of PCOS and contributes to the possibility of long-term observation of these patients.

This study has provided an important contribution to scientific community by including risk group patients in the sample of the study. These are patients who have the potential to develop PCOS, but currently their symptoms are insufficient to confirm the diagnosis. It is essential to identify this group in order to plan appropriate follow-up and early treatment options.

This is one of the few studies in adolescent age group, that is using the latest evidence based ESHRE diagnostic criteria for PCOS. These criteria precisely define both the parameters of each diagnostic criterion (oligomenorrhea, amenorrhea, clinical and biochemical hyperandrogenism) and the criteria necessary to confirm the diagnosis specifically in adolescents with PCOS. The introduction of these criteria into more studies in the future will also help to better understand the results of our research and its importance in the overall understanding of PCOS.

Study participants were included in the study at the CCUH, which is the only specialised children's hospital in Latvia. It provides both hospital and out-patient medical services. This is also the main institution in Latvia, visited by most children and adolescent gynaecology patients. The gynaecologist is available free of charge and without referral, so the services are available to patients from different regions and socio-economic groups. The participants in the study were subjected to a comprehensive clinical and laboratory examination, as well as an examination of relevant previous medical data in the hospital's electronic database, which keeps records from the last 10–15 years. At the same time, questions about other illnesses and medications were asked both to the participants in the study and to their mothers when they were present at the time of the visit. This way, it was possible to avoid the inclusion of participants whose underlying conditions could affect the results of the studies, as much as possible.

PCOS for adolescents in Latvia has not been studied so far, so the results of the study also provide an indirect insight into the situation in the country. However, the sample of the study should be increased to reach a representative sample at national level.

The study also has several weaknesses. The most important is the small sample size of the study. However, it should be noted that different techniques were used to overcome this factor in the course of the study. Special visit times were offered directly to the potential participants in the study (healthy control group representatives), the offer to involve patients in the study was distributed among CCUH colleagues, especially children's endocrinologists, and information was disseminated on social media. However, we did not observe a rapid inflow of participants, as the study provided strict exclusion criteria (other diseases, medication use), as well as several contact episodes with medical staff for patients with PCOS and risk group patients – the first visit, carrying out of analyses, repeated visit and inclusion in the study. Furthermore, in order to be included in the study, control group representatives had to perform blood tests which would otherwise be largely unnecessary and could cause significant psychological discomfort in adolescence (there was a proportion of potential participants who refused to participate for that reason). The mothers of patients included in section I also had to undergo venopunction to take blood, so some of them also refused to take part in the study. The difficulty in including patients was also caused by the fact that the presence of the patient's mother or, at least, the legal representative, was mandatory during the inclusion visit, whereas the Patients' Rights Act provides that the patient may visit a doctor alone from the age of 14. Moreover, given the range of issues with which patients usually turn to a gynaecologist, they often do not want to discuss the visit and its conduct with their parents or legal representatives. A partial solution to this problem was found in the involvement of the control group – the Central Medical Ethics Committee authorised the involvement of patients from 16 years of age only with the

agreement of the participants themselves, and without the presence of parents. In addition, it was ensured that genetic material analysis is initiated only if consent is not withdrawn by the age of 18 years and 3 months.

Taking into account the peculiarities of PCOS (the wide variations in the manifestation of symptoms), the very complex pathogenesis (interaction of various endocrine and environmental factors), the contradictory genetic results of previous studies, it is not surprising that the exact role of certain genetic variations in the development of PCOS has not been found. Several steps should be taken to verify the accuracy of the results. It would also be important to include a larger number of participants in the study on this issue. It is planned to follow-up a sample of the study in the future. This could change the proportion of participants in the PCOS group and in the risk group, as at least some of the patients in the risk group will have developed full PCOS. An analysis of haplotypes is also planned. The sequencing of the full *CYP21A2* gene would also be useful.

Conclusions and proposals

Conclusions:

1. Clinical symptoms associated with PCOS in adolescents cannot be predicted based on PCOS symptoms in their mothers;
2. The genetic variations analysed (*GNRHR* (rs104893837), *ESR2* (rs4986938), *LHCGR* (rs2293275), *FSHR* (rs6166, rs6165, rs2349415), *CYP21A2* (rs779300117, rs6467, rs387906510, rs6475, rs12530380, rs267606756)) are not associated with the development of PCOS in adolescents with PCOS. The incidence of these variations was also not higher in risk group patients than in healthy control group representatives.
3. Adolescents with PCOS, which are carriers of the *ESR2* rs4986938 minor allele in homozygous state and adolescents with the minor *LHCGR* rs2293275 allele in their genotype, have higher total testosterone in blood than patients who are not carriers of these alleles.
4. The lowest HRQOL results for adolescents with PCOS are in components that reflect worries about body hair and body weight;
5. Binge eating in adolescents with PCOS is not more common than in the control group;
6. The most important factors affecting HRQOL for adolescents with PCOS are the diagnosis of PCOS itself, the result of the binge eating scale and the BMI percentile.

Proposals based on data from the study:

1. Education of healthcare professionals on the intergenerational relationship of PCOS should be continued. PCOS patients should be

informed that their close relatives may have PCOS-related disorders, and therefore special attention should be paid to healthy lifestyle;

2. The use of the PCOSQ should be implemented in the clinical practice in order to improve the understanding of the individual needs of the patient and the ability to influence them;
3. A binge eating scale, which has already been validated in the Latvian population, could be implemented in clinical practice in order to detect binge eating behaviour in different patient groups in a timely manner;
4. Given the different and sometimes conflicting results of the studies, it is necessary to further investigate the intergenerational, genetic and HRQOL aspects of PCOS in larger patient groups and to continue to follow the cohort of participants in this study in the long term.

Publications

Scientific publications in international databases

1. Līdaka, L., Grasmane, A., Lazdane, G., Dzīvīte-Krišāne, I., Gailīte, L., Vīberga, I. Can a Mother's Polycystic Ovary Syndrome (PCOS)-Related Symptoms be Used to Predict the Future Clinical Profile of PCOS in her Adolescent Daughter? A Pilot Study. *EUR J Contracept Reprod Health Care.* 2021; 26(1), 17–22. doi: 10.1080/13625187.2020.1795118.

2. Līdaka, L., Beķere, L., Rota, A., Isakova, J., Lazdāne, G., Ķīvīte-Urtāne, A., Dzīvīte-Krišāne, I., Kempa, I., Dobele, Z., Gailīte, L. Role of Single nucleotide Variants in *FSHR*, *GNRHR*, *ESR2* and *LHCGR* Genes in Adolescents with Polycystic Ovary Syndrome. *Diagnostics (Basel). 11*, 2021; doi 2327: 10.3390/diagnostics11122327.

3. Līdaka, L., Beķere, L., Lazdāne, G., Dzīvīte-Krišāne, I., Ķīvīte-Urtāne, A., Gailīte, L. Non-Classical Congenital Adrenal Hyperplasia-Causing Alleles in Adolescent Girls with PCOS and in Risk Group for PCOS Development. *Diagnostics (Basel).* 2021; 11(6):980. doi: 10.3390/diagnostics11060980.

4. Līdaka, L., Lazdāne, G., Ķīvīte-Urtāne A., Gailīte, L., Dzīvīte-Krišāne, I., Stokenberga, I. Health-Related Quality of Life and Binge Eating Among Adolescent Girls with PCOS. *The Journal of Clinical and Experimental Obstetrics & Gynaecology (CEOG).* 2022; *49*(3). doi: 10.31083/j.ceog4903057

Presentations at international scientific conferences with oral papers or theses

1. 2018 Rīga Stradiņš University Scientific Conference, Riga, Latvia, 22.–23.03.18. (poster presentation)

2. Latvian Congress of Obstetricians and Gynaecologists Riga, Latvia, 20.–21. 04.18. (oral presentation)

3. The 16th Annual Meeting of the Androgen Excess-PCOS Society, Stockholm, Sweden, 23.–25.09.18. (poster presentation)

4. The 57th Annual Meeting of European Society of Paediatric Endocrinology. Athens, Greece 27.–29.09.18. (poster presentation)

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