الجمهورية الجزائرية الديمقراطية الشعبية

**People's Democratic Republic of Algeria**

وزارة التعليم العالي والبحث العلمي

**Ministry of Higher Education and Scientific Research**

جامعة الإخوة منتوري قسنطينة ا
**Frères Mentouri Constantine I University**
**Université Frères Mentouri Constantine I**

**Nature and life sciences faculty**          كلية علوم الطبيعة والحياة
**Animal Biology Department**          قسم بيولوجيا الحيوان

## Thesis presented for the graduation of a Master of Science

**Field:** Natural and Life Sciences
**Sector:** Biological Sciences
**Specialty:** Genetic

**Order N°:**
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## Genetic susceptibility to polycystic ovary syndrome (PCOS): Molecular study of I/D *ACE* gene polymorphism and meta-analysis on the *CYP17A1* (*rs*74357) polymorphism involvement

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**Presented by:**          BENDAOUD Hiba          16/06/2022

EL KHOUR Jihan

**Evaluation jury:**

**Supervisor:**  REZGOUN Mohamed Larbi (MC-A - Frères Mentouri - Constantine 1 University).

**Reviewer 1:**  SEMMAME Ouarda (MC-B - Frères Mentouri - Constantine 1 University).

**Reviewer 2:**  ZIADA Hadia (MC-B - Frères Mentouri - Constantine 1 University).

**Academic year**
**2021 - 2022**

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# Acknowledgment

# *Dedication*

My university career is coming to an end after much effort and suffering, and today I completed writing my thesis at the end of university studies (Master) with courage and vitality.

- I thank God, first and foremost, for giving me the strength and will to finish this humble letter.

- Moreover, I heartily thank with all my heart, and dedicate the fruits of my labor to the person dear to my heart, the one who has illumined my life with her good advice; She who was a pure ocean of love and constant encouraging smiles; She is the one whom God has crowned with prestige and dignity, and who has always encouraged me to continue my studies: my mother **Bougrit Hayet** may God preserve her and give her a long and happy life. I also thank my dear and ideal father, **Bendaoud Mansour**. I pray to God to protect her and to benefit me with his victory for a long time to come. I also extend my heartfelt thanks:

- May God keep my grandfather and grandmother in good health and give them a long life.

- In my heart, may God protect and help my dear sister **Hadjer,** and her daughter **Eline Mayer,** as well as my brother

- To my fiance**, Kerbab Anis,** who has always supported and encouraged me to continue my studies, for his emotional and psychological support, and for his constant presence next to me.

- To every one of my aunts and uncles

- To my heartfelt and devoted friends, **Elkhour Jehan** and **Boukous Safa Raounek**

- I am grateful to Professor **Rezgoun Mohammed Larbi** and **Mrs. Rezgoun-Chellat Djalila** for your invaluable advice and encouragement, which have made a significant contribution. This work could not have been done without them.

- To everyone who assisted and encouraged me in completing my work.

To all those I love and to everyone who participated directly or indirectly in the realization of this work, I extend my sincere thanks to all of you and express my deepest gratitude.

*Hiba*

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My university career is coming to an end after much effort and suffering, and today i completed writing my thesis at the end of university studies (Master) with courage and vitality.

- I thank God, first and foremost, for giving me the strength and will to finish this humble letter.
- Furthermore, I thank God with all my heart and dedicate the fruits of my labor to the person dear to my heart, the one who has illumined my life with her good advice; she who was a pure ocean of love and constant encouraging smiles; she is the one whom God has crowned with prestige and dignity, and who has always encouraged me to continue my studies; my mother **Belil Noura**. I also thank my dear and ideal father, **Elkhour Kassem**. I pray to God to protect her and to benefit me with his victory for a long time to come.
- To my second mother, **Nadia**, the person dear to my heart, the one who lit up my life with her kind advice;  It was she who was a pure ocean of love and constant encouraging smiles.

I also extend my heartfelt thanks:

- To my grandfather **Mohammed** and my grandmother **Charifa**, May God keep them in good health and give them a long life.
- To my dear sisters in my heart, may God protect you for me, my faithful supporters, and help me.
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- To everyone who helped and supported me in completing my work.

To all those I love and to everyone who participated directly or indirectly in the realization of this work, I extend my sincere thanks to all of you and express my deepest gratitude.

**Jehan**

# Abbreviations

**17-OHP:** 17- hydroxyprogesterone

**ACE:** Angiotensin Converting Enzyme

**ASRM:** American Society for Reproductive Medicine

**BET:** Ethidium Bromide

**BMI:** Body Mass Index

**BMP-15:** Bone Morphogenetic Protein 15

**bp:** base pairs

**BPB:** Bromo-Phenol Blue

**C/EBP:** CAAT/Enhancer-Binding Protein

**CI:** Confidence Interval

**DALYs:** Disability-Adjusted Life Years

**DHEA:** dehydroepiandrosterone

**DNA:** Deoxyribonucleic acid

**EDTA:** Ethylene Diamino Tetracetic Acid

**ESHRE:** European Society for Human Reproduction and Embryology

**FSH:** Follicle Stimulating Hormone

**FSHR:** Follicle-Stimulating Hormone Receptor

**GBD:** Global Burden of Diseases

**GDF-9:** Growth Differentiation Factor 9

**GnRH:** gonadotrophin releasing hormone

**GWAS:** Genome Wide Association Study

**IGF-1R:** Insulin-like Growth Factor Receptor 1

**IR:** Insulin Resistance

**IRS-1:** Insulin Receptor Substrate 1

**LH:** Luteinizing Hormone

**LHR:** Luteinizing Hormone Receptor

**mFG:** modified Ferriman-Gallwey

**MW:** Molecular Weight

**NCBI:** National Center for Biotechnology Information

**NIH:** National Institutes of Health

**OCPs:** Oral Contraceptive Pills

**OD:** Optical Density

**OR:** Odds Ratio

**PCOM:** Poly-Cystic Ovary Morphology

**PCOS:** Polycystic Ovary Syndrome

**PCR:** Polymerase Chain Reaction

**RAS:** Renin-Angiotensin System

**SDI:** Socio-Demographic Index

**SDS:** Sodium Dodecyl Sulfate

**SHBG:** Sex Hormone-Binding Globulin

**SNPs:** Single Nucleotide Polymorphisms

**WHO:** World Health Organization

# *Contents*

## Bibliographic Part

### Chapter I: Anatomy and physiology of the ovaries

### Chapter II: Polycystic ovary syndrome

# Chapter III: Genetic of PCOS

# Practical Part

# Patients and methods

# Results and discussion

**Abstracts**

# Index of figures

# Index of tables

Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathies affecting the early reproductive age in women, first reported in 1935 by *Stein I. F* and *Leventhal M. L* **(Goodarzi *et al*., 2016)**. World Health Organization (WHO)'s estimated proportion of PCOS affecting women of reproductive age group worldwide is 116 million (3.6%) **(Kabel *et al*., 2016)**. This disorder is typically characterized by hyperandrogenism and/or hyperandrogenemia, menstrual and ovulatory dysfunction (irregular menstruation), bulky multi follicular ovaries on ultrasonography, and metabolic deviations such as hyperinsulinemia, dyslipidemia, obesity **(Watson, 2019)**. Early diagnoses of PCOS benefit women in treatment and limit the exacerbation of the syndrome **(Patel, 2018)**.

PCOS is the most common reproductive endocrine diseases in women and despite this, diagnostic challenges, delayed diagnosis, and less-than-optimal treatment regimens plague the condition **(Hoeger *et al*., 2021)**. The diagnostic standards for PCOS have been grouped in diverse classifications that have been conflicting for many years. At this time, the classification of Rotterdam is the most used. These criteria include the existence of two out of three of the following criteria: anovulation, clinical or biochemical hyperandrogenism and polycystic ovarian morphology on ultrasonography **(Dewailly, 2016)**.

The diagnosis of PCOS is primarily attained through clinical history and physical findings. The main features are hirsutism or biochemical evidence of excess androgen production and irregular menstrual bleeding instigated by the chronic anovulation. Associated findings include insulin resistance with compensatory hyperinsulinemia and obesity. Screening examinations comprise measurement of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) (and the ratio of LH to FSH), serum total testosterone, dehydroepiandrosterone (DHEA) sulfate, and 17-hydroxyprogesterone. In addition, in the obese individual, determinations of glucose and insulin levels, as well as a lipid profile, are highly recommended **(Azziz *et al*., 2016)**.

The etiopathogenesis of PCOS is not fully explained, but it seems that the hypothalamus-pituitary-ovarian axis, ovarian, and/or adrenal androgen secretion may contribute to developing the disorder. Infertility and poor reproductive health in women's lives are highly associated with elevated levels of androgens **(Kosova & Urbanek, 2013)**. Clinical hyperandrogenemia leads to excessive terminal hair growth on the face or body suggesting masculine features known as hirsutism and leads to cosmetic consequences such as acne and alopecia (male pattern baldness). In contrast, biochemical hyperandrogenism results in excessive production of androgens and insulin resistance. It is also associated with metabolic risk factors including hyperinsulinemia, type II diabetes mellitus, hypertension, dyslipidemia, and cardiovascular disorders **(Chaudhary *et al*., 2021)**.

PCOS is one of the most controversial entities in gynaecological endocrinology. It has been recognized to be a familial condition. Some genetic studies have pointed to an autosomal dominant inheritance while others presented that it was more likely that the disorder is a complex trait with oligogenic basis and that no single gene can completely explain the disease (**Prapas** *et al***., 2009**). Furthermore, candidate gene method (GWAS: Genome Wide Association Study) has not provided conclusive results for any of the susceptible genes. As a consequence, the genetic markers considered thus far could aid in diagnosing the syndrome and its phenotypes, allowing for earlier involvement in comorbidities and more adapted care (**Crespo** *et al***., 2018**). Although the part of genetic factors in PCOS is strongly supported, the genes that are involved in the aetiology have not been fully inspected until now, as well as the environmental influence in their expression (**Diamanti-Kandarakis** *et al***., 2006**). Several genes have revealed altered expression, which means that the genetic abnormality in PCOS affects the signaling pathways that control steroidogenesis, steroid hormones action, gonadotrophin action and regulation, insulin action and secretion, energy homoeostasis, chronic inflammation and others. Several genes have been proposed as playing a role in the etiopathogenesis of PCOS, and the presence of mutations and/or polymorphisms has been discovered, which suggests that PCOS has a heritable vital component (**Chaudhary** *et al***., 2021**).

*CYP17* encodes the enzyme 17-α-hydroxylase /17-20 lyase (P450 17α), which catalyzes the conversion of pregnenolone to 17-hydroxy-pregnenolone and P to 17- hydroxyprogesterone (17-OHP), which are rate-limiting steps in androgen biosynthesis. Augmented activity of this enzyme has been hypothesized to contribute to higher androgen biosynthesis and secretion in PCOS. The gene that codes for cytochrome P450c17 α, located on chromosome 10q24.3. A polymorphism has been found in the regulatory region of the *CYP17* gene, being a T to C substitution-34 base pairs (bp) of Deoxyribonucleic acid (DNA) from the translation initiation point in the promoter region (*rs*74357). It has been proposed that this variation may up-regulate the expression of *CYP17*, subsequent in an increased synthesis of androgens. The Single Nucleotide Polymorphisms (SNPs) of *CYP17* are associated with several diseases in different ethnic groups (**Munawar Lone** *et al***., 2021**).

A number of case-control studies were directed to investigate the association between *CYP17* T/C polymorphisms and PCOS risk in humans. But these studies reported contradictory results. Different methods have been used, but, in particular, most of the studies used a minor sample size and it is consequently not surprising that there has been a lack of replication in the various studies (**Liu** *et al***., 2021; Xu** *et al***., 2021**).

By using all the available published data to increase the statistical power, it was hypothesized that a meta-analysis might allow plausible candidate genes to be excluded and causative genes to be identified with reliability. We have therefore taken a meta-analysis in which all the published case-control studies are processed to confirm whether the T/C polymorphism of *CYP17* gene promoter increased the risk of PCOS.

In the other hand, there is evidence to indicate that the Renin–Angiotensin System (RAS) may influence oocyte maturation, ovulation and steroidogenesis as well as formation of corpus luteum through complex interactions with other systems. ACE, encoded by the *ACE* gene, is one of the components of RAS and can be expressed in multiple tissues including ovaries. I/D polymorphisms of *ACE* are associated with the plasma ACE concentration. Since ACE induces a high blood supply and hypersteroidogenesis in the ovary, it may be associated with PCOS which exhibits hyperplasia, hypervascularity of the ovarian theca interna and stroma, as well as disorderd steroidogenesis **(Bayram *et al*., 2011; Cintra *et al*., 2018 ; Chen *et al*., 2021)**.

Taking this information into account, and to explore several genetic polymorphisms in the development of PCOS, we carried out this research work by setting as objectives:

- Investigate the relevance of polymorphism in Angiotensin Converting Enzyme (*ACE*) gene (OMIM : 106180) insertion/deletion (I/D) polymorphism (*rs*1799752) to the pathophysiology of PCOS in Algerian women. *ACE* I/D gene polymorphism revelation will be performed by Polymerase Chain Reaction (PCR).

- Determine whether the *CYP17* (OMIM: 609300) T/C (*rs*74357) gene polymorphism is an exposure risk for PCOS, by performing a comprehensive meta-analysis summarizing all previous published case-control studies on this topic. All parts of this meta-analysis including: the research methodology adopted, the results obtained (including figures, graphs and tables), the discussion and the conclusions drawn, as well as the bibliographical references used will be presented in the form of a scientific article which will be submitted for publication.

# BIBLIOGRAPHIC PART

# CHAPTER I

## ANATOMY AND PHYSIOLOGY OF THE OVARIES

## 1.   Female genitalia

The female reproductive system includes all of the internal and external organs that help with reproduction. The internal sex organs are the ovaries, which are the female gonads, the fallopian tubes, two muscular tubes that connect the ovaries to the uterus, and the uterus, which is the strong muscular sack that a fetus can develop in. The neck of the uterus is called the cervix, and it protrudes into the vagina. At the opening of the vagina are the external sex organs, and these are usually just called the genitals and they're in the vulva region. They include the labia, the clitoris, and the mons pubis **(Hoare and Khan, 2021)**.

The female reproductive system functions to produce a female egg (gamete), reproductive hormones, support a developing fetus and deliver it into the outside world. Unlike its male counterpart, the female reproductive system is located primarily inside the pelvic cavity **(Gibson and Mahdy, 2019; Ożegowska *et al*., 2019)**.
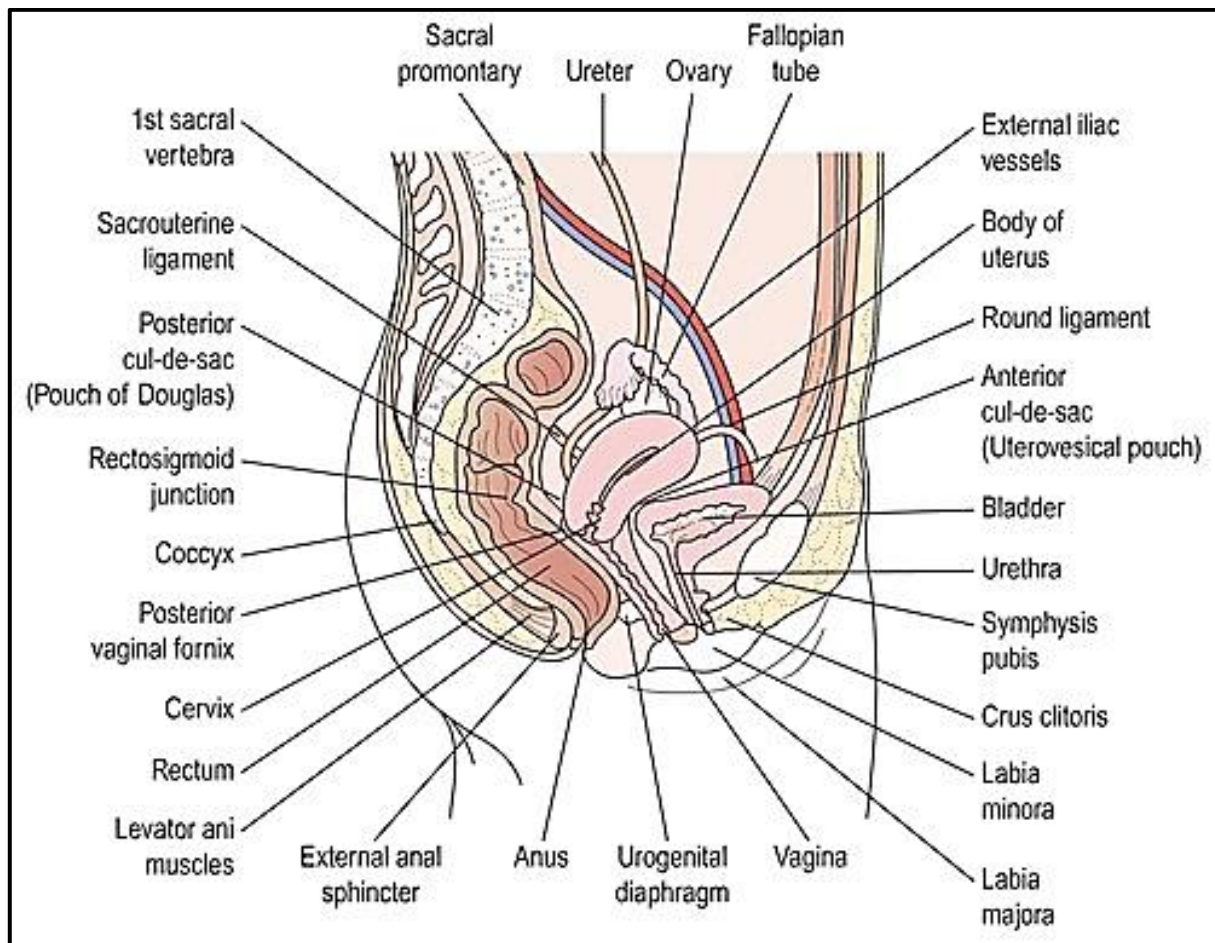


**Figure 01.** Sagittal section of the female reproductive system
**(Knudtson and McLaughlin, 2019)**.

## 2. Structure and function of the ovaries

### 2.1. Structure of the ovaries

Typically, ovaries are found close to the fallopian tubes within the ovarian fossa. The ovarian fossa is composed of the bifurcation of the external iliac artery and the internal iliac artery. Anterior to the ovary is the medial umbilical ligament. Posteriorly is the ureter and internal iliac artery. The ovary has two ligaments. The suspensory ligament carries both of the ovarian artery and vein as well as the sympathetic and parasympathetic plexuses. The proper ligament of the ovary is the remnant of the gubernaculum and does not contain any vessels **(Zhu, 2016)**.

The normal ovary is 2.0 cm in width, 3.5 cm in length and 1.0 cm in thickness. The volume of the ovary changes as females age. At two years old, the volume of the ovary averages 0.7 ml. At 20 years of age, the volume will peak at 7.7 ml. After this, the volume will slowly decrease until menopause, where the average volume is 2.8 ml **(Zık *et al*., 2019)**. The microanatomy of the ovary begins with the outer epithelium; this layer is made of simple cuboidal and is called the germinal epithelium. Underneath this layer is a connective tissue made of collagen and called the tunica albuginea. The next zone contains the ovarian follicles and is called the cortex. Here follicles of different sizes and maturity can be seen. The most central zone is the medulla. It is made of loose connective tissue and contains major blood vessels; this region is also called the hilus **(Petraglia *et al*., 2008)**.

### 2.2. Function of the ovaries

There are two primary functions of ovaries:

The first function of the ovary is hormone production, which changes at puberty. The ovaries will begin to secrete increasing levels of hormones, including estrogen, testosterone, inhibin, and progesterone; in response to rising levels of gonadotropin-releasing hormone (GnRH). This activity creates the Hypothalamus-Pituitary-Ovarian (HPO) axis. **(Owens, Kristensen *et al*., 2019)**. The GnRH is secreted by the hypothalamus, which acts on cells in the anterior pituitary. The anterior pituitary will then produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH has an affinity for granulosa cells; these cells promote follicle growth and maturation. LH will affect theca cells, which produce androgens and precursors for estradiol. This estradiol will become estrogen, and the increase of estrogen at puberty leads to the development of secondary sex characteristics **(Hoare and Khan, 2021)**.

Secondly, the ovary houses the egg cells, or oocytes, which begin developing in utero and pause development until puberty. The ovum matures and are released when the surge of luteinizing hormone gets secreted by the pituitary gland, which is ovulation. The average antral follicle measures between 2 to 9 mm. The average number of follicles is below 25 follicles (when using optimal resolution). Antral follicles enlarge during the menstrual cycle until a dominant follicle forms, while the others degenerate **(Hoare and Khan, 2021)**.
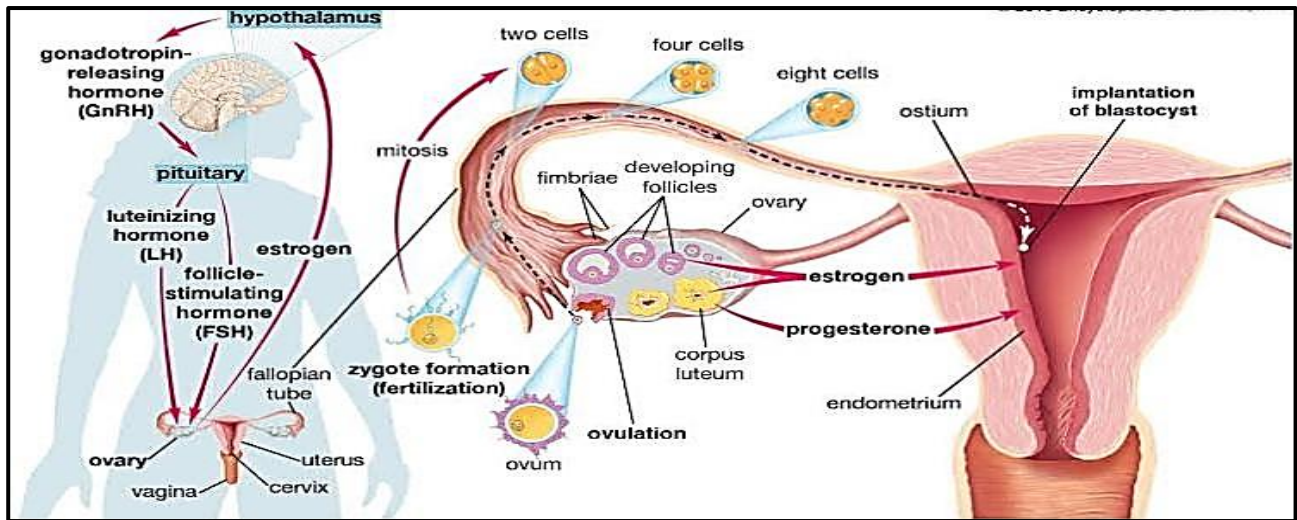


**Figure 02.** Structure and function of the ovaries **(Knight, 2021)**.

## 3.    Oogenesis

Oogenesis is a reproductive system growth process in which the primary egg cell (or ovum) becomes a mature ovum. The egg's development starts before the female that carries it is even born; 8 to 20 weeks after the fetus has started to grow, cells that are to become mature ova have been multiplying, and by the time that the female is born, all of the egg cells that the ovaries will release during the active reproductive years of the female are already present in the ovaries. These cells, known as the primary ova, number around 400,000. The primary ova remain dormant until just prior to ovulation when an egg is released from the ovary. The egg cell remains as a primary ovum until the time of its release from the ovary arrives. The egg then undergoes a cell division. The nucleus splits so that half of its chromosomes go to one cell and half to another. One of these two new cells is usually larger than the other and is known as the secondary ovum; the smaller cell is known as a polar body. The secondary ovum grows in the ovary until it reaches maturation; it then breaks loose and is carried into the fallopian tubes. Once in the fallopian tubes, the secondary egg cell is suitable for fertilization by the male sperm cells **(Wallace and Kelsey, 2004)**.

## 4.     Female ovarian cycle physiology

These are the primary female reproductive organs located in the pelvic cavity. They produce haploid ova (oocytes), which develop in fluid-filled sacs called follicles. Each mature ovary is an irregular, lumpy, almond-shaped structure, typically 3-5cm long and weighing around 5-8g. The developing, and subsequently degenerating, follicles form the primary endocrine tissue in the ovaries that synthesizes and secretes estrogens and progesterone **(Orlowski and Sarao 2018)**.

### 4.1.  Ovarian cycle

The ovarian cycle is the series of cyclical monthly events of follicle development and degeneration occurring in the ovaries. This consists of three distinct phases: follicular phase, ovulation (typically around day 14), and luteal phase **(Orlowski and Sarao 2018)**.

### 4.2.  Uterine cycle

This is the series of changes the endometrium undergoes during each 28-day cycle. Like the ovarian cycle, it has three phases:
- **Menstrual phase (day 1-5):** the endometrium is deprived of progesterone, causing breakdown and shedding of the endometrial lining.
- **Proliferative phase (day 6-14):** the endometrial lining is rebuilt and begins to thicken and mature. This is primarily driven by the estrogens secreted by the developing ovarian follicles **(Burton *et al*., 2007)**.
- **Secretory phase (day 14-28):** as the new endometrial lining matures, progesterone secreted by the corpus luteum stimulates the endometrium to secrete a sticky mucoid material called uterine milk **(Tsutsumi and Webster, 2009)**.

This coats the surface of the endometrium, ensuring it is adherent, which encourages a fertilized ovum (zygote) to stick to it, helping implantation. The term 'uterine milk' is appropriate, as it can provide nutrition before implantation **(Kara, Dupuy *et al*., 2019)**.
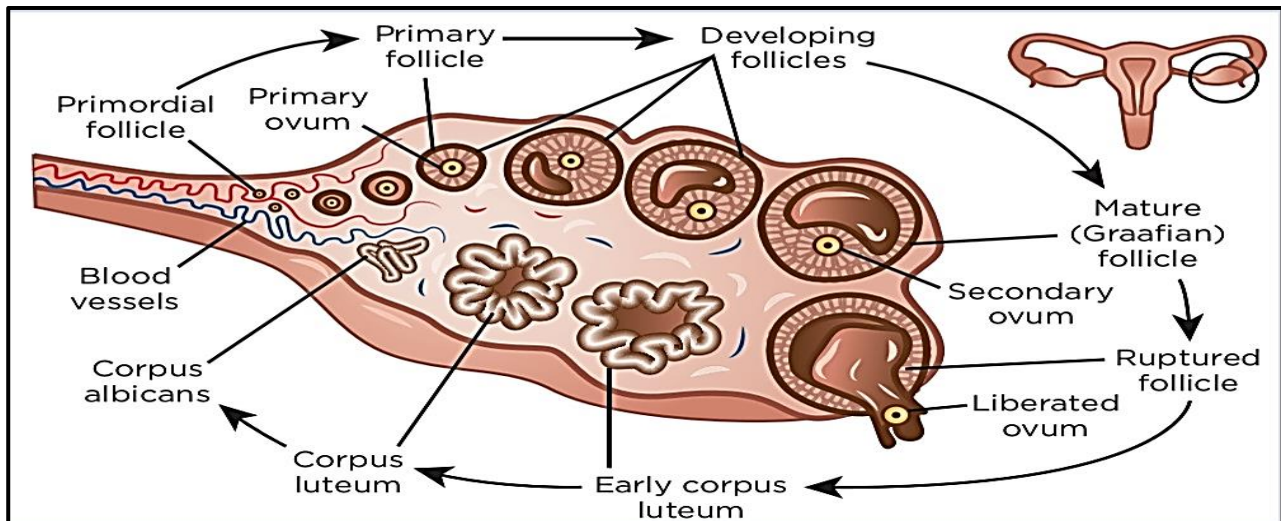
**Figure 03.** Ovarian cycle (**Knight, 2021**).

## 5.    Hormonal regulation of the menstrual cycle

During the second half of the preceding cycle, the elevated level of estradiol and progesterone acting via the hypothalamic-pituitary axis suppresses the production of FSH and LH by the pituitary gland. The declining production of estradiol and progesterone by the corpus luteum at the end of the cycle eliminates this suppression and the level of FSH increase (**Richards, Russell *et al*., 1998**).

The follicles in the ovaries require a threshold of FSH below which no stimulation occurs. Initially FSH values are below this threshold, but slowly increase until the threshold is crossed and a group of follicles are stimulated into active growth. It takes several days of growth before the follicles begin to produce estradiol which is secreted into the bloodstream and reaches the hypothalamus to provide the signal that the threshold has been reached. There is also an intermediate level of FSH production, which must be exceeded before a follicle is brought to its full ovulatory response, and a maximum level, which must not be exceeded, otherwise too many follicles are stimulated and several ovulations occurs (**Kumar and Sait, 2011**).

Near ovulation, the dominant follicle rapidly produces increasing levels of estradiol. This hormone stimulates the production of cervical mucus and also suppresses the production of FSH which goes below the threshold value, thus withdrawing the necessary contribution to the other follicles which are competing in the race for ovulation. The drop in FSH levels also triggers a maturation mechanism within the dominant follicle which makes it receptive to the second pituitary gonadotropin, LH (**Petraglia *et al*., 2008; Kumar and Sait, 2011**).

The high level of estradiol also activates a positive feedback mechanism in the hypothalamus which leads to a massive discharge of LH by the pituitary gland. This LH surge is the trigger that initiates follicle rupture (ovulation) usually 24 to 36 hours after it begins. Ovarian production of estradiol drops sharply between the interval between the LH peak and ovulation. After ovulation, the ruptured follicle is transformed into the corpus luteum and the production of the second ovarian hormone, progesterone, increases rapidly along with that of estradiol. This progesterone causes the abrupt change in the characteristics of the cervical mucus. The disappearance of the corpus luteum (around the 26th day) of the ovarian cycle (in the event of non-fertilization) causes the cessation of progesterone synthesis and induces desquamation of part of the endometrium which extends over a period of 3 to 5 days and is characterized by bleeding. The first day of menstruation is also the first day of the new cycle **(Knudtson and McLaughlin, 2019; Laven, 2019)**.
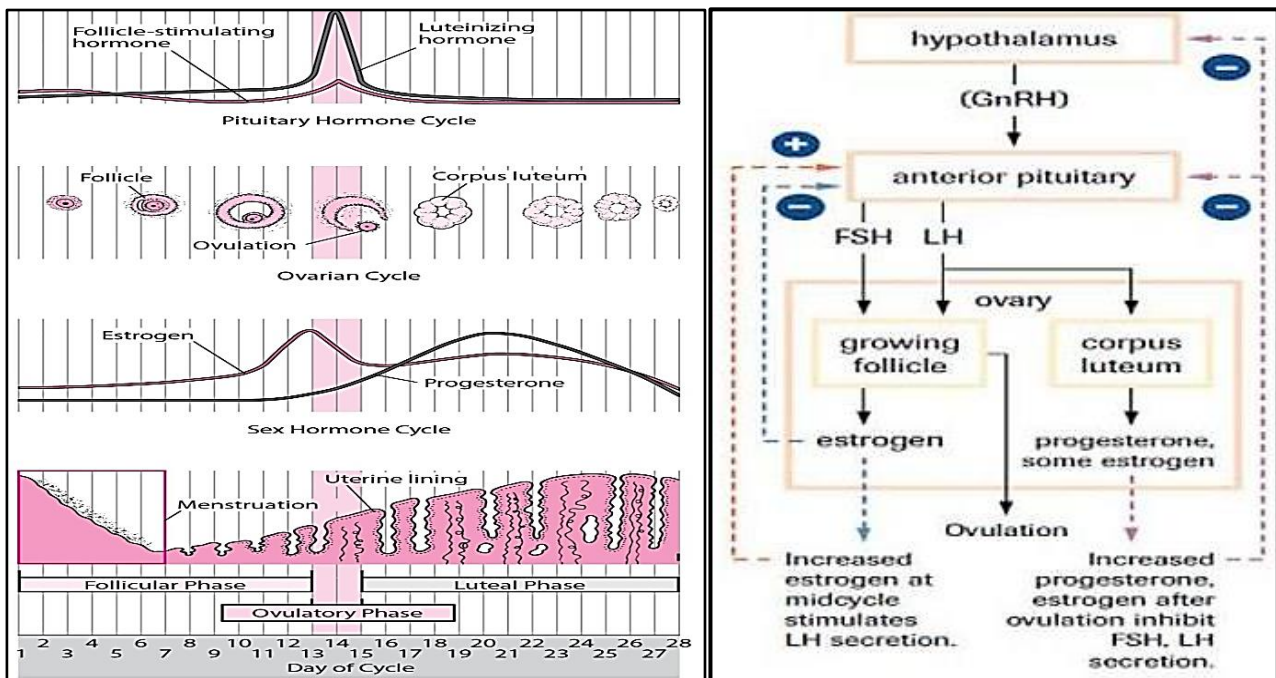


**Figure 04.** Hormonal regulation of the menstrual cycle **(Knudtson and McLaughlin, 2019)**.

# CHAPTER II

# POLYCYSTIC OVARY SYNDROME

## 1. Definition

Polycystic ovary syndrome (POCS) is an endocrine and metabolic disorder that leads to several hormonal and reproductive troubles, characterized by irregular ovulation, oligomenorrhea or amenorrhea, hyperandrogenemia, hirsutism, and infertility (**Zhang *et al*., 2019**).

Stein, I.F., and Leventhal, M.L. described it initially in 1953. It affects 6%-20% of women of reproductive age worldwide, potentially making this condition the most common endocrine and metabolic illness in women of reproductive age. Although, the exact cause of PCOS is unknown, emerging evidence suggests that it is a multigenic illness with substantial epigenetic and environmental impacts, including nutrition and lifestyle variables. PCOS is usually linked to abdominal adiposity, insulin resistance, obesity, metabolic abnormalities, and risk factors for cardiovascular disease (**Escobar-Morreale, 2018**).

Many treatments for PCOS have recently been developed, including hormone medications to reduce the severity of symptoms, but the most effective treatment is still healthy lifestyle (**Patel, 2018**).

## 2. Epidemiology

PCOS is a primary cause of anovulatory infertility in women of reproductive age (15-49 years) and is one of the most frequent endocrine disorders (**Szilagyi and Szabo, 2003 ; Balen *et al*., 2016**). The prevalence of PCOS is estimated to be between 5% and 15% worldwide (**Azziz, 2016**). According to compelling evidence, obesity, dyslipidemia, poor glucose tolerance, and long-term consequences such as diabetes, endometrial cancer, and cardiovascular disease are all linked to PCOS (**Lim *et al*., 2012 ; Peigné and Dewailly, 2014**).

The global age-standardized prevalence of infertility and associated The Disability-Adjusted Life-Years (DALYs) among women increased by 0.370 percent and 0.396 percent per year between 1990 and 2017 (**Sun *et al*., 2019**). Anovulatory infertility in women is the most commonly caused by PCOS (**Balen *et al*., 2016**).

Women of reproductive age were responsible for 1.55 million incident cases of PCOS (95 percent confidence intervals (UIs): 1.19-2.08) and 0.43 million (0.19-0.82) DALYs globally. From 2007 to 2017, the global age-standardized PCOS incidence rate among women of reproductive age increased by 1.45 percent (1.43-1.47%) to 82.44 (1.43-1.47%) per 100 000 people. From 2007 to 2017, the rate of age-standardized DALYs increased by 1.91 percent (1.89-1.93 percent) to 21.96 (12.78-31.15) per 100 000 population. The age-standardized PCOS incidence and DALYs rates increased the most in the middle- Socio-Demographic Index (SDI) and high-middle SDI regions, respectively, across the study period (**Liu *et al*., 2021**).

At the Global Burden of Diseases (GBD) regional level, Andean Latin America had the highest age-standardized incidence and DALY rates in 2017, while Tropical Latin America had the largest percentage increases in both rates from 2007 to 2017. Ecuador, Peru, Bolivia, Japan, and Bermuda had the highest age-standardized incidence and DALYs rates at the national level in both 2007 and 2017. From 2007 to 2017, Ethiopia, Brazil, and China had the largest increases in both age-standardized incidence rates and DALYs rates from 2007 to 2017 (**Liu *et al*., 2021**).

## 3.     Aetiology

Although the aetiology of POCS has yet to be determined, it has been proposed that one of the most prevalent causes of POCS is hyperandrogenism, which is caused by a defect in steroidogenesis and a malfunction of gonadotropins. Metabolic disorders like obesity, insulin resistance, and hyperinsulinemia are some of the reasons involved in the presence of this syndrome. Wrong lifestyle, unhealthy food, various environmental factors, and daily use of numerous chemicals all contribute to the occurrence of POCS (**Glueck and Goldenberg, 2019**). The number of genes, how they are expressed, and the presence of genetic diversity among people is produced by the presence of numerous polymorphisms, dilatation, translocation, and invariant. These are all risk factors for PCOS (**Patel, 2018**).
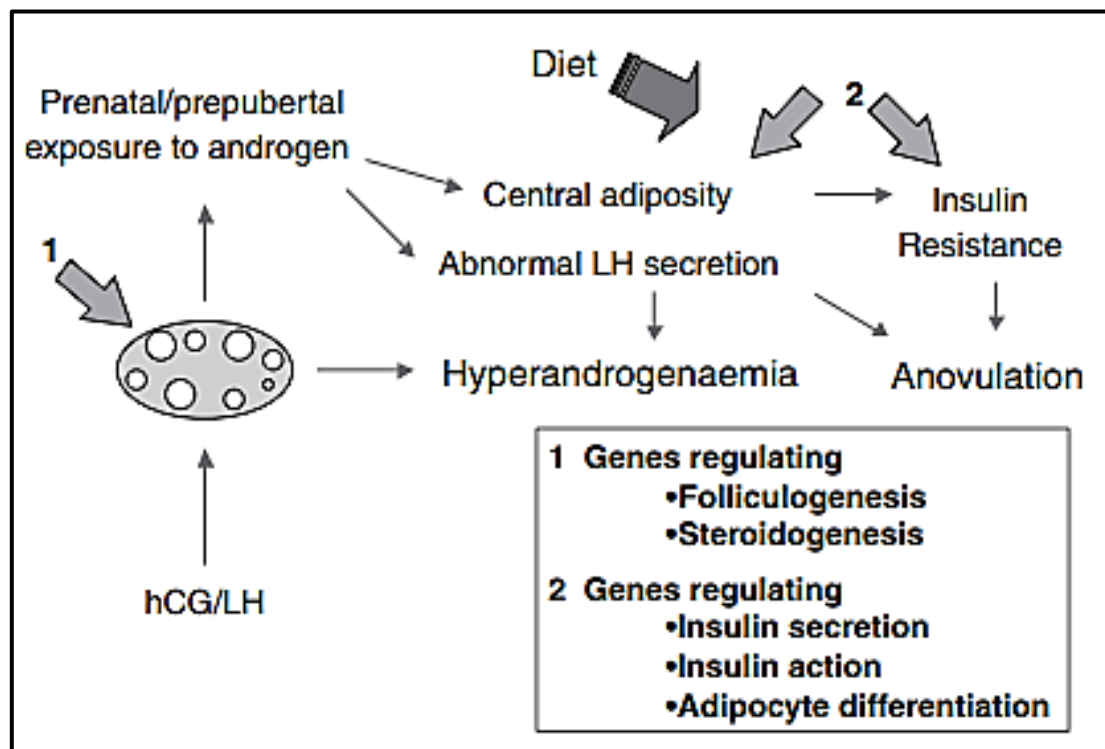


**Figure 05.** Schematic representation of the putative developmental origin of PCOS in women (**Franks *et al*., 2006**).

## 4.    Diagnosis

PCOS is a heterogeneous disorder of unknown aetiology and it's truly a diagnostic challenge. Many features have been associated with the disorder, including ovulatory dysfunction, 'polycystic ovaries' on either ultrasonographic or histopathological examination, hirsutism, hyperandrogenemia, abnormal gonadotrophin concentrations, and most recently insulin resistance and hyperinsulinemia **(Dewailly, 2016)**.

### 4.1.  Diagnosis criteria

In 2003, a professional conference held in Rotterdam and sponsored by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) recommended new and broader criteria for PCOS, and to include the finding of polycystic ovaries on ultrasonography. The meeting proceedings suggested that PCOS be defined when at least two of the following three features were present, after elimination of other aetiologias:

-      Oligo- or anovulation,
-      Clinical and/or biochemical hyperandrogenism, or
-      Poly-Cystic Ovary Morphology (PCOM) on ultrasound **(Dewailly, 2016; ACOG, 2018)**.

These newer criteria for PCOS give rise to new questions. For example, two new phenotypes of PCOS are defined, namely patients who have hirsutism and/or hyperandrogenemia with polycystic ovaries, but who have normal ovulation, and women who have polycystic ovaries and irregular ovulation, but no sign of androgen excess. According to the National Institutes of Health (NIH), to diagnose this syndrome, there should be menstrual irregularity and hyperandrogenism. However, according to the Rotterdam guideline, diagnosis is made on the present of any two of the three criteria **(Kamboj and Bonny, 2017)**.

The diagnostic standards for PCOS have been gathered in different classifications that have been conflicting for many years. Right now, the classification of Rotterdam is the most used, but with varying rate depending on the country and medical specialties. The Rotterdam classification is now >20 years old. Although its fundamental principle (two criteria required out of three) is still valid, each of its three items (oligo-anovulation, hyperandrogenism, and polycystic ovarian morphology) needs to be updated. The definition of biological hyperandrogenism is still unresolved. The criteria used to define oligo- or anovulation are insufficient. The definition of PCOM proposed in 2003 is now obsolete when using the latest generation of ultrasound machines **(Goodarzi *et al*., 2011; Hoeger *et al*., 2021)**.

### 4.2. Clinical diagnosis

PCOS presents in women in a myriad of ways, as this disorder is a spectrum of clinical signs and symptoms. Clinical or biochemical hyperandrogenism, oligo- anovulation and polycystic morphology are the generally accepted diagnostic criteria. Women with PCOS may appear without any symptoms or may have several symptoms such as hirsutism, acne, irregular menses, and infertility. The severity of these symptoms is mostly determined by the woman's age and lifestyle (**Meier, 2018; Patel, 2018**).

### 4.2.1. Ovulatory Dysfunction

Ovarian dysfunction typically results in oligomenorrhea or amenorrhea due to chronic oligo-ovulation or anovulation. Oligo-ovulation is defined as a menstrual cycle > 35 days in length; although some prefer to define oligo-ovulation as <8 menstrual cycles a year, or cycles > 45 days in length. Approximately 70% to 80% of women with PCOS present with oligomenorrhea or amenorrhea. Up to 40% of hirsute women who claim to be eumenorrheic are actually oligo-anovulatory. Menstrual irregularity usually begins in adolescence, but is frequently masked with oral contraceptive pill treatments. It is not until they attempt to reproduce and discontinue the hormonal treatment that they seek medical evaluation and treatment. Women may initially present to their provider complaining of infertility, as absence of ovulation leads to infertility (**Kabel, 2016; Hoeger *et al*., 2021**).

### 4.2.2. Hyperandrogenism

Increased androgen secretion, which manifests as menstrual irregularities, hirsutism, and acne, is one of the most striking features of this syndrome. Theca cell response to luteinizing hormone is amplified, which results in this rise (**Dabadghao 2019**).

Hyperandrogenism typically presents with hirsutism, which is the presence of unwanted terminal hair growth in a male-like pattern. Terminal hairs grow beyond 5 mm in length, are medullated, and often have both pigment and shape. In contrast, vellus hairs are unmedullated, softer, <5 mm in length, are uniform in shape and may or may not be pigmented. Traditionally the amount of hirsutism is graded visually using the modified Ferriman-Gallwey (mFG) score. Nine areas of the body (upper lip, chin and neck, upper chest, upper abdomen, lower abdomen, lower abdomen or male escutcheon, upper back, lower back, upper arms, and thighs) are graded each a score of 0 (no visible terminal hair) to 4 (terminal hair growth consistent with normal adult male) and summed, with a possible total score of A total mFG score > 3 is defined as abnormal body hair, and a score of 6 or more is significant hirsutism (**ACOG, 2018; Watson, 2019**).

There are limitations to the mFG scoring system: there are ethnic differences in hair growth and distribution, women typically treat unsightly hair with removal or shaving, and adolescents may not fully exhibit hair growth until later in development **(Watson, 2019)**.

When evaluating for biochemical hyperandrogenism, a total and free testosterone should be assessed. Acne and alopecia are additional clinical signs of hyperandrogenism, however independently both acne and female alopecia are not specific to hyperandrogenism, especially in the absence of hirsutism **(Azziz *et al*., 2016; Di Guardo *et al*., 2020)**.

### 4.2.3. Ultrasound diagnosis

Ovarian morphology is typically assessed with transvaginal ultrasonography examination. Polycystic ovarian morphology is defined as an abnormal ovary(ies) with a volume > 10 mL$^3$ and/or > 12 follicles measuring between 2 and 9 mm in size in at least 1 ovary **(ACOG, 2018)**.

### 4.2.4. Obesity

In general, women with PCOS have 2 main phenotypes: lean and obese. A small portion of patients with PCOS present with a normal Body Mass Index (BMI; ≤ 25 kg/m2), and are classified as "lean PCOS." Recent research suggests that metabolic, hormonal, and hematological abnormalities are similar to women with "obese PCOS," however they are usually more subtle and less-severe. Obesity causes a rise in other symptoms linked with PCOS, such as metabolic problems and insulin resistance, so the majority of women with PCOS are obese or overweight (38 percent to 88 percent). Furthermore, the global expansion of obesity has resulted in a greater chance of getting PCOS, as obese women are more likely to develop this syndrome **(Barber *et al*., 2019)**.

### 4.2.5. PCOS and infertility

PCOS is the most common cause of anovulatory infertility, and accounts for 90% to 95% of women in infertility clinics. PCOS affects 70% of infertile women and is the most frequent cause of infertility. It causes ovarian dysfunction as well as menstrual irregularities (oligomenorrhea or amenorrhea) and can sometimes lead to anovulation **(Brassard *et al*., 2008; Zhang *et al*., 2019)**.

### 4.2.6. Insulin resistance

Insulin Resistance (IR) is diagnosed in more than 50% of women with polycystic ovary syndrome. PCOS is characterized by metabolic disorders, which can lead to a variety of ovarian disorders such as ovulatory dysfunction and the development of endometrial disorders. Insulin resistance is one of the main causes of obesity in women with PCOS **(He and Li, 2020)**.

## 5. Physiopathology of POCS

The physiopathology of this syndrome includes disorders of one or both ovaries, which are linked to a variety of internal problems such as hyperinsulinemia and pituitary gland disorders, as they cause increased secretion of gonadotrophin releasing hormone (GnRH), which leads to increased secretion of luteinizing hormone (LH), which causes anovulation and hyperandrogenism. Other problems are related to external factors such as lifestyle, nutrition, and environmental factors (**Tsilchorozidou** *et al.*, **2004; Zhu** *et al.*, **2019**).

The abnormalities in folliculogenesis that characterize PCOS are thought to be the cause of anovulation. Growth factors such as Growth Differentiation Factor 9 (GDF-9) and Bone Morphogenetic Protein 15 (BMP-15) stimulate the transition from the primordial to the primary stage of folliculogenesis, while FSH regulates the stages of folliculogenesis, leading to the selection of the dominant follicle in normal folliculogenesis. Androgens and insulin perform a synergistic function with LH during folliculogenesis, with LH exerting its main action in the middle to late follicular stages. Aromatase activity may be influenced by the balance of FSH and AMH during and after the selection of the dominant follicle. AMH levels rise when there are too many small follicles, interfering with follicular reactivity to FSH. Insulin increases the expression of LH receptors, causing premature luteinization (**Diamanti-Kandarakis, 2008**).
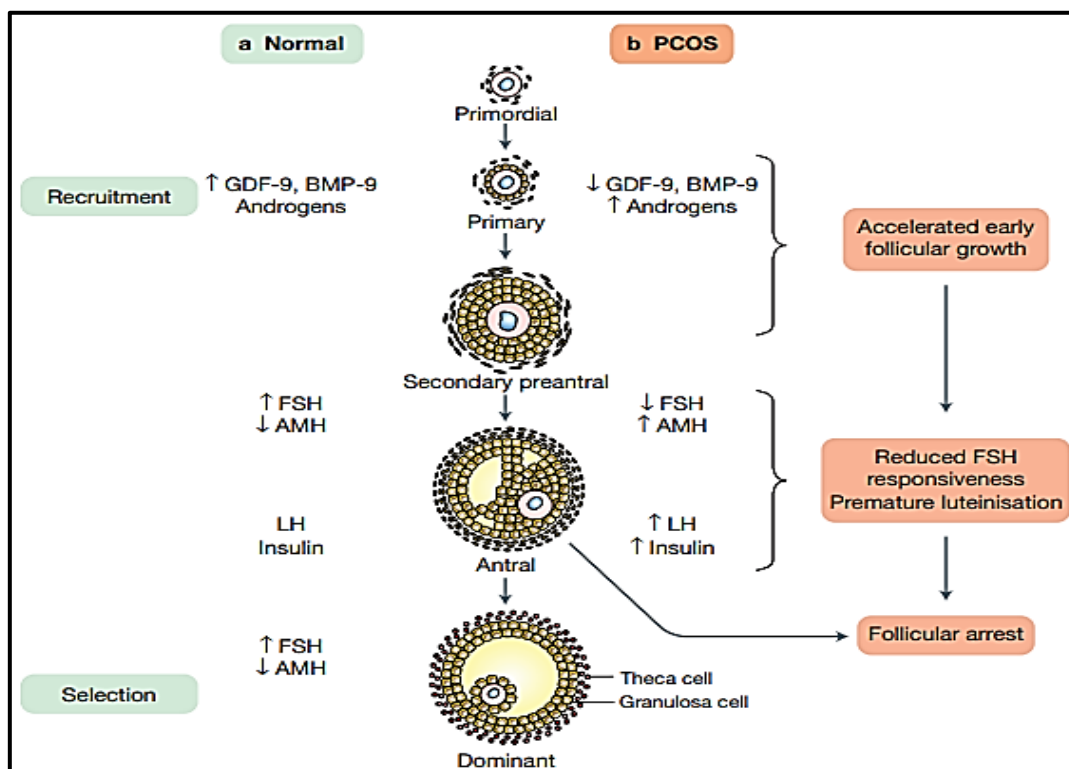


**Figure 06.** Normal folliculogenesis and the follicular defect in PCOS
(**Diamanti-Kandarakis, 2008**).

## 6.     Treatment

Because the main cause of PCOS is unidentified, treatment is focused at the symptoms. Few treatment approaches improve all aspects of the syndrome, and the patient's wish for fertility may prevent her from seeking treatment despite the presence of symptoms. Treatment goals must include correcting anovulation, inhibiting androgens action on target tissues, and reducing insulin resistance. Weight decrease for obese patients with PCOS is beneficial in many ways. Weight loss benefits to decrease androgen, luteinizing hormone, and insulin levels. It also helps to normalize ovulation, thereby improving the potential for pregnancy. Laparoscopic ovarian drilling is an outpatient surgical intervention in which multiple perforations are created in the ovarian surface and stroma. It is believed that this intervention destroys androgen-producing tissue, which should lead to diminished androgen levels **(Patel, 2018; Hoeger *et al*., 2021)**.

Treatment should target specific manifestations and individualized patient goals. When choosing a treatment regimen, physicians must take into account comorbidities and the patient's desire for pregnancy. Lifestyle modifications should be used in addition to medical treatments for optimal results **(Bjekić-Macut *et al*., 2021)**.

### 6.1.   Oral contraceptive pills

The Oral Contraceptive Pills (OCPs) containing estrogen and progesterone should be regarded as first-line therapy for the control of hyperandrogenism symptoms such as hirsutism and acne,  as well as the regularization of menstrual periods **(Dabadghao, 2019)**.

### 6.2.   Weight loss

Given the unfavorable consequences of weight gain in PCOS, attempting to reduce 5-10% of one's body weight will result in a general improvement in the syndrome's symptoms, both at the level of ovulation and at the level of androgen excess symptoms. This strategy is considered an effective treatment method for PCOS when combined with a healthy lifestyle and exercise commitment **(Dabadghao, 2019)**.

### 6.3.   Antiandrogens

Antiandrogen therapy is used to treat hyperandrogenism symptoms like hirsutism and acne. In general, there are two types of antiandrogens used to treat PCOS: reductase inhibitors, like finasteride, and androgen receptor blockers, such as spironolactone and flutamide. However, due to their toxic effects on the liver and the risk of pregnancy, these drugs are only used in limited circumstances **(Tehrani and Amiri, 2019)**.

# CHAPTER III

## GENETIC OF PCOS

## 1.    Family studies

There is no doubt about the contribution of the genetic component to the presence of PCOS among families, but there is no convincing study so far of the genetic pattern of the transmission of this syndrome. This is because the number of families studied was small and, therefore, the parental phenotypes were not determined or the male phenotype is uncertain. However, most empirical studies on the mode of inheritance in familial PCOS have found that the autosomal-dominant pattern is the most common, as 55-60% of families support the autosomal dominant inheritance theory of PCOS (**Legro and Strauss, 2002; Prapas *et al*., 2009**).

Twin studies in small cohorts of monozygotic and dizygotic twin pairs revealed that PCOS is an X-linked polygenic disorder, not an autosomal dominant or monogenic disease. Furthermore, twin studies discovered that genetics accounts for 72% of the variation in PCOS risk, emphasizing the genetic component (**Khan *et al*., 2019**).

## 2.    Molecular abnormalities involved in PCOS

The fact that numerous genes have changed expression patterns shows that the genetic aberration of PCOS has a considerably greater impact on the signal transduction pathways that control the expression of a gene family than on the expression of a single gene. A single steroidogenic enzyme is encoded by this gene. Cytogenetic research has failed to reveal prevalent karyotypic abnormalities, which supports this viewpoint. The location of a causal gene would be shown by a consistently detected aberration with a specified breakpoint. Because aberrant steroidogenesis is a key symptom of PCOS, researchers have been looking for a link or connections between PCOS and the genes involved in androgen biosynthesis or metabolic pathways related to insulin action for a long time. Linkage analysis is used to show that a genetic variant and a disease locus co-segregate (**Urbanek and Spielman, 2002; Rani and Chandna, 2022**).

PCOS is a complex condition that is brought on by a variety of factors. PCOS is linked to all genes/mutations that impact the ovaries, either directly or indirectly. These gene groups and their effects on PCOS are described in detail lower down (**Khan *et al*., 2019**).

### 2.1.  Gene involved in the synthesis of glucocorticoids

### 2.1.1. *CYP11A* gene

*CYP11A1* named as Cytochrome P450, family 11, subfamily A, member 1. It encodes a superfamily of cytochrome p450 his gene is located on chromosome 15q24.1. The main function is in the catalysis of cholesterol to pregnenolone, It also plays a vital role in the steroid synthesis pathway (**Ajmal *et al*., 2019**).

The polymorphism of the *CYP11A1* gene is a sign of susceptibility to PCOS, as it is considered to be the culprit in the increase in the level of androgens through the regulation of Luteinizing hormone in various genotypes. The increased risk of PCOS depends on the interaction of these polymorphism with environmental and genetic factors (**Ajmal *et al*., 2019**).

## a. *CYP17* gene

Located on chromosome 10 q24-q25, the cytochrome P450 family 17 gene play an essential function in the steroid production process. The enzyme cytochrome P450 17a-hydroxylase - 17, 20-lyase is encoded by this gene. SNPs in the 50-UTR, 34 bp upstream from the translational initiation point, cause a T to C change, which is thought to provide an extra Sp1-type promoter site (CCACC box). This alteration is thought to upregulate the expression of the *CYP17* gene, resulting in androgen overproduction. The majority of research shows that these polymorphisms in the cyp17 gene contribute to the risk of developing PCOS (**Ashraf *et al*., 2021**).

## b. *CYP21* gene

Cytochrome P450 21-hydroxylase is a gene found on chromosome 6p21.3. The *CYP21* gene is involved in the generation of steroid hormones and encodes an enzyme that converts 17-hydroxyprogesterone to 11-deoxycortisol. Several studies have resulted in an increased frequency of heterozygosity for *CYP21* gene mutation in women. It leads to symptoms similar to those of PCOS, such as hyperandrogenism. In general, this gene does not play a major role in the presence of PCOS, but contributes secondary to it (**Khan *et al*., 2019**).

## c. SHBG gene

The Sex Hormone-Binding Globulin (SHBG) gene is found on chromosome 17p13.1. It regulates the availability of target tissues to androgens and controls the amount of sex hormones in the circulation. RSHBG is primarily expressed in sex-steroid-dependent cells such as the ovaries, endometrium, colon, prostate, hypothalamus, breast, placenta, liver, epididymis, immune cells, and cardiomyocytes. The main cause of PCOS is an increase in androgens, which causes a variety of problems, including inhibition of hepatocyte synthesis, which is the primary site for SHBG production, and hence a decrease in SHBG concentration in the bloodstream. Low SHBG concentration is a sign of PCOS that leads to hyperandrogenism symptoms. On the other hand, polymorphisms in the *SHBG* gene alter SHBG levels and hence contribute to the occurrence of PCOS. Several studies have found a link between the length of TAAAA repeats polymorphism and a low level of SHBG, which therefore contributes genetically to PCOS. (**Chaudhary *et al*.. 2021**).

## 2.2.  Genes involved in carbohydrate metabolism

### 2.2.1. Insulin receptor gene

The human insulin receptor gene contains 22 exons, and occupies in excess of 150 kilobase pairs of DNA on the short arm of chromosome 19 (bands pl3.2→pl3.3). Typical of a "housekeeping" type of promoter, the promoter is GC-rich, lacks a TATA box, but contains several Spl binding sites. In addition, an enhancer element has been identified 410–481 base pairs (bp) upstream from the initiator AUG codon. Several potential CAAT/Enhancer-Binding Protein (C/EBP) binding sites have been identified in the human insulin receptor gene, two in the 5'-flanking domain and one in the first intron **(Taylor, 1992)**.

The insulin receptor is always present in normal and affected cells. There is structural and functional homology between IGF and the insulin receptor, whose IGF-I receptor is also present in the ovary, and its ligand, IGF-I, is synthesized by the ovary. Insulin Receptor Substrate 1 (IRS-1) is an intracellular signaling adapter protein that integrates and coordinates multiple biologically key extracellular signals within the cell. It is also a key central receptor in insulin signaling, and plays a focal role in maintaining essential cellular capabilities, e.g., survival, development, and digestion system. IRS1 is essentially found in the cytoplasm. But localization in the nucleus may occur in some cell types and under certain stimuli. **(Fadhil and Mousa Abo Almaali, 2021)**.

### 2.2.2. Insulin gene

The majority of the islet-restricted (BETA2, PDX-1, RIP3b1-Act/C1) and ubiquitous (E2A, HEB) insulin-binding proteins have been characterized. Transcriptional regulation results not only from specific combinations of these activators through DNA-protein and protein-protein interactions, but also from their relative nuclear concentrations, generating a cooperativeness and transcriptional synergism unique to the insulin gene. Their DNA binding activity and their transactivating potency can be modified in response to nutrients (glucose, NEFA) or hormonal stimuli (insulin, leptin, glucagon-like peptide-1, growth hormone, prolactin) through kinase-dependent signaling pathways (PI3-K, p38MAPK, PKA, CaMK) modulating their affinities for DNA and/or for each other **(Melloul _et al_., 2002)**.

Insulin can bind to the IGF-I receptor and activate it, and IGF-I can bind to the insulin receptor and activate it as well. The action of ovarian insulin on steroidogenesis is thus preserved, despite the resistance to the metabolic actions of insulin in PCOS. Increased insulin levels in synergy with that of LH can trigger premature expression of the LH receptor in a subpopulation of small follicles, leading to premature terminal granulosa differentiation and growth arrest  follicles which may contribute to anovulation **(Diamanti-Kandarakis and Dunaif, 2012)**.

Insulin is well known to play a prominent role in PCOS and cross-reacts with Insulin-like Growth Factor Receptor 1 (IGF-1R) to enhance ovarian and adrenal steroidogenesis by activating phosphorylation tyrosine kinase and several intracellular signaling cascades. Insulin resistance is largely due to different mutations in the insulin receptor gene **(Chehin *et al*., 2020)**.

### 2.2.3. Insulin receptor substrate protein gene

Insulin receptor substrate proteins are essential for insulin signal transduction in cells. Polymorphisms in genes encoding IRS-1 (Gly972Arg) and IRS-2 (Gly1057Asp) have been associated with susceptibility to type 2 diabetes. **(Dewailly, 2005)**. The discovery of insulin receptor substrate (IRS) proteins and their role in linking cell surface receptors to the intracellular signaling cascades is a key step to understanding insulin and insulin-like growth factor (IGF) action. Moreover, IRS-proteins coordinate signals from the insulin and IGF receptor tyrosine kinases with those generated by proinflammatory cytokines and nutrients. The IRS2-branch of the insulin/IGF signaling cascade has an important role in both peripheral insulin response and pancreatic beta-cell growth and function. Dysregulation of IRS2 signaling to cause the failure of compensatory hyperinsulinemia during peripheral insulin resistance. IRS protein signaling is suppressed by serine phosphorylation or proteasome-mediated degradation, which could be a key mechanism of insulin resistance during acute injury and infection, as well as chronic stress associated with aging or obesity **(Lee and White, 2004)**.

### 2.2.4. Calpain gene

The human calpain-10 gene, a member of the calpain cysteine protease family, is located on chromosome 2q37.3 and consists of 15 exons and 14 introns, covering a region of approximately 31 kb. Genetic variations in the calpain-10 gene can lead to impaired glucose metabolism and cause insulin resistance, thus affecting individual susceptibility to PCOS. Calpain-10 transcriptional activity is elevated in pancreatic islet cells, muscle, and the liver, suggesting that it is involved in the regulation of insulin secretion and action and in the production of hepatic glucose. Polymorphisms of the gene codon for calpain 10 may be involved in the pathogenesis of hyperandrogenic disorders and PCOS **(Shen *et al*., 2013)**.

### 2.3. Genes involved in the action and regulation of gonadotropins

### 2.3.1. LH gene and its receptor

The Luteinizing Hormone Receptor (LHR) plays a pivotal role during follicular development and the thecal production of androgens. Consequently, its expression pattern is of major importance for research and has clinical implications **(Yung *et al*., 2014)**.

Structural analysis of the LH b subunit gene reveals the existence of Trp8Arg and Ile15Thr polymorphisms responsible for structural variants of LH. These abnormalities were found with the same frequency in women with PCOS and in normal subjects. It is interesting to recall that inactivating mutations of the LH receptor have been identified in girls with a normal karyotype (XX), having primary-secondary amenorrhea with an elevated level of LH and polycystic ovaries on ultrasound (**Dewailly, 2005; Yung *et al*., 2014**).

### 2.3.2. FSH gene and its receptor

The Follicle-Stimulating Hormone Receptor (FSHR) is found exclusively on granulosa cells from as early as the two-layer or primary stage of folliculogenesis (**Findlay and Drummond, 1999**). Granulosa cells secrete FSH to play a key role in follicular maturation and estrogen secretion. In patients with PCOS, there is an arrest of follicular maturation, which suggests probable abnormalities of the FSH gene and/or its receptor resulting from a C76T polymorphism on exon 3 (**Dewailly, 2005**).

### 2.3.3. Follistatin gene

Follistatin has been reported as a candidate gene for PCOS through linkage and association studies. Affecting the development of ovarian follicles and acting as an antagonist to aromatase activity, alterations in follistatin function or expression may result in key features of PCOS such as reduced serum FSH, impaired ovarian follicle development, and augmented ovarian androgen production (**Jones *et al*., 2007**).

### 2.3.4. Dopamine receptor genes

Dopamine inhibits the secretion of GnRH and prolactin. Polymorphisms associated with the PCOS phenotype have been identified at the level of the dopamine D2 and D3 receptor genes (**Dewailly 2005**).

## 2.4. Detoxification genes

*GSTM1* and *GSTT1*, located at 1q13.35 and 22q11.26, respectively, are members of the GST family (**Azevedo *et al*., 2020**). GSTs are involved in the metabolism of a wide range of xenobiotics, including carcinogens, pollutants, and drugs. Polymorphisms in the *GSTM1* and *GSTT1* genes that are prevalent among individuals give different susceptibilities to developing diseases. Polycystic ovary syndrome is one of these diseases. (**Sharma, Pandey *et al*., 2012**).

Where the study was conducted on the existence of a relationship between polymorphic deletion and the incidence of polycystic ovary syndrome (**Azevedo *et al*., 2020**).

### 3. Epigenetic of PCOS

The genetic loci associated with PCOS so far account for only ~10% of its heritability, which is estimated at 70%. Nevertheless, rising evidence suggests that altered epigenetic and developmental programming subsequent to hormonal dysregulation of the maternal uterine environment contributes to the pathogenesis of PCOS. Male as well as female relatives of women with PCOS are also at an amplified risk of developing PCOS-associated reproductive and metabolic syndromes. Although PCOS phenotypes are extremely heterogenous, hyperandrogenism is thought to be the principal driver of this condition **(Stener-Victorin and Deng, 2021)**.

Current research has shown that the interaction of susceptible and protective genomic variants under the influence of environmental factors can modify the clinical presentation via epigenetic modifications. MicroRNA (miRNA) are regulators of gene expression. Altered miRNA expression has been associated with various diseases such as diabetes, insulin resistance, inflammation, and cancer. Several miRNA have been identified in PCOS **(Ilie and Georgescu, 2015)**.

### 4. Genes of interest

### 4.1. *CYP17* gene and PCOS

One of the reasons of ovarian hyperandrogenism in PCOS is thought to be a deregulated P450 CYP17 enzyme, Whereas, the increased expression of cyp17 enzyme led to an increase in androgens in PCOS. Any CYP 17 activity in the ovary's theca cells contributed to the existence of ovaries, according to studies published in 2004. Many studies conducted around the world have discovered a link between polymorphism of the *CYP17* gene and elevated androgen levels in PCOS, the polymorphism was determined in three different single nucleotides in the *CYP17* gene: a first change in the single base-pair (C → A transition) in the intron 6 of the *CYP17* gene at nucleotide 5471; a second change SNP (G → A) at nucleotide 1951 in the promoter of the *CYP17* gene and the last change that has been extensively scrutinized in the 5′-untranslated region (5′-UTR), a single base-pair change (T-C) in the promoter region, 34 base pairs upstream from the translational initiation point **(Liu *et al*., 2021; Xu *et al*., 2021)**.

Through all these studies, a relationship was concluded between hyperandrogenism in PCOS and increased Cyp17 enzyme production, and a link between single nucleotide polymorphisms of the *CYP17* gene and PCOS **(Ashraf *et al*., 2019)**.

## 4.2. *ACE* gene and PCOS

Angiotensin Converting Enzyme (ACE) is a zinc metallopeptidase that functions largely as a dipeptidyl-carboxy-peptide synthase on the cell surface (**Marcous *et al*., 2004**). ACE has a role in the homeostatic regulation of blood pressure and electrolyte balance, and it has been related to a range of cardiovascular and renal illnesses (**Laraqui, 2006 ; Shen *et al*., 2014**). Additionally, this enzyme is involved in the Renin-Angiotensin System (RAS) by converting angiotensin I into angiotensin II, a powerful vasoconstrictor, and metabolic inactivation of bradykinin, a vasodilator peptide (**Corvol *et al*., 2004**). ACE is found in large amounts throughout the body and in bodily fluids. It can be found vascularly in the lungs, proximal tubular epithelium of the kidney, small intestine, and choroid plexus, or tissue wise in the lungs, proximal tubular epithelium of the kidney, small intestine, and choroid plexus. It can be found in the lungs, kidney's proximal tubular epithelium, small intestine, and choroid plexus, as well as tissue in the kidney, heart, and brain (**Nguyen, 2014**). In humans, two types of ACE have been identified: somatic ACE, which is the most abundant isoenzyme and is found in a membrane form (endothelial, epithelial, and neuroepithelial cells) and more specifically in the beds capillaries of the lungs, with a Molecular Weight (MW) of 160 kDa, and soluble ACE, which is slightly smaller and freely circulating in plasma, cerebrospinal fluid. The germinal for  of ACE, a testicular version of PM 90 kDa seen only in sperm, is also present (**Laraqui, 2006**).

This enzyme is encoded by a gene on chromosome 17q23 that is approximately 21 Kb long and has 26 exons and 25 introns. Exons 1 to 26 except exon 13 are used to make a somatic ACE, which is extensively dispersed in the organism, and exons 13 to 26 are used to make a testicular ACE, which is essential for male fertility (**Sayed-Tabatabaei *et al*., 2006**). **Rigat *et al*.,** initially revealed *ACE* gene polymorphism in 1990 in a study on the involvement in the genetic control of plasma ACE levels (**Rigat *et al*., 1990**). More than 160 genetic variations have been identified for this gene, the majority of which are single nucleotide polymorphisms, according to the National Center for Biotechnology Information (NCBI). Only 34 of these polymorphisms are in coding areas, with 18 of them being missense mutations (**Sayed-Tabatabaei *et al*., 2006**).

Ovarian tissues contain all the elements for the production of angiotensin, including prorenin/ renin, angiotensinogen and ACE. Angiotensin II is implicated to play a role in ovulation, steroidogenesis, follicular atresia and hyperandrogenic syndromes (**Yoshimura, 1997**). Previous studies indicated that the RAS might be involved in the development of PCOS. It has been reported that the ovarian RAS may be up-regulated in the ovaries of women with PCOS. Considering the central role of ACE in the RAS, it is reasonable to hypothesize that ACE may be a potential biological candidate for PCOS (**Ramanathan *et al*., 2021**).

# Practical part

# Patients and methods

## 1. Study Framework

We carried out between March 01 and May 31, 2021, a statistical and molecular, cross-sectional, descriptive study with prospective, multicentric recruitment at the level of several private gynecology practices in the city of Constantine. This study aims to evaluate the impact of a genetic variant (I/D polymorphism of the *ACE* gene) in the determinism of PCOS.

## 2. Inclusion and exclusion criteria

The enrollment of women with PCOS is done after the confirmation of the diagnosis by the gynecologist. This confirmation is done by referring to the Rotterdam criteria (**ESHRE/ASRM Consensus, 2004**). According to these criteria, we selected any patient presenting for consultation during the period mentioned above and who presents at least 2 of the following 3 clinical-biological characteristics: oligo or anovulation, clinical and/or biological hyperandrogenism and finally an ultrasound of OPK (of at least 12 follicles of 2 to 9 mm in diameter per ovary and/or ovarian volumes greater than 10 ml per ovary).

Following the same consensus, exclusion criteria were rigorously established. Indeed, in front of clinical signs of hyperandrogenism or android obesity, it is necessary to evoke the diagnosis of polycystic ovary syndrome while taking care to eliminate pathologies which present the same clinical manifestations, associated with menstrual irregularities and major signs. Virilization (hoarseness of the airway, major alopecia). These pathologies can be: Cushing's syndrome, hyperprolactinemia, congenital adrenal hyperplasia called "non-classical", androgen-secreting adrenal tumors or possible androgen-secreting ovarian tumors. Iatrogenic causes can also lead to confusion.

All the women included in the statistical study, after explanations on site, gave us their consent, thus authorizing us to use their clinical and biological data. These women responded to a questionnaire aimed at collecting data related to the dysfunction studied (**Appendix I**).

## 3. Molecular study

We set up a cross-sectional case-control study to assess the difference in the distribution of a given genetic variant (I/D of the *ACE* gene) between a population of cases, made up of women diagnosed with PCOS, and a population of controls, assumed to be healthy, selected from the general population and who are not carriers of the disorder studied. The objective is to verify, on a "representative sample" of the Algerian population, data published in the literature which associate (or not) this polymorphism with an increased risk of developing PCOS.

## 3.1. Patients

The people included in our study are all women diagnosed with PCOS and recruited from several private gynecology practices in the Constantine region. All patients who participated in this molecular study, after reading and exhaustive explanations, signed an informed consent authorizing us to use their clinical and biological data, as well as their genetic material (DNA) for this molecular study as well as further examinations in the future **(Appendix II)**.

In the molecular component of this study, we included a total of patients meeting the sole inclusion criterion of having PCOS confirmed by a medical specialist. We excluded from this survey patients who refused to take the sample.

## 3.2. Controls

Our control population comes from a previous study carried out as part of a doctoral thesis entitled "Identification of biological and genetic risk factors for coronary atherosclerosis in the Algerian population", presented and supported by Dr SEMMAME-BENSAKESLI Ouarda in 2017. The cohort of healthy controls recruited in this study is considered to be representative of the distribution of the polymorphism of interest (I/D of the *ACE* gene), subject of our study, in the Algerian population. This control population comprises 31 female subjects, apparently healthy, after completion of a questionnaire. Were excluded from this cohort of controls male subjects **(Semmame-Bensakesli *et al*., 2017)**.

## 3.3. Genetic analysis

After recruiting the patients, the DNA extraction, as well as the molecular analysis which followed for the study of the Ins/Del polymorphisms (*rs*1799752) of the *ACE* gene (OMIM: 106180) were carried out at the level of the laboratory of molecular biology pedagogy of the SNV faculty - Constantine 1 University.

### 3.3.1. DNA extraction from whole blood

### 3.3.1.1. The blood samples

The blood sample (5 to 10 ml) intended for DNA extraction is collected under sterile conditions by venipuncture, in a vacutainer tube containing EDTA (Ethylene Diamino Tetracetic Acid) as anticoagulant.

### 3.3.1.2.    DNA extraction

The DNA extraction technique used on a whole blood sample uses an inorganic solvent, NaCl, known as Miller's method. The extraction is done in three steps; the preparation of the leukocytes, the actual DNA extraction and finally the solubilization (**Miller *et al*., 1988**). After taking a blood sample of 5 to 10 ml in EDTA tubes, the DNA extraction is launched immediately or if the conditions do not allow it within 3 days of taking the sample stored at +4°C. DNA extraction is done in 3 steps:

- **Preparation of leukocytes:** the leukocytes are separated from the blood by hypotonic lysis of the cells in a Tris-EDTA buffer (20 mM Tris, 5 mM EDTA, pH 7.5) (TE) 20:5 for 10 minutes on ice. After washing, the pellet is resuspended in TE 20:5.

- **DNA extraction:** is done by adding a lysis buffer (400 mM NaCl, 2 mM EDTA, 10 mM Tris, pH 8.2), 10% Sodium Dodecyl Sulfate (SDS) and 10 mg/ml proteinase K. The tubes are rotated on a wheel, at 27° C., overnight, and are cooled the following day in ice for 5 minutes. 1 ml of 4M NaCl is then added to allow the release of the nuclear DNA in the lysate as well as the digestion and the elimination of the proteins which are associated with it by precipitation with this inorganic solvent. The DNA pellet is formed in the supernatant by precipitation with pure ethanol. Once the ball of DNA has been recovered with a Pasteur pipette, it is rinsed twice in 70% ethanol and then placed in a 1.5 ml Nunc® tube.

- **Solubilization:** the DNA thus obtained is dissolved in the aqueous phase, by adding between 300 and 1000 μl of bi-distilled water depending on the size of the ball. The mixture is left overnight on a rotator-stirrer at 37° C., then at ambient temperature until complete dissolution. This operation lasts between 1 and 2 days.

### 3.3.2. Determination of purity and quality of extracted DNA

The purity as well as the concentration of the DNA are determined by UV spectrophotometry. This is a spectrophotometer that does not require the use of a cuvette. A volume of 5 μl of the sample is deposited directly at the end of an optical cuvette. The absorbance at different wavelengths is recorded.

The DNA absorbs at 260 nm whereas the proteins which represent the contamination controls absorb at 280 nm. Absorption (absorbance or Optical Density (OD)) is measured at two different wavelengths (260 and 280). Subsequently, the 260/280 ratio is established to assess the purity of the DNA is determined by checking for possible contamination by proteins or by RNA. It is considered that: the DNA is sufficiently pure when the ratio R = DO 260/280 is between 1.6 and 2 ($1.6 < R \leq 2$), the DNA is contaminated by proteins if $R < 1, 6$ and that the DNA is contaminated by RNA if $R > 2$.

### 3.3.3. Genotyping

In order to genotype our population for the polymorphism of interest (I/D) of the *ACE* gene, we had to perform a simple PCR technique. This technique is based on the difference in size of the amplicon (the product of amplification) between the I allele and the D allele. Indeed, the expected size of the fragments is 490 bp in the case of insertion (allele I) and 190 bp in the case of deletion (allele D), which allows us to identify the three possible genotypes: II (homozygous I), ID (heterozygous) and DD (homozygous D).

### 3.3.3.1.    Amplification of the region of interest

To prospect for the I/D polymorphism (*rs*4646903) of the *ACE* gene (OMIM: 106180), we PCR amplified a region of intron 16 using a pair of specific primers.

**Table I.** Sequences of the primers used for the amplification of the region of interest.

| Primers | Sequence (5'→3') | Region Size amplified (pb) |
|---------|------------------|----------------------------|
| *ACE*(F) | 5'-CTGGAGACCACTCCCATCCTTTCT-3' | 490 bp (I allele) |
| *ACE*(R) | 5'-GATGTGGCCATCACATTCGTCAGAT-3' | 190 bp (D allele) |

Primer solution prepared separately for (F) and (R) by a 1/6th dilution from the stock solution: 10µl (F) or (R) + 50µl of bi-distilled water.

The reagents used for the preparation of the PCR reaction medium as well as the quantities necessary for each tube are mentioned in the table below. The preparation of the PCR reaction medium is done in ice.

**Table II.** Composition of the PCR reaction medium for amplification
of the region of interest

| Reagent | Vol/tube (µl) |
|---|---|
| DNA (~100ng) | 1 |
| 10X buffer (without MgCl$_2$) | 1 |
| dNTP 2mm | 1.60 |
| MgCl2 50mm | 0.30 |
| Taq Polymerase (Bioline® 250U Kit) | 0.08 |
| Bi-distilled H$_2$O | 4.02 |
| Primers (F) | 1 |
| Primers (R) | 1 |
| **Total** | **10** |

**Table III.** Thermal cycler program for amplification
of the region of interest (duration: 52 minutes).

| Process | Temperature (°C) | Duration | Cycles |
|---|---|---|---|
| Initial denaturation | 95 | 6 minutes | 1 |
| Denaturation | 95 | 30 seconds | 30 |
| Hybridization | 65 | 30 seconds | |
| Elongation | 72 | 30 seconds | |
| Final elongation | 72 | 1 minute | 1 |

PCR products are stored at 4°C until use.

### 3.3.3.2.     Migration on agarose gel

The migration of PCR products stained with Bromo-Phenol Blue (BPB) (diluted to ½ in TBE1X) is done on a 2% agarose gel (UltraPure™ Agarose) prepared with Ethidium Bromide (BET). The migration takes place under a current at 100 V for 30 minutes and in parallel with the size marker XIV (Marker XIV - 100 bp, Roche®).

The electrophoresis bands made it possible to identify three genotypes: the profiles with a single band of 490 bp or 190 bp correspond respectively to the homozygotes of genotype II and DD. The profile with the two bands 190 bp and 490 bp visualized corresponds to the heterozygous genotype of genotype ID **(figure 07)**.
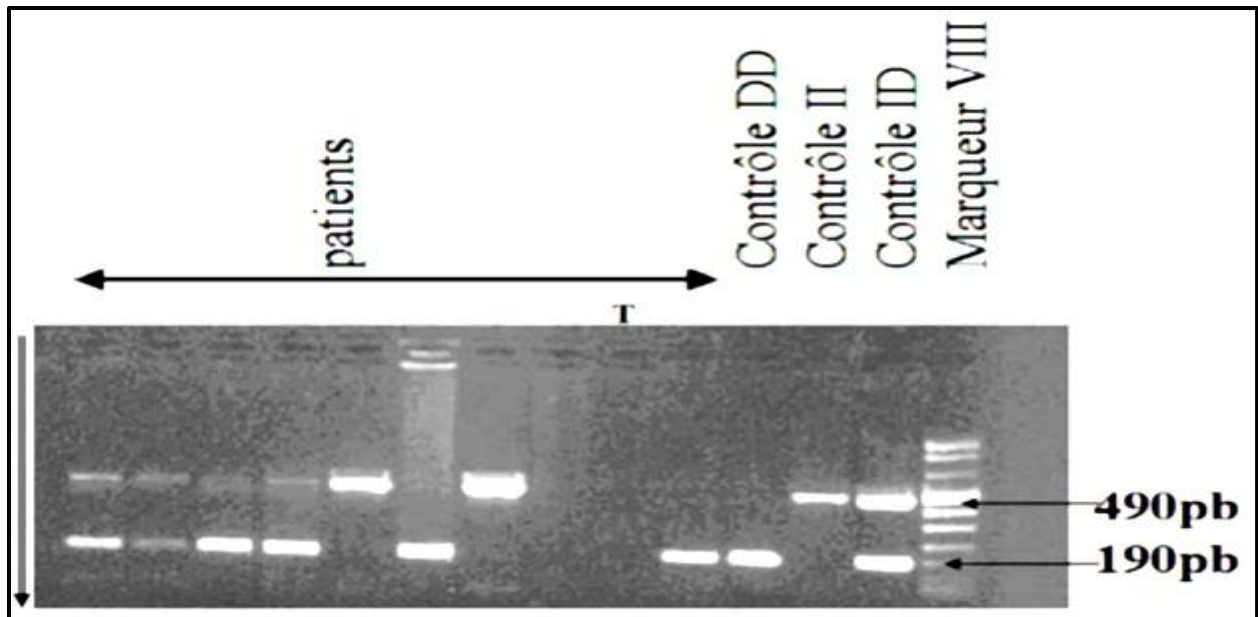
**Figure 07.** Expected electrophoretic migration profile of PCR products and different genotypes of patients. The 490 bp and 190 bp bands correspond respectively to the presence of the insertion (I) and the deletion (D) (**Mehri** *et al*., **2005**).

### 3.3.4. Statistical analysis

Statistical analysis is based on comparisons of genotypic and allelic frequencies between patients and healthy controls, using the $\chi2$ test from the free access software Epi-info® (6.0): http://www.epiconcept.fr.

Prior to any statistical analysis, we performed a *Hardy-Weinberg* Equilibrium (HWE) evaluation to avoid large errors due to genotyping or selection bias. To verify that our population is in HWE, we used the standard $\chi2$ test. This classic evaluation of the $\chi2$ is possible when the counts are greater than 5. Otherwise, it is necessary to use the corrected $\chi2$, either with the Yates correction (count less than 5) or with the Fisher correction (count less than 3). This was done online at: http://analysis.bio-x.cn/SHEsisMain.htm.

Genotyping results for the studied polymorphism of all our patients and controls were processed by Excel (Microsoft Office® 2016) and compared by Epi-info® software (6.0) to assess the significance of the association between the factor studied risk and susceptibility to PCOS. To do this, we use a typical 2×2 cross contingency table:

**Table IV.** Crossed contingency table.

|  | Patients | Controls | Total |
|---|---|---|---|
| Presence of the presumed genetic risk factor for the pathology | has | B | **a+b** |
| Absence of the presumed genetic risk factor for the pathology | vs | D | **c+d** |
|  | **a+c** | **b+d** | **a+b+c+d** |

The OR (Odds Ratio) and the 95% confidence intervals (Confidence Interval: CI) were calculated taking into account the allele at risk or the genotypes containing the allele at risk for our polymorphism. A particularity for this variant is that the D and I alleles of the *ACE* gene are codominant. The evaluation of the degree of significance (*p*-value) of the differences in frequencies of each genotype between patients and controls corresponds to the probability that the overall difference is attributable solely to fluctuations of chance. When the probability *p* is equal to or less than 0.05 (5%), there is less than a 5 in 100 chance that the distribution results from chance. Thus, the difference in distribution between the patient and control populations for a given marker is deemed to be statistically significant and the genetic marker studied, in this context, can be considered to be associated with PCOS. We analyzed 4 possible effects of the I and D alleles on the patients in comparison with our controls.

**Table V.** Formulation of different comparison models for the study
of the effect of alleles I and D for the polymorphism of the *ACE* gene.

| Analyzed effect | Comparison model |
|---|---|
| **Dominant effect** | D/D *vs*. D/I + I/I |
| **Recessive effect** | I/I *vs*. D/I + D/D |
| **Heterozygote effect** | ID *vs*. DD + II |
| **Allelic effect** | I *vs*. D |

## 4.     Metanalysis

To determine whether the *CYP17* (OMIM: 609300) T/C (*rs*74357) gene polymorphism is an exposure risk for PCOS, we performed a comprehensive meta-analysis summarizing all previous published case-control studies on this topic. This meta-analysis was carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines **(Page *et al*., 2021) (Appendix III)**. PRISMA extensions can be reached at their website: www.prisma-statement.org. The methodology used in detail for the realization of our genetic meta-analysis including case-control studies on the subject is developed in the article presented in this thesis.

# Results and discussion

# Results and discussion
# Part I

*ACE* gene I/D polymorphism:
a case-control study

After extracting the DNA, carrying out a PCR and subjecting the amplification products of the region of interest to migration on an agarose gel, we obtained the following electrophoretic profile:
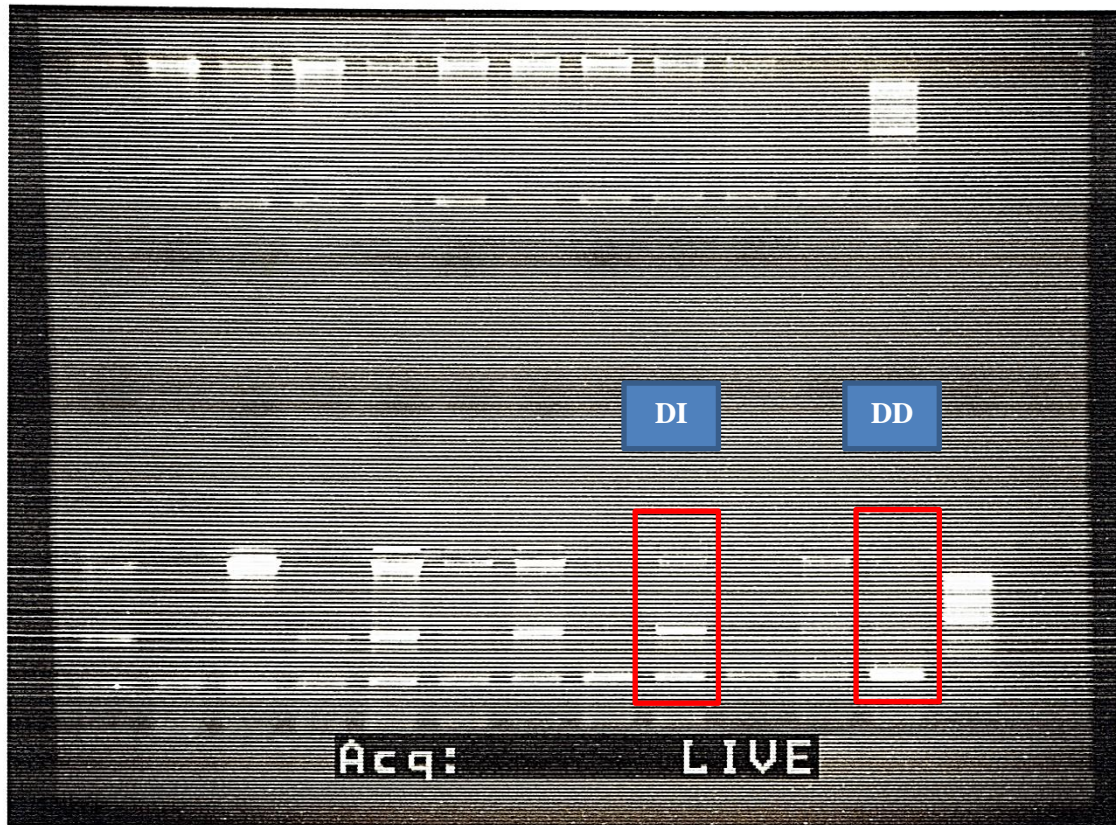


**Figure 08.** Electrophoresis profile of PCR-amplified fragments after migration.
M: marker DD: homozygous deletion II: homozygous insertion ID: heterozygous I/D

It should be emphasized that we submitted 12 samples from women with PCOS to genetic testing in order to discover the *ACE* gene's I/D polymorphism. However, two samples were damaged when they were placed in the wheel at 37 degrees Celsius because they were not firmly sealed and only 9 patients' genotypes were disclosed by the electrophoretic profile. We were unable to obtain interpretable results for one subject. We were unable to redo the technique.

The rigorous reading of the electrophoretic profiles obtained allowed us to establish the genotypes of our 09 patients and to calculate the genotypic and allelic frequencies. As a reminder, our control population (defined in the patients and methods section) comes from a previous study which was carried out on our gene variant of interest in association with another dysfunction. By the way, we just noted the genotypic and allelic frequencies mentioned in the study. The genotypic and allelic frequencies of the I/D polymorphism of the *ACE* gene in our study population are detailed in the table below (**table VI**).

**Table VI.** Genotypic and allelic frequencies of the I/D polymorphism of the *ACE* gene in our study population.

| PCOS | | | | | Controls | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **DD** | **ID** | **II** | **D** | **I** | **DD** | **ID** | **II** | **D** | **I** |
| 06 66.67% | 03 33.33% | 00 00.00% | 15 83.33% | 03 16.67% | 16 51.61% | 08 25.81% | 07 22.58% | 40 64.52% | 22 35.48% |
| 09 100% | | 18 100% | | | 31 100% | | 62 100% | | |

Comparison of genotypic frequencies between the two cohorts of women with PCOS and controls. The genotypic frequencies of women with PCOS and controls were compared, and there were significant differences. The DD genotype was the most prevalent in both cohorts, with 66.67% for SPOKs and 51.61% for controls, respectively. In terms of the heterozygous genotype, the two groups had nearly identical frequencies: 33.33% for the sick and 25.81% for the controls. The largest significant difference in genotype distribution between the two groups is homozygote II. Indeed, the latter was absent in our study's women with PCOS, while it was present at a rather high frequency of 22.58% in the controls (**figure 09**).
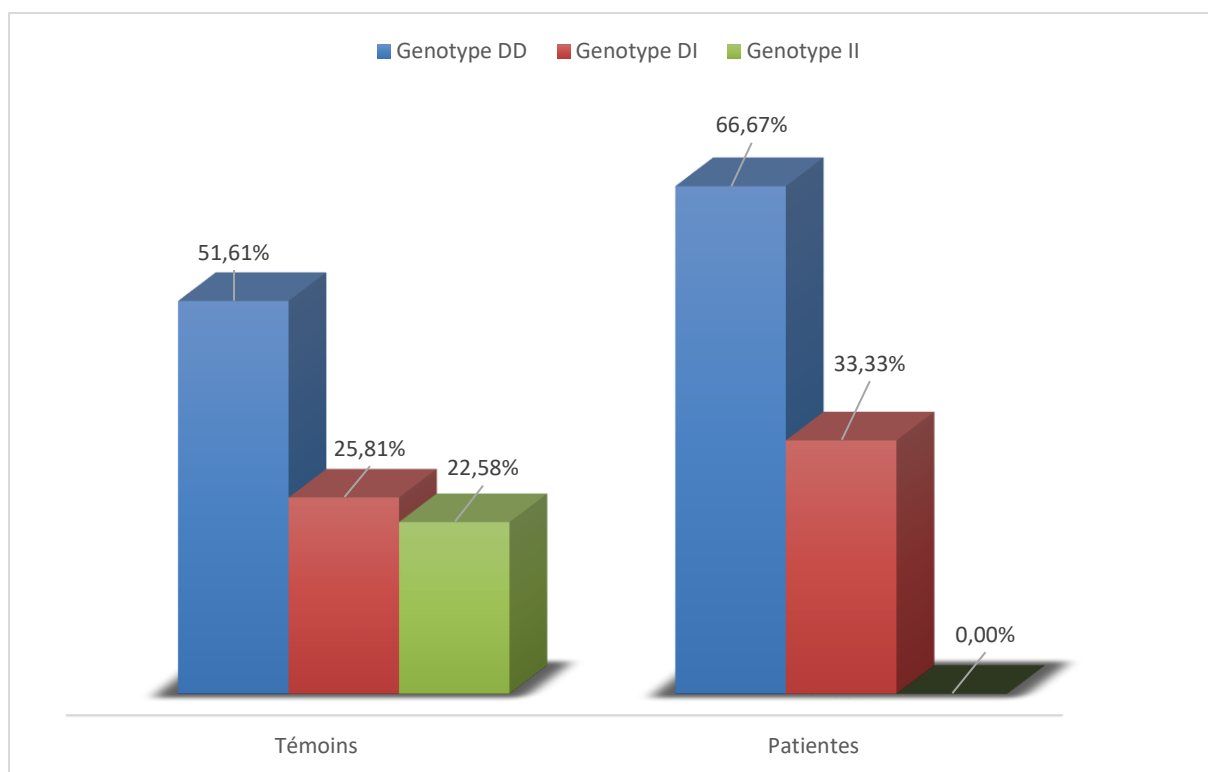
**Figure 09.** Genotypic frequencies.

For allele frequencies, the distribution of the D and I alleles in our two series was more or less heterogeneous with a more marked difference between the frequency of the D allele and the I allele in the patients than in the controls. However, in both cohorts, the D allele was the most frequent with proportions of 83.33% in PCOS and 64.52% in controls **(figure 10)**.
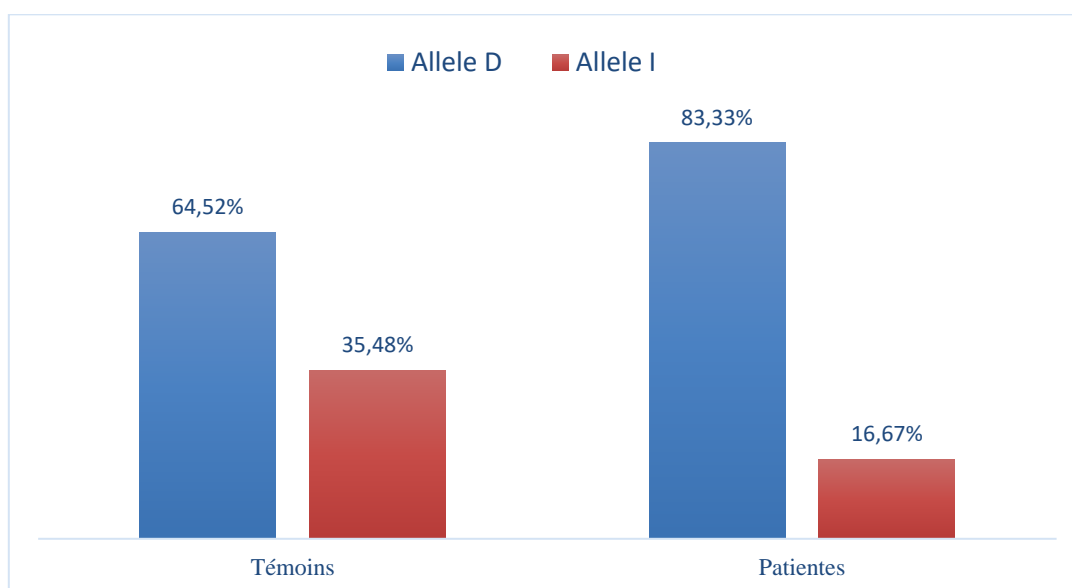


**Figure 10.** Allele frequencies.

To assess the real significance of this observed heterogeneity in genotypic and allelic frequencies between the two cohorts, we conducted a statistical case-control study. However, before proceeding with the statistical analysis, we subjected the values of the distribution of the different genotypes in the patient cohort to the test aimed at determining whether a study population is indeed in *Hardy-Weinberg* equilibrium.

We got a *p*-value = 0.0151; value less than 0.05 which suggests that our population is not in equilibrium. This observation determines the reliability of the results obtained at the end of the statistical study.

The genotyping results for the investigated variant in the *ACE* gene demonstrate a variation in genotype distribution between patients and controls. The differences in genotype distribution are statistically non-significant, according to the heterozygous comparison model, with a value of *p* respectively of 0.099 asymptotically equal to the significance level set at 0.05. Homozygous genotype II was found to be non-existent in the OPK cohort but prevalent in the control group with a frequency of 22.58 %. This difference in distribution is statistically significant, according to the dominant model, with a *p*-value of 0.262 (> 0.05). The analysis of allele frequencies also revealed a difference in the distribution of the least frequent allele (I) statistically  significant between patients (16.6703%) and controls (35.4822%).  Indeed, we obtained a p-value of 0.036 for the allelic comparison model **(Table VII).**

**Table VII.** Results of statistical analysis of the effect of polymorphism
I/D of the *ACE* gene established by the χ2 test.

| | Controls % | n | PCOS % | n | OR (95% CI) | *p-value* |
|---|---|---|---|---|---|---|
| **DD vs DI+II (Dominant model)** | 51.61 | 16 | 66.67 | 06 | 0.5333 [0.1777; 1.6011] | **0.262** |
| **ID vs. DD+II (Co-dominant model)** | 25.81 | 08 | 33.33 | 03 | 1.4375 [0.4629; 4.4639] | **0.530** |
| **II vs DI+DD (Recessive pattern)** | 22.58 | 07 | 00.00 | 00 | 0.1719 [0.0212; 1.3934] | **0.099** |
| **D allele** | 64.52 | 40 | 83.33 | 15 | 0.3636 [0.1406; 0.9408] | **0.036** |
| **I allele** | 35.48 | 22 | 16.67 | 03 | | |

Although the data obtained suggest that the I/D polymorphism of the *ACE* gene plays some role in the risk of developing PCOS and that carriers of the heterozygous ID genotype present a significantly increased risk compared to those carrying the homozygous DD and homozygous II genotype , faced with the extremely small number of patients in the cohort (9 cases), these results do not allow us to draw clear and definitive conclusions as to the degree of incrimination of the polymorphism studied in the genesis of PCOS in Constantinian women.

In recent years, a significant amount of research has been undertaken to clarify the effect of the I/D polymorphism of the *ACE* gene in the pathogenesis of SPOK. The study of this association was initiated for the first time in **1999** by **Cao *et al*.**, and the latest in **2021** conducted by **Ramanathan *et al*.** All these studies were carried out in different countries (China, Turkey, Greece, India, Poland, Brazil, Pakistan and Algeria) and on different ethnic groups (Caucasians, Afro-Americans and Asians). These 15 studies prior to ours that investigated the association between the I/D polymorphism of the *ACE* gene and the risk of developing PCOS have reported rather contradictory results.

Of these studies, 11 of them reported a positive association and suggest that the D allele is indeed a risk factor for the dysfunction studied (**Cao** *et al*.**, 1999 ; Cao** *et al*.**, 2002 ; Li** *et al*.**, 2008 ; Che** *et al*.**, 2009 ; Bayram** *et al*.**, 2011 ; Koika** *et al*.**, 2012 ; Deepika** *et al*.**, 2012 ; Ożegowska** *et al*.**, 2016 ; Cintra** *et al*.**, 2018 ; Nazeer** *et al*.**, 2021 ; Dif and Lebrima, 2021**).

Only four studies, including two conducted in the Turkish population, have ruled out the presence of such an association and report that there is no statistically positive difference in the distribution of genotypic and allelic frequencies between PCOS patients and healthy controls (**Sun** *et al*.**, 2010 ; Karabulut** *et al*.**, 2010 ; Celik** *et al*.**, 2010 ; Ramanathan** *et al*.**, 2021**).

The Algerian study carried out last year by **Dif and Lebrima** as part of a Master's thesis in PCPP concluded with a positive association. This study built according to the same model was carried out on a series of 18 patients with PCOS and highlighted statistically significant differences in distribution according to the recessive ($p = 0.02943$) and allelic ($p = 0, 0468$) models.

The results obtained from all these studies are grouped in the **table VIII** and illustrated in **figures 11** and **12**.

**Table VIII.** Collection of genotypic and allelic frequencies reported in various case-control studies on the involvement of the I/D polymorphism of the *ACE* gene in the development of PCOS.

**S:** statistically significant association      **NS:** statistically non-significant association

| N° | Author and year | Country | Association | Cases (PCOS) | | | | | | | | | | | Controls | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cohort | Genotype DD | DD (%) | Genotype DI | DI (%) | Genotype II | II (%) | Allele D | D (%) | Allele I | I (%) | Cohort | Genotype DD | DD (%) | Genotype DI | DI (%) | Genotype II | II (%) | Allele D | D (%) | Allele I | I (%) |
| 1 | **Cao *et al*., 1999** | China | S | 56 | 16 | 28,57 | 15 | 26,79 | 25 | 44,64 | 47 | 41,96 | 65 | 58,04 | 30 | 3 | 10,00 | 10 | 33,33 | 17 | 56,67 | 16 | 26,67 | 44 | 73,33 |
| 2 | **Cao *et al*., 2002** | China | S | 50 | 14 | 28,00 | 13 | 26,00 | 23 | 46,00 | 41 | 41,00 | 59 | 59,00 | 30 | 3 | 10,00 | 10 | 33,33 | 17 | 56,67 | 16 | 26,67 | 44 | 73,33 |
| 3 | **Li *et al*., 2008** | China | S | 102 | 52 | 50,98 | 23 | 22,55 | 27 | 26,47 | 127 | 62,25 | 77 | 37,75 | 101 | 23 | 22,77 | 17 | 16,83 | 61 | 60,40 | 63 | 31,19 | 139 | 68,81 |
| 4 | **Che *et al*., 2009** | China | S | 346 | 98 | 28,32 | 160 | 46,24 | 88 | 25,43 | 356 | 51,45 | 336 | 48,55 | 236 | 77 | 32,63 | 107 | 45,34 | 52 | 22,03 | 261 | 55,30 | 211 | 44,70 |
| 5 | **Sun *et al*., 2010** | China | NS | 142 | 47 | 33,10 | 67 | 47,18 | 28 | 19,72 | 161 | 56,69 | 123 | 43,31 | 107 | 26 | 24,30 | 52 | 48,60 | 29 | 27,10 | 104 | 48,60 | 110 | 51,40 |
| 6 | **Karabulut *et al*., 2010** | Turkey | NS | 30 | 19 | 63,33 | 7 | 23,33 | 4 | 13,33 | 45 | 75,00 | 15 | 25,00 | 33 | 15 | 45,45 | 14 | 42,42 | 4 | 12,12 | 44 | 66,67 | 22 | 33,33 |
| 7 | **Celik *et al*., 2010** | Turkey | NS | 32 | 16 | 50,00 | 12 | 37,50 | 4 | 12,50 | 44 | 68,75 | 20 | 31,25 | 31 | 7 | 22,58 | 20 | 64,52 | 4 | 12,90 | 34 | 54,84 | 28 | 45,16 |
| 8 | **Bayram *et al*., 2011** | Turkey | S | 100 | 56 | 56,00 | 24 | 24,00 | 20 | 20,00 | 136 | 68,00 | 64 | 32,00 | 100 | 28 | 28,00 | 47 | 47,00 | 25 | 25,00 | 103 | 51,50 | 97 | 48,50 |
| 9 | **Koika *et al*., 2012** | Greece | S | 801 | 313 | 39,08 | 395 | 49,31 | 93 | 11,61 | 1021 | 63,73 | 581 | 36,27 | 266 | 109 | 40,98 | 112 | 42,11 | 45 | 16,92 | 330 | 62,03 | 202 | 37,97 |
| 10 | **Deepika *et al*., 2012** | India | S | 259 | 100 | 38,61 | 97 | 37,45 | 62 | 23,94 | 297 | 57,34 | 221 | 42,66 | 315 | 97 | 30,79 | 162 | 51,43 | 56 | 17,78 | 356 | 56,51 | 274 | 43,49 |
| 11 | **Ożegowska *et al*., 2016** | Poland | S | 138 | 79 | 57,25 | 52 | 37,68 | 7 | 5,07 | 210 | 76,09 | 66 | 23,91 | 110 | 29 | 26,36 | 49 | 44,55 | 32 | 29,09 | 107 | 48,64 | 113 | 51,36 |
| 12 | **Cintra *et al*., 2018** | Brazil | S | 97 | 53 | 54,64 | 24 | 24,74 | 20 | 20,62 | 130 | 67,01 | 64 | 32,99 | 94 | 51 | 54,26 | 29 | 30,85 | 14 | 14,89 | 131 | 69,68 | 57 | 30,32 |
| 13 | **Nazeer *et al*., 2021** | Pakistan | S | 161 | 91 | 56,52 | 12 | 7,45 | 58 | 36,02 | 194 | 60,25 | 128 | 39,75 | 90 | 35 | 38,89 | 15 | 16,67 | 40 | 44,44 | 85 | 47,22 | 95 | 52,78 |
| 14 | **Dif et Lebrima, 2021** | Algeria | S | 18 | 12 | 66,67 | 6 | 33,33 | 0 | 0,00 | 30 | 83,33 | 6 | 16,67 | 31 | 16 | 51,61 | 8 | 25,81 | 7 | 22,58 | 40 | 64,52 | 22 | 35,48 |
| 15 | **Ramanathan *et al*., 2022** | India | NS | 100 | 15 | 15,00 | 55 | 55,00 | 30 | 30,00 | 85 | 42,50 | 115 | 57,50 | 100 | 60 | 60,00 | 30 | 30,00 | 10 | 10,00 | 150 | 75,00 | 50 | 25,00 |
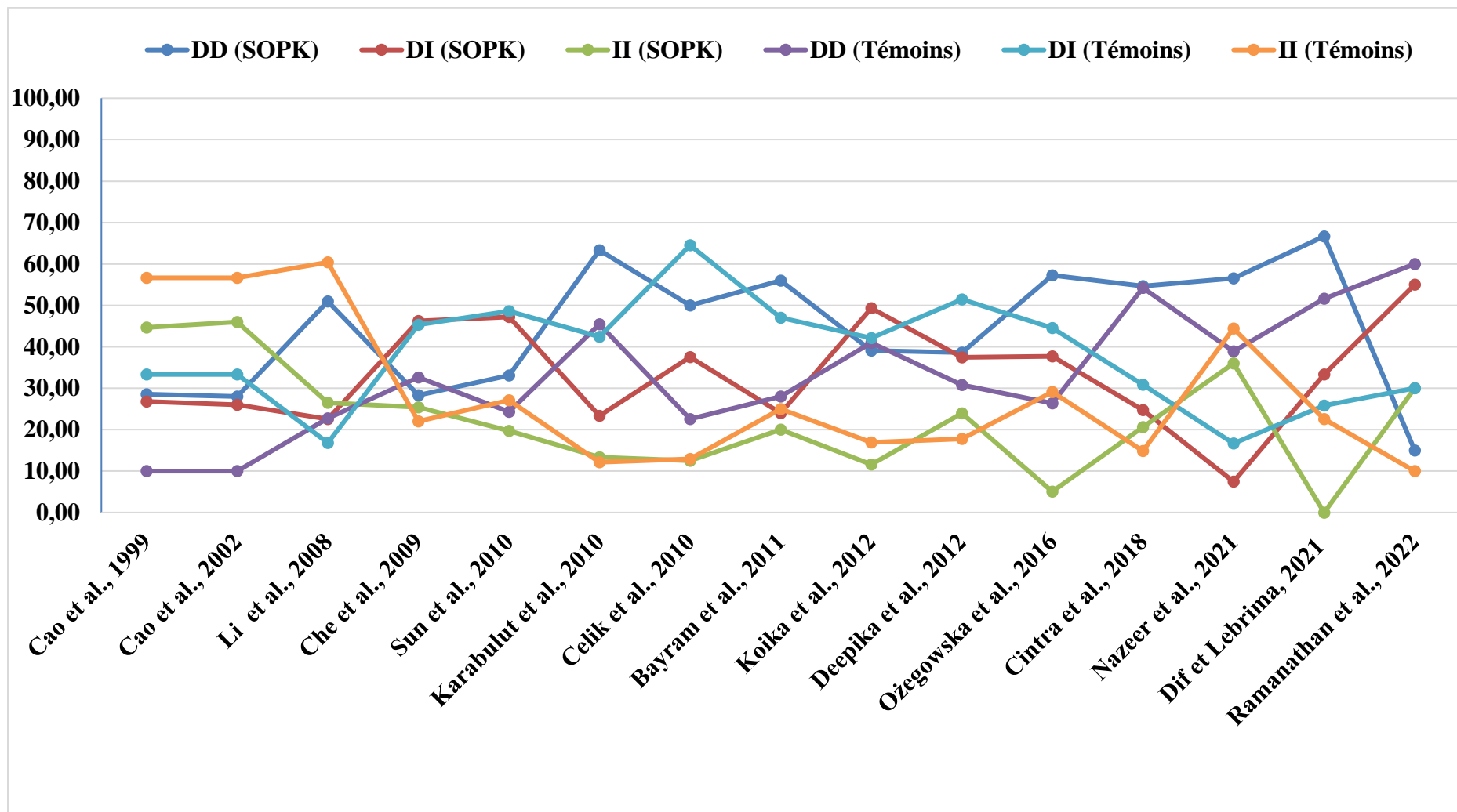
**Figure11.** Graphical representation of genotypic frequencies reported in different case-control studies on the involvement of the I/D polymorphism of the *ACE* gene in the development of PCOS.
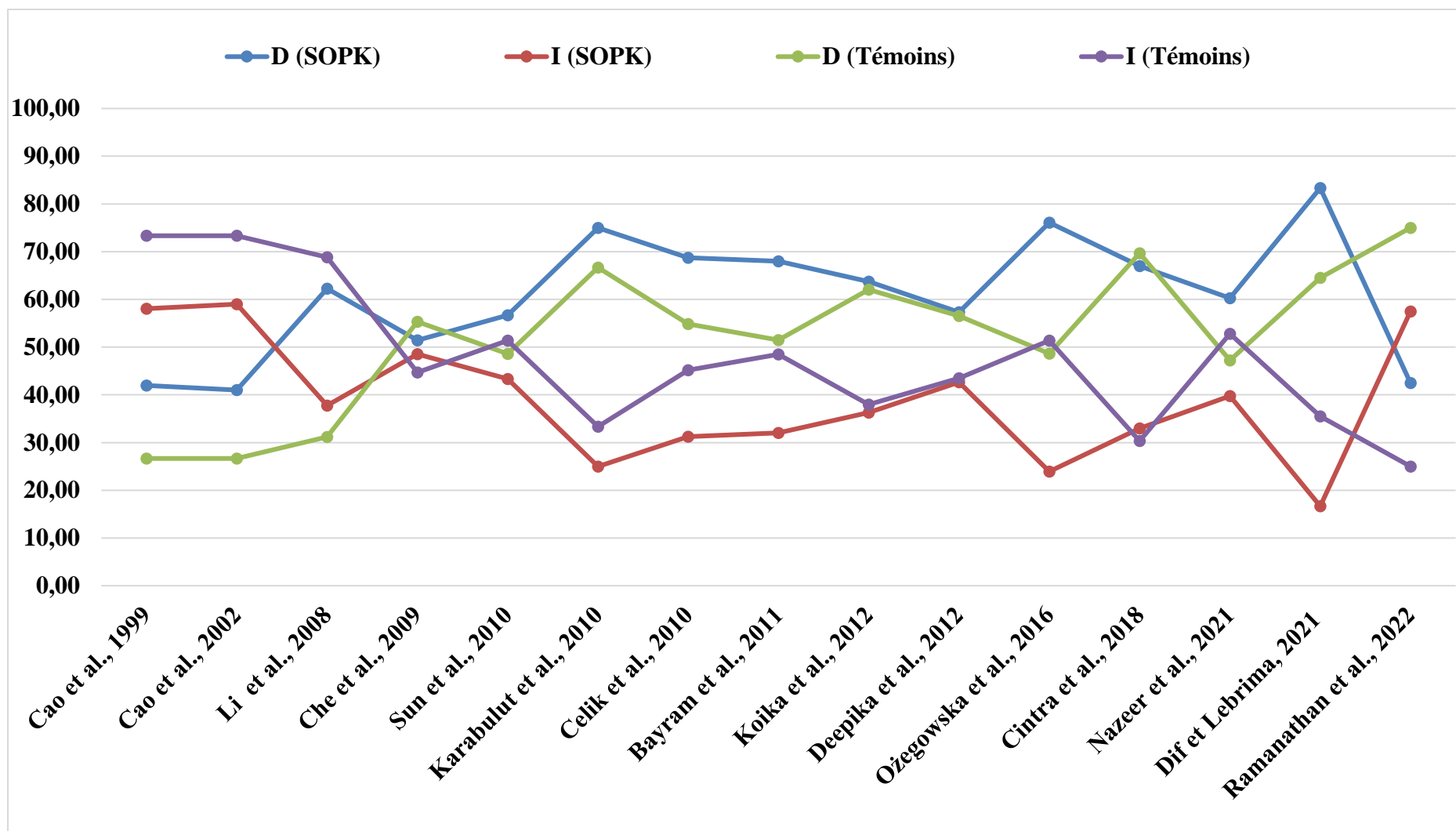
**Figure 12.** Graphical representation of allelic frequencies reported in different case-control studies on the involvement of the I/D polymorphism of the *ACE* gene in the development of PCOS.

According to our study, women with one or two copies of the I allele had a lower risk of PCOS than those with the DD genotype, and the I allele has a lower activity (low risk) than the D allele, which has a higher activity (high risk). Indeed, we identified statistically significant differences between the two cohorts of women with PCOS and healthy controls, according to allelic models, with *p* value = 0.036. The results of our study suggest the relevance of the *ACE* gene's I/D polymorphism in the incidence of PCOS, but the molecular processes behind these dysfunctions remain unknown. The I/D polymorphism of the *ACE* gene is thought to play a substantial impact in the development of PCOS, with carriers of the homozygous DD genotype having a significantly higher risk than those with the heterozygous ID and homozygous II genotypes.

ACE or Angiotensin Converting Enzyme is a key component of the renin-angiotensin system. It is a zinc metalloprotease that can convert angiotensin I to angiotensin II, which is the main effector peptide of the system. It exists both in a form anchored to the membrane, on the surface of endothelial and epithelial cells, and in a circulating form in the plasma **(Karabulut *et al*., 2010)**. Given the central role of ACE in RAS, it is reasonable to assume that this enzyme could be a potential candidate to explain the pathophysiology of PCOS **(Schwentner *et al*., 2011)**.

The *ACE* gene, located on chromosome 17q23, contains a polymorphism based on the presence (Insertion, I) or absence (Deletion, D) in intron 16 of a 287-base pair Alu repeat sequence. This results in 3 genotypes: two homozygous, DD and II, and one heterozygous ID genotype **(Deepika *et al*., 2013)**. Plasma ACE levels vary with polymorphism; individuals homozygous for the D allele have the highest levels of the enzyme, those homozygous for the I allele have the lowest, and heterozygous subjects have an intermediate level **(Giacchetti *et al*., 2005)**. The D allele has been shown to be associated with several disease processes, such as coronary heart disease and hypertension **(Jia *et al*., 2013)**.

Ovarian tissues contain all the elements necessary for the production of angiotensin, including pro-renin/renin, angiotensinogen, and ACE. It is now accepted that the enzyme ACE plays an important role in the renin-angiotensin system which regulates blood pressure, and which participates in the angiogenesis of the ovarian epithelium, follicle growth, steroidogenesis and inflammation **(Cintra *et al*., 2018)**.

The *ACE* gene insertion/deletion polymorphism is associated with changes in the plasma concentration of this enzyme. The presence of the D allele leads to elevated plasma levels, which subsequently results in elevated angiotensin II levels and alterations in steroid hormone synthesis **(Cintra *et al*., 2018)**.

The ovarian renin-angiotensin system may have important actions in the ovary, ranging from ovulation regulation to ovarian dysfunction, such as hyperandrogenism syndromes in women, suggesting that the potentially functional effect of the I/D polymorphism may be of particular importance in PCOS (**Nemeth** *et al*.**, 1994**). These effects are even greater after the activation of the SRA system, particularly in women with PCOS, as several authors have pointed out (**Palumbo** *et al*.**, 1993 Hacihanefioglu** *et al*.**, 2000**). Previous studies suggest that insulin resistance upregulates ovarian ARS through various mechanisms, which contribute to the pathogenesis of PCOS (**Celik** *et al*.**, 2010**).

A study reported that ovarian RAS activates insulin resistance by angiotensin-II and blocking the effects of the intracellular signal transduction system of insulin and by oxidative stress, the effects of which are mediated by angiotensin II (**Celik** *et al*.**, 2010; Deepika** *et al*.**, 2013**).

Also, it has been suggested that the ARS could influence the hypothalamic-pituitary axis. Evidence from animal experiments suggests that endogenous or exogenous angiotensin II in the brain may stimulate LH and GnRH secretion, which in turn modulates ovarian function, such as ovulation (**Bayram** *et al*.**, 2011; Koika** *et al*.**, 2012**). Second, as all components of RAS such as pro-renin, renin, ACE and angiotensinogen have been identified in ovarian tissues, it is now proven and accepted that ovarian RAS is involved in the development follicular, ovulation and steroidogenesis. Moreover, products of this system have been shown to be massively upregulated in women with PCOS, and this increased activity has even been suggested to be linked to hyperandrogenism (**Ożegowska** *et al*.**, 2016; Cintra** *et al*.**, 2018**). Third, accumulating evidence indicated that RAS was a potential contributor to insulin resistance, which in turn played a central role in the pathogenesis of PCOS (**Palumbo** *et al*.**, 2016**). The most recent studies on this topic have demonstrated that angiotensin II can decrease insulin sensitivity not only by altering insulin signaling pathways, but also by decreasing blood flow to muscles (**Nazeer** *et al*.**, 2021**).

Pharmacological inhibition of RAS by angiotensin converting enzyme inhibitor could improve insulin sensitivity and, consequently, attenuate the phenotypic expression of certain symptoms associated with this condition (**Ramanathan** *et al*. **, 2022**).

The study by Sun et al., 2010, which evaluated the effect of this polymorphism on 142 patients and 100 Chinese controls, did not observe any differences between the groups and found no association between the polymorphism and the PCOS. However, a Turkish population-based study that analyzed 100 PCOS patients and 100 controls (**Bayram *et al*., 2011**), and another Polish study with 138 patients and 110 controls showed differences between groups using the same analysis (**Ożegowska *et al*., 2016**), indicating that suppression may be a risk factor for PCOS.

The work of **Sun *et al*., 2010** did not show an association of the I/D polymorphism of the *ACE* gene with PCOS. Nevertheless, in this study conducted on a series of Chinese women, differences in testosterone concentration between the three genotypes were observed in patients and controls.

The existence of an association between the *ACE* I/D polymorphism and PCOS is controversial. Several studies have reported no contribution of this polymorphism to PCOS susceptibility, while others have found a relationship with insulin resistance, hyperandrogenism, and worsened clinical manifestations of PCOS (**Celika *et al*., 2010; Karabulut *et al*., 2010**).

A meta-analysis performed to clarify the effect of this polymorphism in the genesis of PCOS was conducted by **Jia *et al*., 2012** and reported a significant relationship between this polymorphism and the risk of PCOS in Caucasians, but not in Caucasians. Asians.

Peripheral insulin resistance has a crucial role in the pathogenesis of PCOS. Many women with PCOS also exhibit insulin resistance and hyperinsulinemia, which may contribute to the clinical and laboratory abnormalities that characterize this dysfunction (**Diamanti-Kandarakis, 2008**). A significant increase in serum insulin concentration and HOMA-IR index in women with PCOS with *ACE* DD genotype were observed. For precision, the HOMA-IR method or HOMA-IR index (Homeostasis Model Assessment - Insulin Resistance) is currently the most widely used and best validated means for the evaluation of insulin sensitivity (**Marcondes *et al*., 2007**).

The DD genotype was associated with higher insulin concentration than the ID or II genotype, implying that *ACE* gene I/D polymorphisms may directly or indirectly contribute to insulin resistance in PCOS subjects. In contrast, no association between *ACE* ID, II polymorphisms and insulin resistance or other corresponding metabolic profiles were observed in PCOS subjects. This suggests that *ACE* ID and II genotypes may not be a critical factor in determining serum insulin concentration and insulin resistance in these patients (**Marcondes *et al*., 2007**).

The most likely reason may be that *ACE* and I/D polymorphisms are only one of the cofactors that affect the etiopathogenesis of PCOS by interacting with other factors. The DD genotype is more sensitive to hyperinsulinemia. The increased activity of the D allele can alter the expression of insulin receptors by a pre- or post-receptor mechanism **(Echiburu *et al*., 2008)**.

The two most recent studies on this topic confirm this trend **(Nazeer *et al*., 2021; Ramanathan *et al*., 2022)**. Indeed, in a study designed to explore the association of *ACE* gene I/D polymorphism with PCOS in Pakistani women of reproductive age, reported that this polymorphism was significantly associated with an atypical LH/FSH ratio in patients' Pakistani people PCOS. Therefore, the presence of the D allele is likely to affect the process of steroidogenesis, which in turn can trigger the development of PCOS in women of childbearing age. This study concluded that this polymorphism may play a role in the pathogenesis of the disease, but is not the primary etiological factor in PCOS **(Nazeer *et al*., 2021)**.

As for the latest study published in 2022, it was conducted by **Ramanathan *et al*.,** on a population of 100 OPK women and 100 controls from the southern region of India. Although there were notable differences between the genotypic frequencies of patients and controls, no statistically significant differences were detected. Indeed, the *p*-value obtained was 0.504. What is interesting in this study is that, in the control cohort, the homozygous wild-type DD genotype was the most frequent, whereas in OPK patients, the heterozygous DI genotype was the most frequent. This notable difference is consistent with what we found in our study.

The latest meta-analysis on this subject which included a total of 12 published case-control studies with 2248 patients and 1759 controls, reported a very significant increased risk, according to the four genetic models of comparison, in women with the D allele (DD and DI genotypes) **(Chen *et al*., 2021)**.

The vast majority of studies published in the literature provide evidence that the I/D polymorphism of the *ACE* gene plays a more or less important role in the development of PCOS, both in Asians and Caucasians, independently of other risk factors. Even if the exact mechanisms are not yet clearly identified, its involvement in the pathophysiology of this dysfunction is increasingly argued.

# Results and discussion

# Part II

*CYP17A1* gene rs74357 polymorphism:
a meta-analysis

# *CYP17A1* (*rs*74357) polymorphism and polycystic ovary syndrome risk:
# A meta-analysis

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## Abstract

PCOS (Polycystic Ovarian Syndrome) is a common endocrine condition that is the leading cause of infertility and hirsutism. Overproduction of androgens in theca cells causes it. In the ovary, androgen synthesis is regulated by 17 -hydroxylase/17,20-lyase enzyme complex containing P450c17. Cytochrome P450 family 17 (CYP17) is associated with hyperandrogenism in women and the association between *CYP17* gene polymorphism and the risk of polycystic ovary syndrome is not clear. In order to determine whether the *CYP17* T/C (*rs*74357) gene polymorphism is an exposure risk for PCOS, a comprehensive meta-analysis summarizing 24 studies including 3462 PCOS and 2898 controls was performed. Summary odds ratios (ORs) and 95% confidence intervals (95% CIs) for *CYP17* T/C polymorphism and PCOS were calculated in a fixed-effect model and a random-effect model. The pooled ORs were performed under 7 genetic models: for the recessive model (CC *vs*. CT+TT), dominant model (CC+CT *vs*. TT), over-dominant model (CT *vs*. CC+TT), CC *vs*. TT model, CC *vs*. CT model, CT *vs*. TT model, and the allele contrast (C *vs*. T). Subgroup analyses were performed by ethnicity, country, *Hardy-Weinberg* equilibrium (HWE) in controls and study sample size.

The overall results validated that the 17 *CYP17* T/C (*rs*74357) gene polymorphism was significantly associated with PCOS risk in 5 genetic models: recessive model (fixed and random effect), dominant model (random effect), CC *vs*. TT (fixed effect), CT *vs*. TT (fixed effect), and allele contrast (random effect). Stratified analyses by ethnicity/country also detected significant association between Asian and Caucasian under the recessive, dominant, CC *vs*. TT, CC *vs*. CT, and the allele contrast models.

These results suggested that the *CYP17* T/C (*rs*74357) gene polymorphism played a crucial role in increasing the susceptibility of PCOS when carrying the recessive C allele, which can be proposed as a predictive factor for the risk of PCOS or an important pathway in PCOS associated metabolic and hormonal dysregulation especially insulin resistance.

# 1.    Introduction

PCOS, or polycystic ovarian syndrome, is one of the most common endocrinopathy, affecting around 5% to 10% of women of reproductive age. On ultrasound examination, cystic ovaries are present, as well as amenorrhea, oligomenorrhea, obesity, hyper-androgenism, and anovulation infertility **(Pusalkar *et al*., 2009)**. The main cause of PCOS is CYP17A1 dysregulation by P450 17α-related steroid hormone synthesis. *CYP17A1* gene is located on chromosome 10q24.3 and has 8 exons and 7 introns. The *CYP17A1* gene encodes the key enzyme 17-α-hydroxylase/17-20 lyase (P450 17α) that contributes to the androgen synthesis pathway and biosynthesis pathways of the ovary and adrenal **(Xu *et al*., 2021)**. The promoter 5 'untranslated region of the *CYP17* MSP AI (T-34C/ *rs*743572) has a polymorphism that affects gene expression regulation. The presence of this polymorphism may result in enhanced androgen synthesis. There are conflicting studies on the role of the *CYP17* MSP Al polymorphism in PCOS susceptibility **(Razavi *et al*., 2012; Rahimi and Mohammadi, 2019)**. Over the last two decades, a number of case-control studies were conducted to investigate the association between *CYP17* T/C polymorphisms and PCOS risk in humans. But these studies reported conflicting results. Some researchers concluded that this C substitution of the *CYP17A1* gene might be associated to the high risk of PCOS and maybe marked as a pathogenic gene of PCOS **(Kaur *et al*.,2018; Pusalkar *et al*., 2009)**, whereas others found the contradictory result **(Park *et al*., 2008; Unsal *et al*., 2009; Dasgupta *et al*., 2014; Ashraf *et al*., 2021)**. In addition, some scholars issued that the association of *rs*7432592 with PCOS is uncertain **(Echiburú *et al*., 2008)**, as this SNP may indirectly affect PCOS through the association between testosterone level and insulin resistance **(Li *et al*., 2015)**. Different methodologies have been used, but, in particular, most of the studies used a small sample size and it is therefore not surprising that there has been a lack of replication in various studies.

Based on the dissimilarity of case-control results and the ambiguous pathological mechanism of PCOS, an updated meta-analysis was designed to characterize better the relationship between *CYP17A1* SNP *rs*743572 and PCOS risk.

## 2.    Materials and methods

This systematic review and meta-analysis followed the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) **(Page *et al*., 2021)**. As this was a meta-analysis, ethical approval was not required.

### 2.1.  Publication search

Studies were searched on Web of Science, Embase, PubMed, and Google Scholar databases for all articles on the association between *CYP17* T/C polymorphisms and PCOS risk. The following key words were used: "polycystic ovary syndrome" or "PCOS" or "Stein-Leventhal syndrome" or "multi-pouch ovary syndrome", "17α-hydroxylase" or "CYP17", and "SNP" or "polymorphisms" or "mutation" or "genotype" or "variant". The search was without restriction on language, conducted on human subjects. The reference lists of reviews and retrieved articles were hand searched at the same time. If more than one article was published by the same author using the same case series, we selected the study where the most individuals were investigated.

### 2.2.  Inclusion and exclusion criteria

Eligible studies were involved if they met many criteria. We first screened by reading the title and abstract and then reviewed the full text according to the following criteria for the second screen: (i). The papers should adopt widely recognized and representative diagnostic criteria for PCOS: NIH criteria **(Franks *et al*., 2001)** or Rotterdam criteria **(ESHRE, 2004)** which case-control studies were conducted to evaluate the association between *CYP17* T/C polymorphism and PCOS risk; (ii). sufficient genotype data were presented to calculate the odds ratios (ORs) and 95% confidence intervals (CIs); (iii) the paper should clearly describe PCOS diagnoses and the sources of cases and controls. Major reasons for exclusion of studies were: (1) duplicate data; (2) abstract, comment, review and editorial; (3) no sufficient data were reported.

## 2.3. Data extraction

Data were extracted from all eligible articles separately. Included papers were organized and the following information was obtained: (i). The first author of the research, publication year, source of control, original country, and the ethnicity of subjects. (ii). Evidence of *Hardy-Weinberg* equilibrium. (iii). Genotyping method. (iv). Genotype frequencies of TT, TC, CC of PCOS group, and control group. After that, a rigorous literature evaluation was carried out. Different ethnicity was categorized as Asian, Caucasian. If original genotype frequency data were unavailable in relevant articles, a request was sent to the corresponding author for additional data. Furthermore, the *Hardy-Weinberg* equilibrium test was also calculated and adjusted manually.

## 2.4. Statistical analysis

To begin with, the *p*-value of the control group's *Hardy-Weinberg* equilibrium was calculated online (https://wpcalc.com/en/equilibrium-hardy-weinberg/), and the literature with a *p*-value less than 0.05 could be regarded as not in line with HWE. The strength of the association between PCOS and the *CYP17* T/C polymorphism was estimated using Odds ratio (OR), with the corresponding 95% Confidence Interval (CI) and *p*-value calculated by Comprehensive Meta-Analysis (CMA) Software 3.0 (https://www.meta-analysis.com/). The pooled ORs and *p*-value in a fixed-effect model and a random effect model of the association test were performed under 7 genetic models: for the recessive model (CC *vs*. CT+TT), dominant model (CC+CT *vs*. TT), over-dominant model (CT *vs*. CC+TT), CC *vs*. TT model, CC *vs*. CT model, CT *vs*. TT model, and the allele contrast (C *vs*. T). Forest plot for each model was generated by the CMA software. We also carried out the stratified analyses  by ethnicity, country, HWE in controls and study sample size. Both the Cochran's Q statistics to test for heterogeneity and the $I^2$ statistics to quantify the proportion of the total variation due to heterogeneity were calculated. A *p*-value of more than the nominal level of 0.05 for the Q statistic indicated a lack of heterogeneity across studies, allowing for the use of a fixed-effect model (the Mantel-Haenszel method; otherwise, the random effect model (the DerSimonian and Laird method) was used. To explore sources of heterogeneity across studies, we did logistic meta-regression analyses by CMA Software 3.0.

Several methods were used to assess the potential publication bias. Visual inspection of generated funnel plot asymmetry was conducted. The Begg's rank correlation method and the Egger's weighted regression method were used to statistically assess publication bias and *p*-value ≤0.05 was considered statistically significant. All these analyses were done using CMA software, version 3.0.

## 3. Results

### 3.1. Literature retrieval results and characteristics of studies

According to PRISMA flow diagram guidelines **(Figure 1)**, a total of 88 articles were obtained from the original search after the exclusion of duplicates. The examination of the title and abstract performed on these articles led to the removal of 50 studies and 38 continued to detailed assessment. After screening the full text of these publications, 14 articles were excluded for not meeting the inclusion criteria. Ultimately, 24 eligible case-control studies were included in this review: **Diamanti-Kandarakis *et al*., 1999; Cao *et al*., 1999; Marszalek *et al*., 2001; Kahsar-Miller *et al*., 2004; Tan *et al*., 2005; Ding *et al*., 2007; Luo *et al*., 2007; Li *et al*., 2008; Echiburu *et al*., 2008; Park *et al*., 2008; Prez *et al*., 2008; Unsal *et al*., 2009; Pusalkar *et al*., 2009; Liu *et al*., 2011; Zaho *et al*., 2011; Cirilo *et al*., 2012; Dasgupta *et al*., 2014; Li *et al*., 2015; Banerjee *et al*., 2016; Wu *et al*., 2017; Kaur *et al*., 2018; Rahimi *et al*., 2019; Ashraf *et al*., 2021; Munawar *et al*., 2021**. There were 17 studies of Asian patients and 7 studies of Caucasian patients. Studies had been carried out in China, Korea, India, Turkey, the USA, Poland, Greece, Mexico, Afghanistan, Belgium and Republic of Chile. The retrieval results and detailed characteristics were shown in **Table 1**. The genotypic and allelic frequencies reported in these studies are graphically represented in **Figures 02 and 03**. Adjusted HWE was reported in **Table 2**.

The 24 studies had been conducted in various countries and ethnicity with 3462 PCOS patients and 2898 control groups involved. 62,5% of the included studies took the Rotterdam criteria, and the remaining 37,5% took NIH criteria (studies performed before 2008). All studies extracted DNA from peripheral blood. Twenty-two of the 24 studies used the classic PCR-RFLP method, and the other two studies used different molecular genotyping methods, such as Taqman, PCR-SSCP.

### 3.2. Quantitative analysis

The main results of this meta-analysis are listed in **Table 3** (association test results), and **Table 4** (heterogeneity tests). Forest plot of meta-analysis comparisons models are presented in **Figure 4, 5, 6, 7, 8, 9 and 10**.

The overall results validated that the 17 *CYP17* T/C (*rs*74357) gene polymorphism was significantly associated with PCOS risk in 5 genetic models: the recessive model (CC *vs*. CT+TT) fixed (OR = 1.2214, 95%CI = [1.0655; 1.4001], *p*-value = 0.0041005483) and random effect (OR = 1.2214, 95%CI = [1.0655; 1.4001], *p*-value = 0.0041005483), dominant model (CC+CT *vs*. TT) random effect (OR = 1.3780, 95%CI = [1.1078; 1.7141], *p*-value = 0.0039888692), CC *vs*. TT (CT *vs*. CC+TT) fixed effect (OR = 1.3024, 95%CI = [1.1042; 1.5361], *p*-value = 0.0017042182), CT *vs*. TT fixed effect (OR = 1.2531, 95%CI = [1.1147; 1.4087], *p*-value = 0.0001576509), and allele contrast (C *vs*. T) random effect (OR = 1.2658, 95%CI = [1.1083; 1.4458], *p*-value= 0.0005097745). However, the variant genotypes (CC and TC) were not associated with PCOS risk, compared with the wild-type TT homozygote under two comparison models: over-dominant model (CT *vs*. CC+TT) fixed (OR = 1.1036, 95%CI = [0.9966; 1.2222], *p*-value = 0.0582285538) and random (OR = 1.1718, 95%CI = [0.9654; 1.4223], *p*-value = 0.1087244792), CC *vs*. CT model fixed (OR = 1.1704, 95%CI = [1.0127; 1.3527], *p*-value = 0.2314677357) and random effect (OR = 1.1704, 95%CI = [1.0127; 1.3527], *p*-value = 0.2314677357).

On the basis of the potential overestimation of the true effect of the polymorphism on the PCOS risk, we stratified these studies according to ethnicity, country, HWE in controls and study sample size. Stratified analyses by ethnicity/country also detected significant association under the five genetic models described above in Asian and Caucasian populations: recessive model (Asian: OR = 1,1811, 95%CI = [1.0169; 1.3718], *p*-value = 0,02926627 and Caucasian: OR = 1,4432, 95%CI = [1.0336; 2.0151], *p*-value = 0,031244366), dominant model (Asian: OR = 1,4304, 95%CI = [1.0725; 1.9079], *p*-value = 0,014846193 and Caucasian: OR = 1,2768, 95%CI = [1.0146; 1.6069], *p*-value = 0,037220539), CC *vs*. TT (Asian: OR = 1,3177, 95%CI = [1.0019; 1.7330], *p*-value = 0,048453792 and Caucasian: OR = 1,5304, 95%CI = [1.0634; 2.2025], *p*-value =0,02196287), and allele contrast (Asian: OR = 1,2728, 95%CI = [1.0722; 1.5109], *p*-value = 0,00582851 and Caucasian: OR = 1,2421, 95%CI = [1.0566; 1.4602], *p*-value = 0,008596616) **(Table 5)**.

However, on the CT *vs*. TT comparison model, significant difference was found only in Asian (OR = 1,3954, 95%CI = [1.0325; 1.8856], *p*-value = 0,03012493).

### 3.3. Heterogeneity analysis

Statistical analysis shows high heterogeneity under 5 genetic comparisons model: dominant model (tau^2 = 0.19, H = 1.85, I^2 = 0.71, Q = 78.45%, *p*-value = 0.00), over-dominant model (tau^2 = 0.14, H = 1.78, I^2 = 0.68, Q = 72.71%, *p*-value = 0.00), CC *vs*. TT (tau^2 = 0.10, H = 1.27, I^2 = 0.38, Q = 35.24%, *p*-value = 0.04), CT *vs*. TT (tau^2 = 0.21, H = 1.83, I^2 = 0.70, Q = 77.40%, *p*-value = 0.00), allele contrast (tau^2 = 0.06, H = 1.66, I^2 = 0.64, Q = 63.09%, *p*-value = 0.00). Due to this high heterogeneity, we conducted logistic meta-regression and subgroup analysis to explore the potential sources of heterogeneity with the following covariates: ethnicity (Asian, Caucasian), country (China or other), HWE in controls (yes or not), diagnostic criteria (Rotterdam criteria, NIH criteria) and genotyping approaches (PCR-RFLP or others). After estimating each covariate's potential contribution to heterogeneity by logistic meta-regression under CMA software, we found that all the *p*-value were > 0.05, which meant the heterogeneity could be attributed to none of the factors above. However, subgroup analysis indicated significantly decreasing heterogeneity in the Caucasian and NIH criteria subgroups. Thus, it was deduced that ethnicity and diagnosis criteria might be the main source of high heterogeneity.

### 3.4. Sensitivity analysis and Publication bias

Begg's Funnel plot (**Figure 11, 11, 12, 13, 14, 15, 16 and 17**) and Egger's test were performed to evaluate publication bias of the literature on PCOS. Displayed a funnel plot that examined the *CYP17* T/C polymorphism and overall PCOS risk included in the meta-analysis in the dominant model. The shape of funnel plots did not reveal any evidence of funnel plot asymmetry. The statistical results still did not show publication bias using the seven genetic models: recessive model (Egger's test *p*-value = 0.1669), dominant model (Egger's test *p*-value = 0.3003), over-dominant model (Egger's test *p*-value = 0.2432), CC *vs*. TT model (Egger's test *p*-value = 0.0748), CC *vs*. CT model (Egger's test *p*-value = 0.9185), CT *vs*. TT model (Egger's test *p*-value = 0.3992), and the allele contrast (C *vs*. T) (Egger's test *p*-value = 0.067).

To assess sensitivity and the effect of individual study on the overall meta-analysis estimate, we excluded one study at a time **(Figure 18, 18, 19, 20, 21, 22, 23 and 24)**, and the exclusion of any single report did not alter the significance of the final decision, suggesting that the outcomes were robust. Finally, the sensitivity analysis demonstrated any individual article did not constitute the source of heterogeneity since removing any single article would not affect the stability of the overall estimate.

## 4.    Discussion

Polycystic ovarian syndrome is a multifaceted disorder caused by anomalies in genetics, metabolism, endocrine function, and environmental factors. Obesity-related health complications such as diabetes, hypertension, cardiovascular disorders, anovulation, infertility, trouble in conception, and unfavorable pregnancy outcomes are widely established in PCOS women **(Delitala *et al*., 2017)**. The indication from family-based and association case-controls studies suggests that PCOS has a substantial genetic foundation, although the genes prompting to PCOS have yet to be clearly defined. The candidate genes predisposing to PCOS comprise those intricated in the regulation of ovarian steroidogenesis and also those genes that influence body mass index (BMI) and adiposity **(Vollmert *et al*., 2007)**. It has been proposed that an amplified activity of ovarian P450c17α, a key enzyme in the biosynthesis of androgens, is the fundamental disorder in the ovarian hyperandrogenism observed in this syndrome **(Ashraf *et al*., 2021)**. Consequently, the initial investigations focused on the possible role of *CYP17*, the gene that codes for cytochrome P450c17α, located on chromosome 10q24.3. A polymorphism has been found in the regulatory region of the *CYP17* gene, being a T to C substitution -34 bp from the translation start point in the promoter region. It has been proposed that this modification may up-regulate the expression of *CYP17*, resulting in an increased synthesis of androgens **(Munawar *et al*., 2021)**. The obvious contribution of the genetic factor to this syndrome was observed, and the involvement of the *CYP17* gene polymorphism in raising the probability of PCOS was noted through multiple case-controls and meta-analysis studies. However, several studies have shown that the T to C substitution at 34 bp upstream the 5' promoter region of the *CYP17* gene was associated with PCOS; while some have found the opposite **(Xu *et al*., 2021)**.

The present meta-analysis integrated the updated published studies of the *CYP17A1* gene through comprehensive literature retrieval as well as systematic analysis and explored the relationship between the *CYP17A1* gene and PCOS. To the best of our knowledge, *CYP17* encodes the enzyme 17-α-hydroxylase/17–20 lyase (P45017α), which is a rate-limiting enzyme in androgen synthesis. **Diamanti-Kandarakis *et al*., 1999** was the first to propose that *CYP17* T/C gene polymorphism could be responsible for the dysregulation of gene *CYP17* expression, which aggravated hyperandrogenemia of PCOS, which was later supported by **Pusalkar *et al*., 2009**, who described a strong association of *CYP17* T/C gene polymorphism with PCOS. In the current study, more frequencies of the polymorphic C allele and CC genotype were discovered in women with PCOS than in controls, which supported the hypothesis that the significance of the association was found to be more significant compared with controls. It was hypothesized that this polymorphism could generate an additional sp1 binding site near the promoter, which enhanced transcription activity of *CYP17A1* expression and produced hyperandrogenism. However, experimental studies have not confirmed this finding (**Xu *et al*., 2021**). This meta-analysis results validated that the 17 *CYP17* T/C (*rs*74357) gene polymorphism was significantly associated with PCOS risk in 5 genetic models: recessive model (fixed and random effect), dominant model (random effect), CC *vs*. TT (fixed effect), CT *vs*. TT (fixed effect), and allele contrast (random effect). Stratified analyses by ethnicity/country also detected significant association between Asian and Caucasian under the recessive, dominant, CC *vs*. TT, CC *vs*. CT, and the allele contrast models. All these data suggest a very strong implication of the studied polymorphism independent of ethnic factors.

This meta-analysis does, however, have certain limitations. First, the number of studies included in the meta-analysis and the number of cases and controls in the studies included in specific subgroups were both limited. Second, because not all published studies offered adjusted ORs, or when they did, the ORs were not adjusted for the same possible confounders, such as age, ethnicity, and exposures, our meta-analysis was based on unadjusted OR estimates. The limited information for data analysis could result in substantial confounding bias. Third, investigations of the polymorphism showed high between-study variability, and the genotype distribution included deviated from *HWE*: Diamanti *et al*., 1999, Kahsar-Miller *et al*., 2004 and Ding *et al*., 2007 case-control study.

Despite these disadvantages, our meta-analysis has certain advantages. First and foremost, a rigorous search. The use of a computer-assisted search method allowed as many eligible studies to be included as possible. Second, the case control studies included in this meta-analysis were of acceptable quality and matched our inclusion criteria. Furthermore, the meta-analysis approach was well-designed before it was started, with specific methods for research selection, data extraction, and data analysis (PRISMA). Also, the meta-analysis was performed using the latest version (3.0) of the reference software for performing meta-analysis in genetics (Comprehensive Meta Analysis).

Furthermore, more research evaluating the impact of gene–gene and gene-environment interactions could lead to a more complete understanding of the link between the *CYP17* T/C polymorphism and PCOS risk.

## 5. Conclusion

The current findings in our meta-analysis result suggest that gene polymorphisms influence the expression and production of *CYP17* and the *CYP17* T/C (*rs*74357) gene polymorphism plays an important role in increasing the susceptibility of PCOS when carrying the C allele (genotype TC and CC). Despite the undoubted connection of *CYP17* gene polymorphism to PCOS, the range to which *CYP17* gene polymorphism contributes to metabolic dysfunction in PCOS is unidentified and needs further study Meanwhile, due to the strong correlation between PCOS and *CYP17A1 rs*7435742 polymorphism, it could be used as a genetic marker for PCOS, and might supply another tool for assessing women's susceptibility. Likewise, the *CYP17A1* could be applied to the treatment of PCOS as a potentially feasible target. However, more research with a larger sample size and more detailed information is required.

## Authors' Roles

Hiba BENDAOUD and Jihan EL KHOUR was designed to research and collect data, Mohamed Larbi REZGOUN intended to analysed data. The manuscript was written, reviewed and edited Mohamed Larbi REZGOUN, Hiba BENDAOUD and Jihan EL KHOUR. The manuscript was revised and commented by Dr Djalila CHELLAT.

## Declarations

Ethical approval and consent to participate: this is a meta-analysis study and ethical approval is not required. All participants provided informed consent before enrolment.

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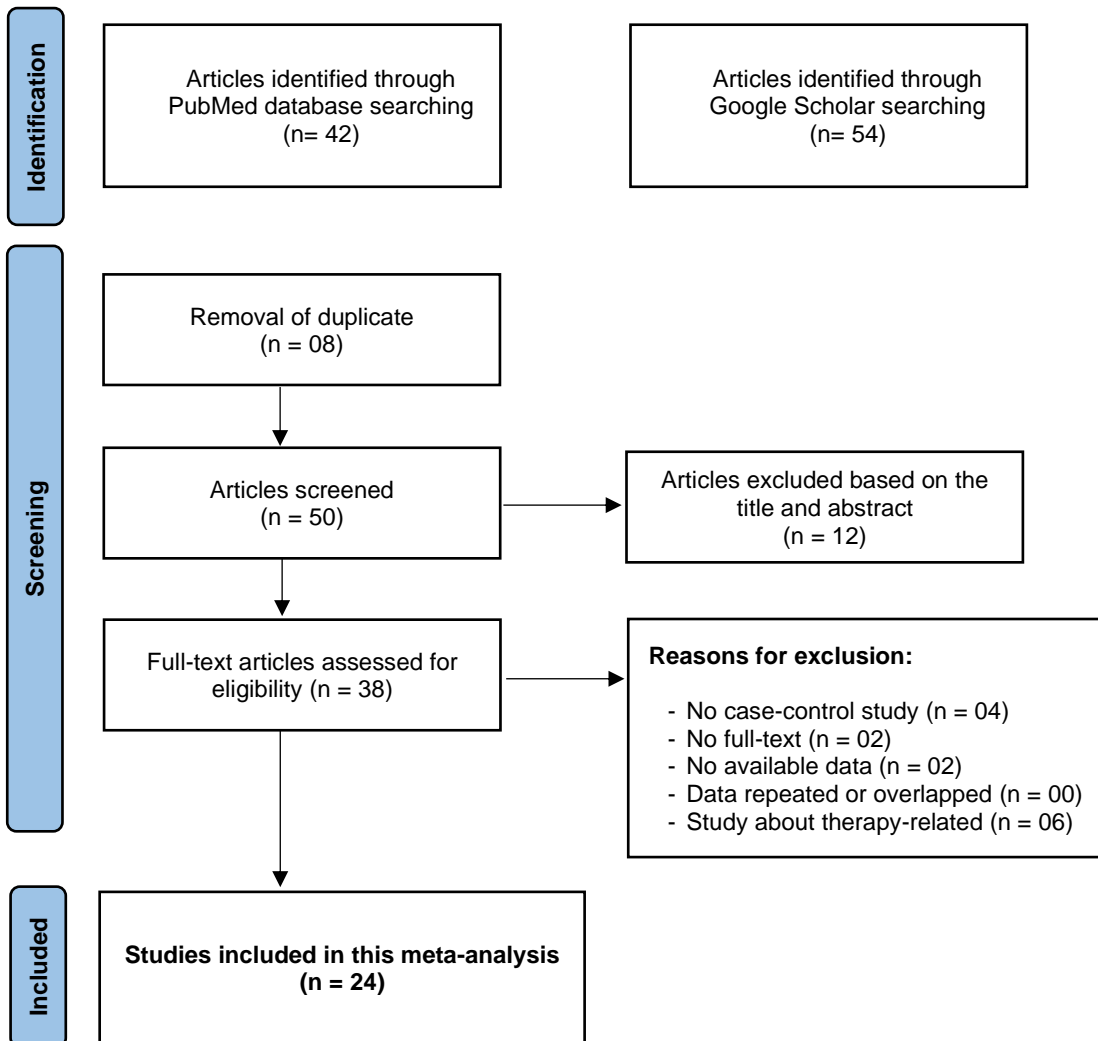
**Figure 1.** Prisma flow diagram.

**Table 1.** Characteristics of studies included in this meta-analysis.

| # | Author and year | Presence or absence of association | Country (ethnicity) | Diagnostic criteria | Genotyping method | Hardy–Weinberg equilibrium (HWE) | p for HWE | Case | | | | | | | | | | | Control | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Cohort | TT | TT (%) | TC | TC (%) | CC | CC (%) | T | T (%) | C | C (%) | Cohort | TT | TT (%) | TC | TC (%) | CC | CC (%) | T | T (%) | C | C (%) |
| 1 | Diamanti et al., 1999 | Yes | Greece | NIH | RFLP | No | 0,01 | 50 | 17 | 34,00 | 29 | 58,00 | 4 | 8,00 | 63 | 63,00 | 37 | 37,00 | 50 | 22 | 44,00 | 28 | 56,00 | 0 | 0,00 | 72 | 72,00 | 28 | 28,00 |
| 2 | Cao et al., 1999 | Yes | China | NIH | RFLP | Yes | 0,72 | 56 | 17 | 30,36 | 17 | 30,36 | 22 | 39,29 | 51 | 45,54 | 61 | 54,46 | 30 | 8 | 26,67 | 14 | 46,67 | 8 | 26,67 | 30 | 50,00 | 30 | 50,00 |
| 3 | Marszalek et al., 2001 | No | Poland | NIH | RFLP | Yes | 0,48 | 55 | 17 | 30,91 | 27 | 49,09 | 11 | 20,00 | 61 | 55,45 | 49 | 44,55 | 56 | 20 | 35,71 | 29 | 51,79 | 7 | 12,50 | 69 | 61,61 | 43 | 38,39 |
| 4 | Kahsar-Miller et al., 2004 | No | USA | NIH | RFLP | No | 0,01 | 259 | 79 | 30,50 | 142 | 54,83 | 38 | 14,67 | 300 | 57,92 | 218 | 42,08 | 161 | 50 | 31,06 | 94 | 58,39 | 17 | 10,56 | 194 | 60,25 | 128 | 39,75 |
| 5 | Tan et al., 2005 | Yes | China | NIH | RFLP | Yes | 0,64 | 118 | 12 | 10,17 | 66 | 55,93 | 40 | 33,90 | 90 | 38,14 | 146 | 61,86 | 106 | 21 | 19,81 | 55 | 51,89 | 30 | 28,30 | 97 | 45,75 | 115 | 54,25 |
| 6 | Ding et al., 2007 | No | China | NIH | RFLP | No | 0,01 | 329 | 55 | 16,72 | 145 | 44,07 | 129 | 39,21 | 255 | 38,75 | 403 | 61,25 | 275 | 30 | 10,91 | 151 | 54,91 | 94 | 34,18 | 211 | 38,36 | 339 | 61,64 |
| 7 | Luo et al., 2007 | Yes | China | NIH | RFLP | | 0,8 | 74 | 38 | 51,35 | 33 | 44,59 | 3 | 4,05 | 109 | 73,65 | 39 | 26,35 | 27 | 16 | 59,26 | 10 | 37,04 | 1 | 3,70 | 42 | 77,78 | 12 | 22,22 |
| 8 | Li et al., 2008 | Yes | China | NIH | RFLP | Yes | 0,18 | 61 | 11 | 18,03 | 32 | 52,46 | 18 | 29,51 | 54 | 44,26 | 68 | 55,74 | 45 | 14 | 31,11 | 18 | 40,00 | 13 | 28,89 | 46 | 51,11 | 44 | 48,89 |
| 9 | Echiburú et al., 2008 | No | Chili | NIH | RFLP | Yes | 0,17 | 159 | 59 | 37,11 | 81 | 50,94 | 19 | 11,95 | 199 | 62,58 | 119 | 37,42 | 93 | 43 | 46,24 | 36 | 38,71 | 14 | 15,05 | 122 | 65,59 | 64 | 34,41 |
| 10 | Park et al., 2008 | No | South Korea | Rott | Taqman | Yes | 0,10 | 133 | 40 | 30,08 | 61 | 45,86 | 32 | 24,06 | 141 | 53,01 | 125 | 46,99 | 99 | 25 | 25,25 | 41 | 41,41 | 33 | 33,33 | 91 | 45,96 | 107 | 54,04 |
| 11 | Prez et al., 2008 | Yes | Argentina | Rott | RFLP | Yes | NI | 64 | 23 | 35,94 | 26 | 40,63 | 15 | 23,44 | 72 | 56,25 | 56 | 43,75 | 57 | 16 | 28,07 | 30 | 52,63 | 11 | 19,30 | 62 | 54,39 | 52 | 45,61 |
| 12 | Unsal et al., 2009 | Yes | Turkey | Rott | RFLP | Yes | 0,77 | 44 | 15 | 34,09 | 19 | 43,18 | 10 | 22,73 | 49 | 55,68 | 39 | 44,32 | 50 | 20 | 40,00 | 24 | 48,00 | 6 | 12,00 | 64 | 64,00 | 36 | 36,00 |
| 13 | Pusalkar et al., 2009 | Yes | India | Rott | SSCP | Yes | 0,13 | 100 | 44 | 44,00 | 42 | 42,00 | 14 | 14,00 | 130 | 65,00 | 70 | 35,00 | 100 | 62 | 62,00 | 30 | 30,00 | 8 | 8,00 | 154 | 77,00 | 46 | 23,00 |
| 14 | Liu et al., 2011 | No | China | Rott | RFLP | Yes | 0,45 | 55 | 19 | 34,55 | 23 | 41,82 | 13 | 23,64 | 61 | 55,45 | 49 | 44,55 | 50 | 17 | 34,00 | 22 | 44,00 | 11 | 22,00 | 56 | 56,00 | 44 | 44,00 |
| 15 | Zaho et al., 2011 | Yes | China | Rott | RFLP | Yes | 0,74 | 177 | 18 | 10,17 | 100 | 56,50 | 59 | 33,33 | 136 | 38,42 | 218 | 61,58 | 159 | 32 | 20,13 | 81 | 50,94 | 46 | 28,93 | 145 | 45,60 | 173 | 54,40 |
| 16 | Cirilo et al., 2012 | Yes | Brazil | Rott | RFLP | Yes | NI | 117 | 53 | 45,30 | 46 | 39,32 | 18 | 15,38 | 152 | 64,96 | 82 | 35,04 | 105 | 65 | 61,90 | 32 | 30,48 | 8 | 7,62 | 162 | 77,14 | 48 | 22,86 |
| 17 | Dasgupta et al., 2014 | Yes | India | Rott | RFLP | Yes | NI | 60 | 15 | 25,00 | 26 | 43,33 | 19 | 31,67 | 56 | 46,67 | 64 | 53,33 | 54 | 18 | 33,33 | 22 | 40,74 | 14 | 25,93 | 58 | 53,70 | 50 | 46,30 |
| 18 | Li et al., 2015 | No | China | Rott | RFLP | Yes | 0,33 | 318 | 158 | 49,69 | 139 | 43,71 | 21 | 6,60 | 455 | 71,54 | 181 | 28,46 | 306 | 137 | 44,77 | 141 | 46,08 | 28 | 9,15 | 415 | 67,81 | 197 | 32,19 |
| 19 | Banerjee et al., 2016 | Yes | India | Rott | RFLP | Yes | 0,73 | 75 | 20 | 26,67 | 33 | 44,00 | 22 | 29,33 | 73 | 48,67 | 77 | 51,33 | 73 | 18 | 24,66 | 35 | 47,95 | 20 | 27,40 | 71 | 48,63 | 75 | 51,37 |

| № | Author | | Country | Criteria | Method | HWE | HWE value | Total | TT | TT% | TC | TC% | CC | CC% | T | T% | C | C% | Total | TT | TT% | TC | TC% | CC | CC% | T | T% | C | C% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | Wu et al., 2017 | No | China | Rott | RFLP | Yes | 0,29 | 260 | 90 | 34,62 | 109 | 41,92 | 61 | 23,46 | 289 | 55,58 | 231 | 44,42 | 237 | 81 | 34,18 | 104 | 43,88 | 52 | 21,94 | 266 | 56,12 | 208 | 43,88 |
| 21 | Kaur et al., 2018 | Yes | India | Rott | RFLP | Yes | 0,28 | 250 | 107 | 42,80 | 118 | 47,20 | 25 | 10,00 | 332 | 66,40 | 168 | 33,60 | 250 | 146 | 58,40 | 94 | 37,60 | 10 | 4,00 | 386 | 77,20 | 114 | 22,80 |
| 22 | Rahimi et al., 2019 | No | Iran | Rott | RFLP | Yes | NI | 50 | 35 | 70,00 | 15 | 30,00 | 0 | 0,00 | 85 | 85,00 | 15 | 15,00 | 109 | 92 | 84,40 | 17 | 15,60 | 0 | 0,00 | 201 | 92,20 | 17 | 7,80 |
| 23 | Ashraf et al., 2021 | Yes | Kashmir | Rott | RFLP | Yes | 0,23 | 394 | 115 | 29,19 | 209 | 53,05 | 70 | 17,77 | 439 | 55,71 | 349 | 44,29 | 306 | 108 | 35,29 | 156 | 50,98 | 42 | 13,73 | 372 | 60,78 | 240 | 39,22 |
| 24 | Munawar et al., 2021 | No | Pakistan | Rott | RFLP | Yes | NI | 204 | 88 | 43,14 | 112 | 54,90 | 4 | 1,96 | 288 | 70,59 | 120 | 29,41 | 100 | 86 | 86,00 | 12 | 12,00 | 2 | 2,00 | 184 | 92,00 | 16 | 8,00 |

**TOTAL**

| 1145 TT | 1650 TC | 667 CC | 3940 T | 2984 C | 1147 TT | 1276 TC | 475 CC | 3570 T | 2226 C |
|---|---|---|---|---|---|---|---|---|---|
| | 3462 | | | | | 2898 | | | |

**NI:** value of HWE not indicated

**NIH:** National Institutes of Health diagnosis criteria for PCOS

**Rott:** Rotterdam diagnosis criteria for PCOS

**RFLP:** polymorphism reveled using PCR-Restriction Fragment Length Polymorphism

**SSCP:** polymorphism reveled using PCR-Single Strand Conformation Polymorphism
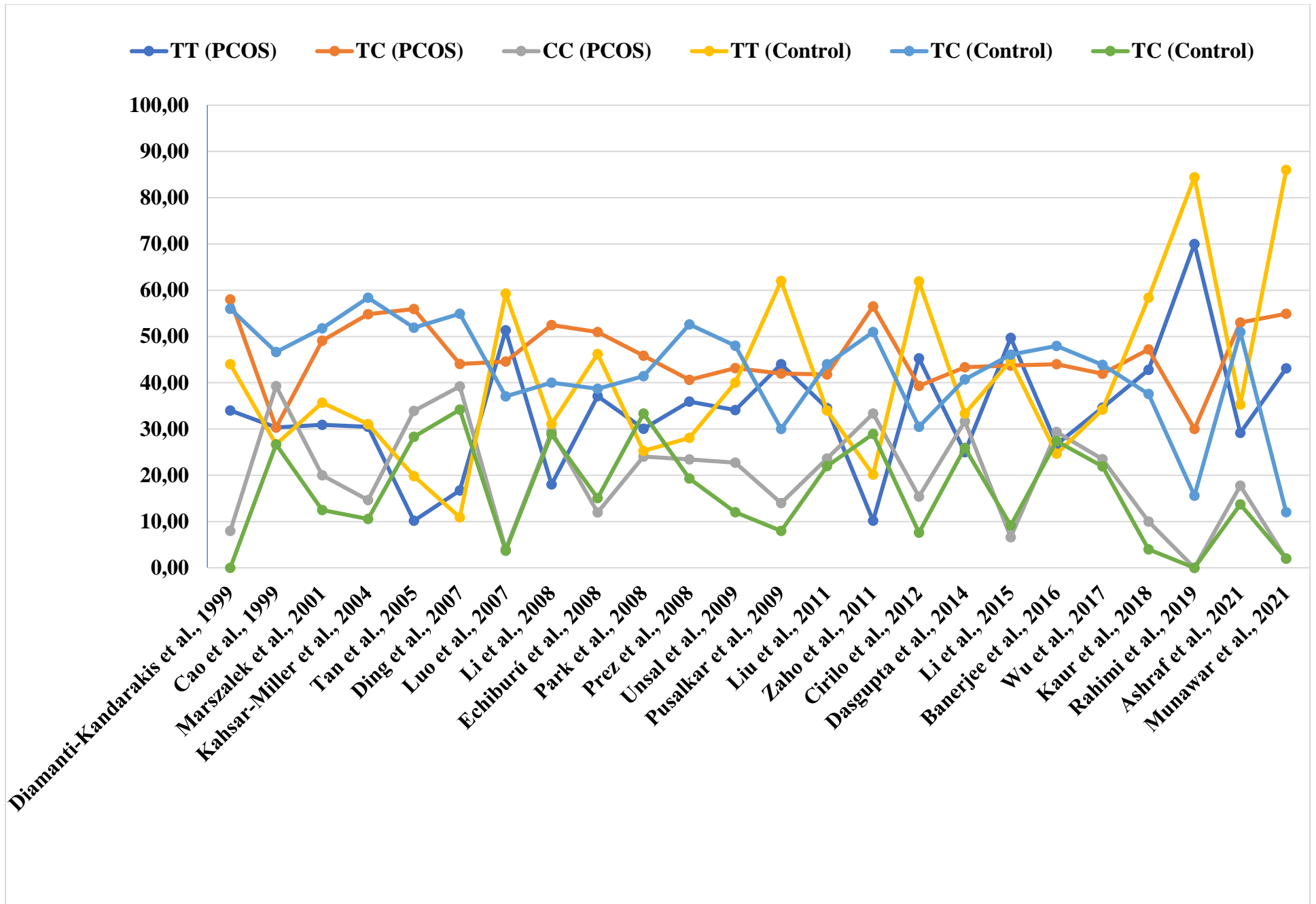
**Figure 2.** Graphic presentation of studies genotypic frequencies included in this meta-analysis.
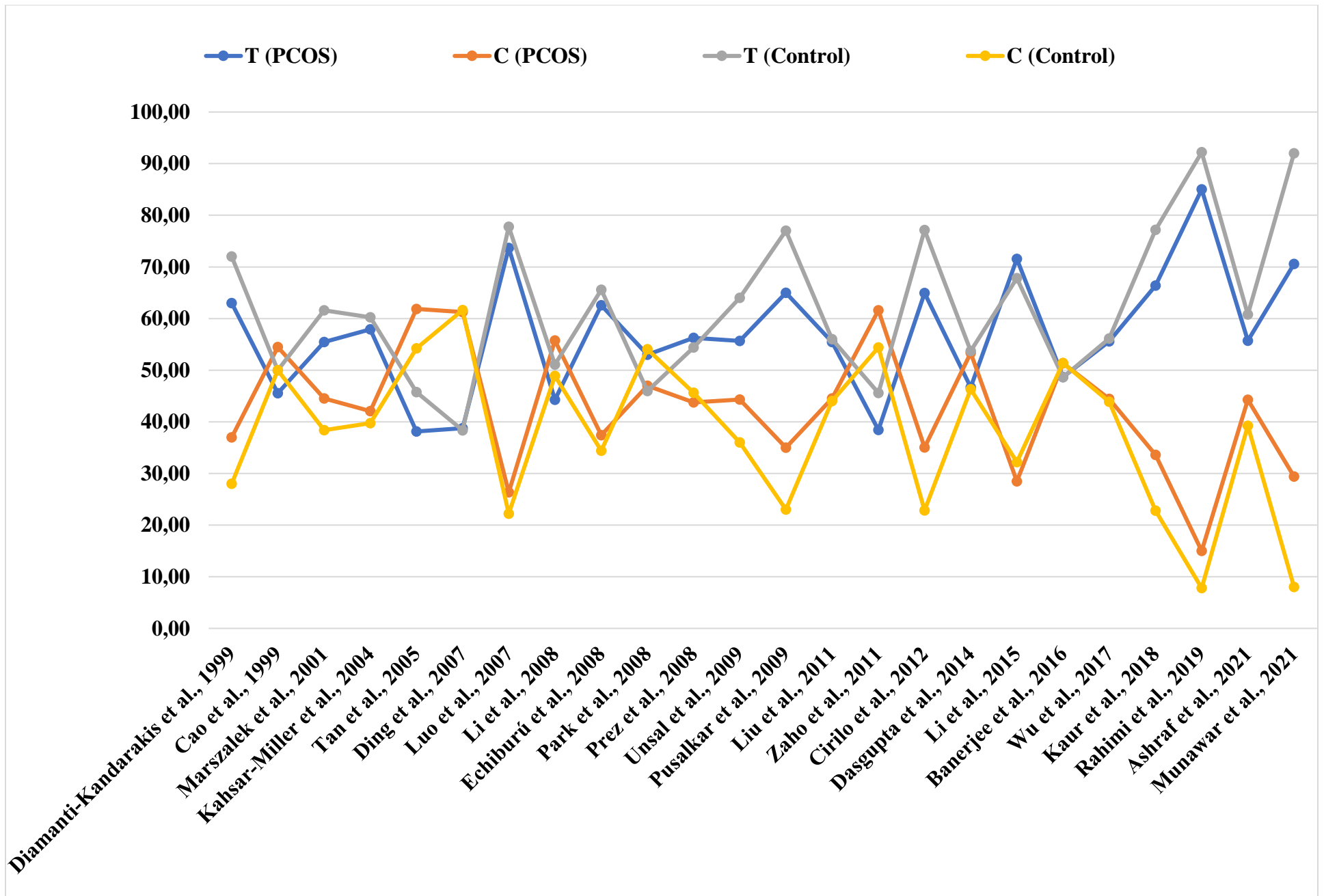
**Figure 3.** Graphic presentation of studies allelic frequencies included in this meta-analysis.

**Table 1.** Characteristics of studies included in this meta-analysis.

| # | Author and year | Presence or absence of association | Country (ethnicity) | Diagnostic criteria | Genotyping method | Hardy–Weinberg equilibrium (HWE) | p for HWE | Case Cohort | TT | TT (%) | TC | TC (%) | CC | CC (%) | T | T (%) | C | C (%) | Control Cohort | TT | TT (%) | TC | TC (%) | CC | CC (%) | T | T (%) | C | C (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Diamanti et al., 1999 | Yes | Greece | NIH | RFLP | No | 0,01 | 50 | 17 | 34,00 | 29 | 58,00 | 4 | 8,00 | 63 | 63,00 | 37 | 37,00 | 50 | 22 | 44,00 | 28 | 56,00 | 0 | 0,00 | 72 | 72,00 | 28 | 28,00 |
| 2 | Cao et al., 1999 | Yes | China | NIH | RFLP | Yes | 0,72 | 56 | 17 | 30,36 | 17 | 30,36 | 22 | 39,29 | 51 | 45,54 | 61 | 54,46 | 30 | 8 | 26,67 | 14 | 46,67 | 8 | 26,67 | 30 | 50,00 | 30 | 50,00 |
| 3 | Marszalek et al., 2001 | No | Poland | NIH | RFLP | Yes | 0,48 | 55 | 17 | 30,91 | 27 | 49,09 | 11 | 20,00 | 61 | 55,45 | 49 | 44,55 | 56 | 20 | 35,71 | 29 | 51,79 | 7 | 12,50 | 69 | 61,61 | 43 | 38,39 |
| 4 | Kahsar-Miller et al., 2004 | No | USA | NIH | RFLP | No | 0,01 | 259 | 79 | 30,50 | 142 | 54,83 | 38 | 14,67 | 300 | 57,92 | 218 | 42,08 | 161 | 50 | 31,06 | 94 | 58,39 | 17 | 10,56 | 194 | 60,25 | 128 | 39,75 |
| 5 | Tan et al., 2005 | Yes | China | NIH | RFLP | Yes | 0,64 | 118 | 12 | 10,17 | 66 | 55,93 | 40 | 33,90 | 90 | 38,14 | 146 | 61,86 | 106 | 21 | 19,81 | 55 | 51,89 | 30 | 28,30 | 97 | 45,75 | 115 | 54,25 |
| 6 | Ding et al., 2007 | No | China | NIH | RFLP | No | 0,01 | 329 | 55 | 16,72 | 145 | 44,07 | 129 | 39,21 | 255 | 38,75 | 403 | 61,25 | 275 | 30 | 10,91 | 151 | 54,91 | 94 | 34,18 | 211 | 38,36 | 339 | 61,64 |
| 7 | Luo et al., 2007 | Yes | China | NIH | RFLP | Yes | 0,8 | 74 | 38 | 51,35 | 33 | 44,59 | 3 | 4,05 | 109 | 73,65 | 39 | 26,35 | 27 | 16 | 59,26 | 10 | 37,04 | 1 | 3,70 | 42 | 77,78 | 12 | 22,22 |
| 8 | Li et al., 2008 | Yes | China | NIH | RFLP | Yes | 0,18 | 61 | 11 | 18,03 | 32 | 52,46 | 18 | 29,51 | 54 | 44,26 | 68 | 55,74 | 45 | 14 | 31,11 | 18 | 40,00 | 13 | 28,89 | 46 | 51,11 | 44 | 48,89 |
| 9 | Echiburú et al., 2008 | No | Chili | NIH | RFLP | Yes | 0,17 | 159 | 59 | 37,11 | 81 | 50,94 | 19 | 11,95 | 199 | 62,58 | 119 | 37,42 | 93 | 43 | 46,24 | 36 | 38,71 | 14 | 15,05 | 122 | 65,59 | 64 | 34,41 |
| 10 | Park et al., 2008 | No | South Korea | Rott | Taqman | Yes | 0,10 | 133 | 40 | 30,08 | 61 | 45,86 | 32 | 24,06 | 141 | 53,01 | 125 | 46,99 | 99 | 25 | 25,25 | 41 | 41,41 | 33 | 33,33 | 91 | 45,96 | 107 | 54,04 |
| 11 | Prez et al., 2008 | Yes | Argentina | Rott | RFLP | Yes | NI | 64 | 23 | 35,94 | 26 | 40,63 | 15 | 23,44 | 72 | 56,25 | 56 | 43,75 | 57 | 16 | 28,07 | 30 | 52,63 | 11 | 19,30 | 62 | 54,39 | 52 | 45,61 |
| 12 | Unsal et al., 2009 | Yes | Turkey | Rott | RFLP | Yes | 0,77 | 44 | 15 | 34,09 | 19 | 43,18 | 10 | 22,73 | 49 | 55,68 | 39 | 44,32 | 50 | 20 | 40,00 | 24 | 48,00 | 6 | 12,00 | 64 | 64,00 | 36 | 36,00 |
| 13 | Pusalkar et al., 2009 | Yes | India | Rott | SSCP | Yes | 0,13 | 100 | 44 | 44,00 | 42 | 42,00 | 14 | 14,00 | 130 | 65,00 | 70 | 35,00 | 100 | 62 | 62,00 | 30 | 30,00 | 8 | 8,00 | 154 | 77,00 | 46 | 23,00 |
| 14 | Liu et al., 2011 | No | China | Rott | RFLP | Yes | 0,45 | 55 | 19 | 34,55 | 23 | 41,82 | 13 | 23,64 | 61 | 55,45 | 49 | 44,55 | 50 | 17 | 34,00 | 22 | 44,00 | 11 | 22,00 | 56 | 56,00 | 44 | 44,00 |
| 15 | Zaho et al., 2011 | Yes | China | Rott | RFLP | Yes | 0,74 | 177 | 18 | 10,17 | 100 | 56,50 | 59 | 33,33 | 136 | 38,42 | 218 | 61,58 | 159 | 32 | 20,13 | 81 | 50,94 | 46 | 28,93 | 145 | 45,60 | 173 | 54,40 |
| 16 | Cirilo et al., 2012 | Yes | Brazil | Rott | RFLP | Yes | NI | 117 | 53 | 45,30 | 46 | 39,32 | 18 | 15,38 | 152 | 64,96 | 82 | 35,04 | 105 | 65 | 61,90 | 32 | 30,48 | 8 | 7,62 | 162 | 77,14 | 48 | 22,86 |
| 17 | Dasgupta et al., 2014 | Yes | India | Rott | RFLP | Yes | NI | 60 | 15 | 25,00 | 26 | 43,33 | 19 | 31,67 | 56 | 46,67 | 64 | 53,33 | 54 | 18 | 33,33 | 22 | 40,74 | 14 | 25,93 | 58 | 53,70 | 50 | 46,30 |
| 18 | Li et al., 2015 | No | China | Rott | RFLP | Yes | 0,33 | 318 | 158 | 49,69 | 139 | 43,71 | 21 | 6,60 | 455 | 71,54 | 181 | 28,46 | 306 | 137 | 44,77 | 141 | 46,08 | 28 | 9,15 | 415 | 67,81 | 197 | 32,19 |
| 19 | Banerjee et al., 2016 | Yes | India | Rott | RFLP | Yes | 0,73 | 75 | 20 | 26,67 | 33 | 44,00 | 22 | 29,33 | 73 | 48,67 | 77 | 51,33 | 73 | 18 | 24,66 | 35 | 47,95 | 20 | 27,40 | 71 | 48,63 | 75 | 51,37 |

| # | Author | HWE | Country | Criteria | Method | | HWE value | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | Wu et al., 2017 | No | China | Rott | RFLP | Yes | 0,29 | **260** | 90 | 34,62 | 109 | 41,92 | 61 | 23,46 | 289 | 55,58 | 231 | 44,42 | **237** | 81 | 34,18 | 104 | 43,88 | 52 | 21,94 | 266 | 56,12 | 208 | 43,88 |
| 21 | Kaur et al., 2018 | Yes | India | Rott | RFLP | Yes | 0,28 | **250** | 107 | 42,80 | 118 | 47,20 | 25 | 10,00 | 332 | 66,40 | 168 | 33,60 | **250** | 146 | 58,40 | 94 | 37,60 | 10 | 4,00 | 386 | 77,20 | 114 | 22,80 |
| 22 | Rahimi et al., 2019 | No | Iran | Rott | RFLP | Yes | NI | **50** | 35 | 70,00 | 15 | 30,00 | 0 | 0,00 | 85 | 85,00 | 15 | 15,00 | **109** | 92 | 84,40 | 17 | 15,60 | 0 | 0,00 | 201 | 92,20 | 17 | 7,80 |
| 23 | Ashraf et al., 2020 | Yes | Kashmir | Rott | RFLP | Yes | 0,23 | **394** | 115 | 29,19 | 209 | 53,05 | 70 | 17,77 | 439 | 55,71 | 349 | 44,29 | **306** | 108 | 35,29 | 156 | 50,98 | 42 | 13,73 | 372 | 60,78 | 240 | 39,22 |
| 24 | Munawar et al., 2020 | No | Pakistan | Rott | RFLP | Yes | NI | **204** | 88 | 43,14 | 112 | 54,90 | 4 | 1,96 | 288 | 70,59 | 120 | 29,41 | **100** | 86 | 86,00 | 12 | 12,00 | 2 | 2,00 | 184 | 92,00 | 16 | 8,00 |

**NI:** value of HWE not indicated

**NIH:** National Institutes of Health diagnosis criteria for PCOS

**Rott:** Rotterdam diagnosis criteria for PCOS

**RFLP:** polymorphism reveled using PCR-Restriction Fragment Length Polymorphism

**SSCP:** polymorphism reveled using PCR-Single Strand Conformation Polymorphism
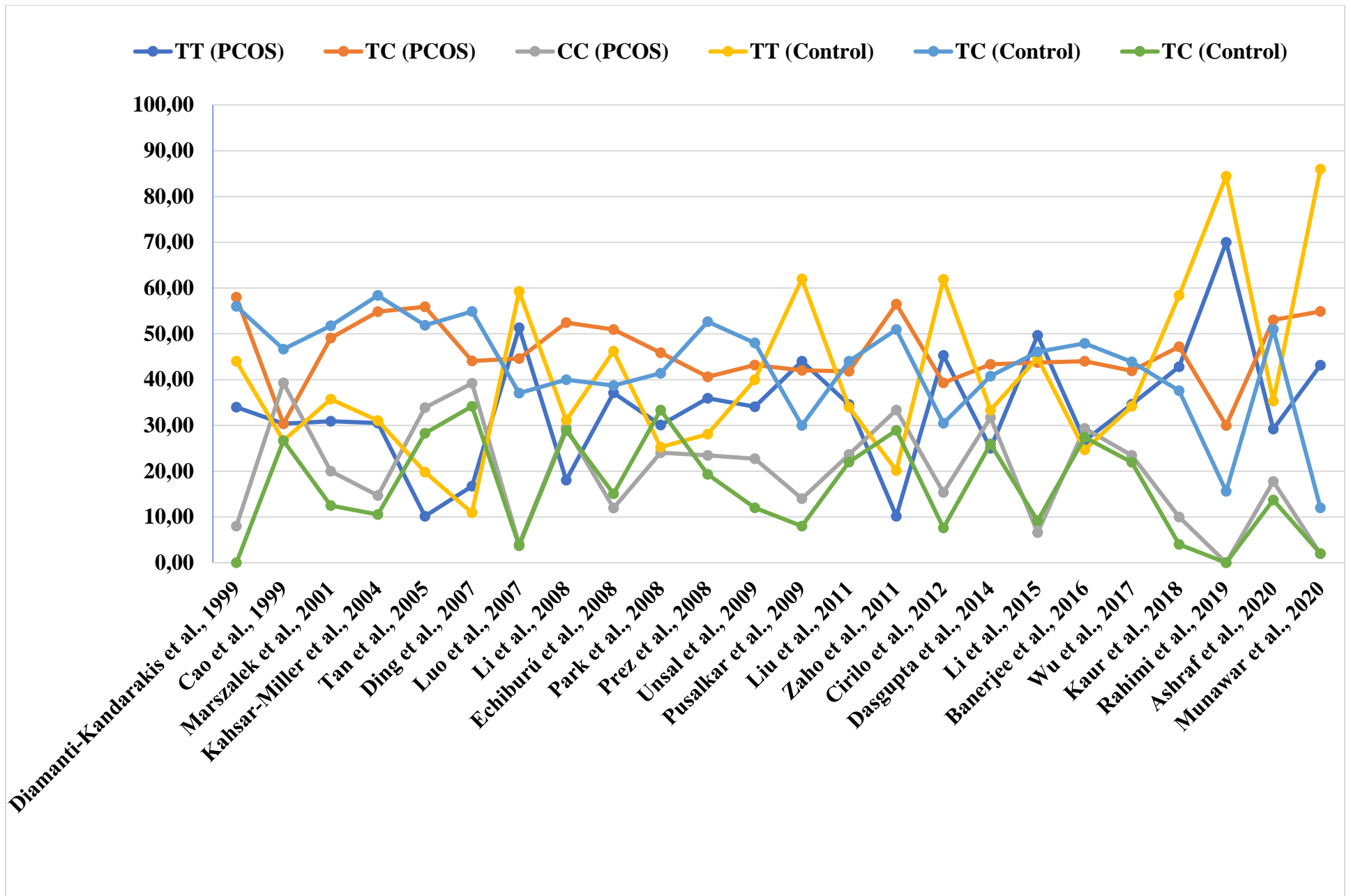
**Figure .** Graphic presentation of studies genotypic frequencies included in this meta-analysis.
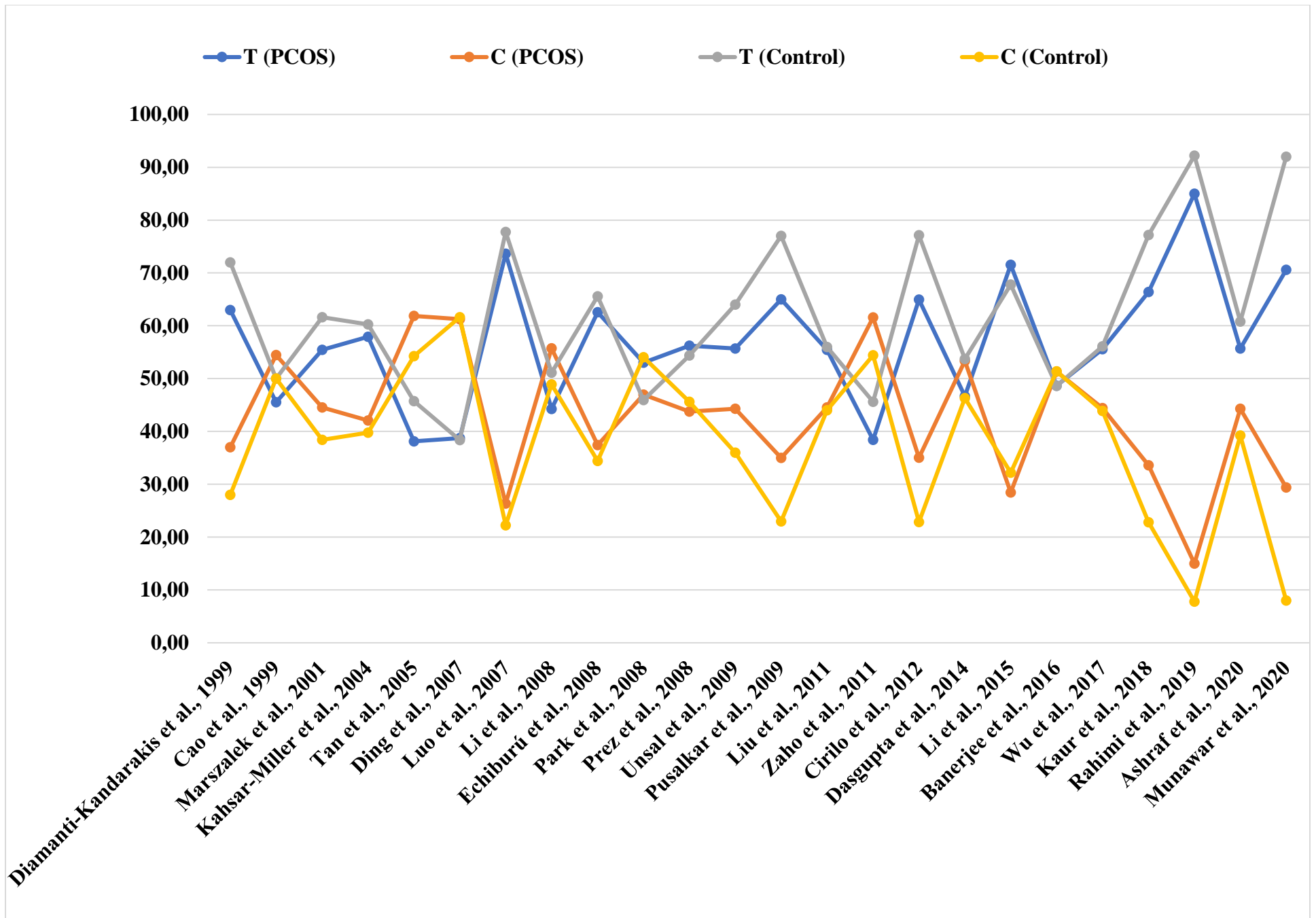
**Figure .** Graphic presentation of studies allelic frequencies included in this meta-analysis.

**Table 2.** data of studies included in this meta-analysis with the Hardy-Weinberg Equilibrium adjusted.

| Author | Ethnicity | TT Cases | TC Cases | CC Cases | TT Controls | TC Controls | CC Controls | HW P value | HW-adjusted P value |
|---|---|---|---|---|---|---|---|---|---|
| **Diamanti-Kandarakis *et al.*, 1999** | Greece (Caucasian) | 17 | 29 | 4 | 22 | 28 | 0 | 0,006 | 0,0608 |
| **Cao *et al.*, 1999** | China (Asian) | 17 | 17 | 22 | 8 | 14 | 8 | 0,715 | 0,7671 |
| **Marszalek *et al.*, 2001** | Poland (Caucasian) | 17 | 27 | 11 | 20 | 29 | 7 | 0,4785 | 0,6755 |
| **Kahsar-Miller *et al.*, 2004** | USA (Caucasian) | 79 | 142 | 38 | 50 | 94 | 17 | 0,0055 | 0,0608 |
| **Tan *et al.*, 2005** | China (Asian) | 12 | 66 | 40 | 21 | 55 | 30 | 0,6411 | 0,7671 |
| **Ding *et al.*, 2007** | China (Asian) | 55 | 145 | 129 | 30 | 151 | 94 | 0,0076 | 0,0608 |
| **Luo *et al.*, 2007** | China (Asian) | 38 | 33 | 3 | 16 | 10 | 1 | 0,7105 | 0,7671 |
| **Li *et al.*, 2008** | China (Asian) | 11 | 32 | 18 | 14 | 18 | 13 | 0,1806 | 0,4019 |
| **Echiburu *et al.*, 2008** | Chili (Caucasian) | 59 | 81 | 19 | 43 | 36 | 14 | 0,1696 | 0,4019 |
| **Park *et al.*, 2008** | South Korea (Asian) | 40 | 61 | 32 | 25 | 41 | 33 | 0,098 | 0,392 |
| **Prez *et al.*, 2008** | Argentina (Caucasian) | 23 | 26 | 15 | 16 | 30 | 11 | 0,6462 | 0,7671 |
| **Unsal *et al.*, 2009** | Turkey (Caucasian) | 15 | 19 | 10 | 20 | 24 | 6 | 0,7683 | 0,7683 |
| **Pusalkar et al., 2009** | India (Asian) | 44 | 42 | 14 | 62 | 30 | 8 | 0,126 | 0,4019 |
| **Liu *et al.*, 2011** | China (Asian) | 19 | 23 | 13 | 17 | 22 | 11 | 0,4487 | 0,673 |
| **Zaho *et al.*, 2011** | China (Asian) | 18 | 100 | 59 | 32 | 81 | 46 | 0,7351 | 0,7671 |
| **Cirilo *et al.*, 2012** | Brazil (Caucasian) | 53 | 46 | 18 | 65 | 32 | 8 | 0,1641 | 0,4019 |
| **Dasgupta *et al.*, 2014** | Asian (India) | 15 | 26 | 19 | 18 | 22 | 14 | 0,1842 | 0,4019 |
| **Li *et al.*, 2015** | Asian (China) | 158 | 139 | 21 | 137 | 141 | 28 | 0,3317 | 0,5686 |
| **Banerjee *et al.*, 2016** | India (Asian) | 20 | 33 | 22 | 18 | 35 | 20 | 0,7301 | 0,7671 |
| **Wu *et al.*, 2017** | China (Asian) | 90 | 109 | 61 | 81 | 104 | 52 | 0,0933 | 0,392 |
| **Kaur *et al.*, 2018** | Asian (India) | 107 | 118 | 25 | 146 | 94 | 10 | 0,2817 | 0,5201 |
| **Rahimi *et al.*, 2019** | Iran (Asian) | 35 | 15 | 0 | 92 | 17 | 0 | 0,3772 | 0,6035 |
| **Ashraf *et al.*, 2020** | Asian (Kashmir) | 115 | 209 | 70 | 108 | 156 | 42 | 0,225 | 0,45 |
| **Munawar *et al.*, 2020** | Asian (Pakistan) | 88 | 112 | 4 | 86 | 12 | 2 | 0,0646 | 0,3876 |

**Table 3.** Association test results.

| Model | | OR | 95%-CI | *p*-value | Adjusted *p*-value |
|---|---|---|---|---|---|
| **Allele contrast (C *vs*. T)** | Fixed effect | 1.1984 | [1.1121; 1.2913] | 2.0761e-06 | 1.45328e-05 |
| | Random effect | 1.2658 | [1.1083; 1.4458] | 0.0005097745 | 0.0035684215 |
| **Recessive model (CC *vs*. CT+TT)** | Fixed effect | 1.2214 | [1.0655; 1.4001] | 0.0041005483 | 0.0287038384 |
| | Random effect | 1.2214 | [1.0655; 1.4001] | 0.0041005483 | 0.0287038384 |
| **Dominant model (CC+CT *vs*. TT)** | Fixed effect | 1.2970 | [1.1608; 1.4492] | 4.3547e-06 | 3.0483e-05 |
| | Random effect | 1.3780 | [1.1078; 1.7141] | 0.0039888692 | 0.0279220844 |
| **Over-dominant model (CT *vs*. CC+TT)** | Fixed effect | 1.1036 | [0.9966; 1.2222] | 0.0582285538 | 0.4075998768 |
| | Random effect | 1.1718 | [0.9654; 1.4223] | 0.1087244792 | 0.7610713547 |
| **CC *vs*. TT** | Fixed effect | 1.3024 | [1.1042; 1.5361] | 0.0017042182 | 0.0119295272 |
| | Random effect | 1.3699 | [1.0958; 1.7127] | 0.0057351129 | 0.0401457903 |
| **CC *vs*. CT** | Fixed effect | 1.1704 | [1.0127; 1.3527] | 0.0330668194 | 0.2314677357 |
| | Random effect | 1.1704 | [1.0127; 1.3527] | 0.0330668194 | 0.2314677357 |
| **CT *vs*. TT** | Fixed effect | 1.2531 | [1.1147; 1.4087] | 0.0001576509 | 0.0011035566 |
| | Random effect | 1.3230 | [1.0520; 1.6637] | 0.0166982268 | 0.1168875874 |

**Details on meta-analytical method:**

- Fixed effect estimate method: Inverse variance,
- Random effect estimate method: DerSimonian-Laird.

**Table 4.** Heterogeneity tests.

| Model | tau^2 | H | I^2 | Q | *p*-value |
|---|---|---|---|---|---|
| **Allele contrast (C *vs*. T)** | 0.06 | 1.66 | 0.64 | 63.09 | 0.00 |
| **Recessive model (CC *vs*. CT+TT)** | 0.00 | 1.00 | 0.00 | 21.84 | 0.47 |
| **Dominant model (CC+CT *vs*. TT)** | 0.19 | 1.85 | 0.71 | 78.45 | 0.00 |
| **Over-dominant model (CT *vs*. CC+TT)** | 0.14 | 1.78 | 0.68 | 72.71 | 0.00 |
| **CC *vs*. TT** | 0.10 | 1.27 | 0.38 | 35.24 | 0.04 |
| **CC *vs*. CT** | 0.00 | 1.00 | 0.00 | 21.45 | 0.49 |
| **CT *vs*. TT** | 0.21 | 1.83 | 0.70 | 77.40 | 0.00 |

**Details on meta-analytical method:**

- Fixed effect estimate method: Inverse variance,
- Random effect estimate method: DerSimonian-Laird.

**tau^2:** estimated standard deviation of underlying effects across studies.
**H:** Heterogeneity.
**I^2:** percentage of variation across studies that is due to heterogeneity rather than chance.
**Q:** Cochran's measure of heterogeneity is, which is calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method.

| Study | Experimental Events | Total | Control Events | Total | Odds Ratio | OR | 95%-CI | Weight (fixed) | Weight (random) |
|---|---|---|---|---|---|---|---|---|---|
| Diamanti-Kandarakis et al., 1999 | 37 | 100 | 28 | 100 | | 1.51 | [0.83; 2.74] | 1.6% | 3.0% |
| Cao et al., 1999 | 61 | 112 | 30 | 60 | | 1.20 | [0.64; 2.24] | 1.4% | 2.8% |
| Marszalek et al., 2001 | 49 | 110 | 43 | 112 | | 1.29 | [0.75; 2.20] | 2.0% | 3.4% |
| Kahsar-Miller et al., 2004 | 218 | 518 | 128 | 322 | | 1.10 | [0.83; 1.46] | 7.0% | 5.5% |
| Tan et al., 2005 | 146 | 236 | 115 | 212 | | 1.37 | [0.94; 1.99] | 3.9% | 4.6% |
| Ding et al., 2007 | 403 | 658 | 339 | 550 | | 0.98 | [0.78; 1.24] | 10.3% | 6.0% |
| Luo et al., 2007 | 39 | 148 | 12 | 54 | | 1.25 | [0.60; 2.62] | 1.0% | 2.3% |
| Li et al., 2008 | 68 | 122 | 44 | 90 | | 1.32 | [0.76; 2.27] | 1.9% | 3.3% |
| Echiburu et al., 2008 | 119 | 318 | 64 | 186 | | 1.14 | [0.78; 1.66] | 3.9% | 4.6% |
| Park et al., 2008 | 125 | 266 | 107 | 198 | | 0.75 | [0.52; 1.09] | 4.1% | 4.7% |
| Prez et al., 2008 | 56 | 128 | 52 | 114 | | 0.93 | [0.56; 1.54] | 2.2% | 3.6% |
| Unsal et al., 2009 | 39 | 88 | 36 | 100 | | 1.41 | [0.79; 2.54] | 1.6% | 3.0% |
| Pusalkar et al., 2009 | 70 | 200 | 46 | 200 | | 1.80 | [1.16; 2.80] | 2.9% | 4.1% |
| Liu et al., 2011 | 49 | 110 | 44 | 100 | | 1.02 | [0.59; 1.76] | 1.9% | 3.3% |
| Zaho et al., 2011 | 218 | 354 | 173 | 318 | | 1.34 | [0.99; 1.83] | 5.9% | 5.3% |
| Cirilo et al., 2012 | 82 | 234 | 48 | 210 | | 1.82 | [1.20; 2.77] | 3.2% | 4.3% |
| Dasgupta et al., 2014 | 64 | 120 | 50 | 108 | | 1.33 | [0.79; 2.23] | 2.1% | 3.5% |
| Li et al., 2015 | 181 | 636 | 197 | 612 | | 0.84 | [0.66; 1.07] | 9.6% | 5.9% |
| Banerjee et al., 2016 | 77 | 150 | 75 | 146 | | 1.00 | [0.63; 1.58] | 2.7% | 3.9% |
| Wu et al., 2017 | 231 | 520 | 208 | 474 | | 1.02 | [0.80; 1.31] | 8.9% | 5.8% |
| Kaur et al., 2018 | 168 | 500 | 114 | 500 | | 1.71 | [1.30; 2.27] | 7.2% | 5.6% |
| Rahimi et al., 2019 | 15 | 100 | 17 | 218 | | 2.09 | [1.00; 4.37] | 1.0% | 2.2% |
| Ashraf et al., 2020 | 349 | 788 | 240 | 612 | | 1.23 | [0.99; 1.53] | 12.1% | 6.2% |
| Munawar et al., 2020 | 120 | 408 | 16 | 200 | | 4.79 | [2.75; 8.33] | 1.8% | 3.2% |
| **Fixed effect model** | | 6924 | | 5796 | | 1.20 | [1.11; 1.29] | 100.0% | -- |
| **Random effects model** | | | | | | 1.27 | [1.11; 1.45] | -- | 100.0% |

Heterogeneity: $I^2 = 64\%$, $\tau^2 = 0.0624$, $p < 0.01$

0.2   0.5   1   2   5

**Figure 2.** Forest plot of meta-analysis in the allele contrast (C *vs*. T).

| Study | Experimental Events | Total | Control Events | Total | Odds Ratio | OR | 95%-CI | Weight (fixed) | Weight (random) |
|---|---|---|---|---|---|---|---|---|---|
| Diamanti-Kandarakis et al., 1999 | 4 | 50 | 0 | 50 | | 9.77 | [0.51; 186.52] | 0.2% | 0.2% |
| Cao et al., 1999 | 22 | 56 | 8 | 30 | | 1.78 | [0.67; 4.70] | 2.0% | 2.0% |
| Marszalek et al., 2001 | 11 | 55 | 7 | 56 | | 1.75 | [0.62; 4.91] | 1.8% | 1.8% |
| Kahsar-Miller et al., 2004 | 38 | 259 | 17 | 161 | | 1.46 | [0.79; 2.68] | 5.0% | 5.0% |
| Tan et al., 2005 | 40 | 118 | 30 | 106 | | 1.30 | [0.74; 2.30] | 5.8% | 5.8% |
| Ding et al., 2007 | 129 | 329 | 94 | 275 | | 1.24 | [0.89; 1.73] | 16.8% | 16.8% |
| Luo et al., 2007 | 3 | 74 | 1 | 27 | | 1.10 | [0.11; 11.04] | 0.4% | 0.4% |
| Li et al., 2008 | 18 | 61 | 13 | 45 | | 1.03 | [0.44; 2.40] | 2.6% | 2.6% |
| Echiburu et al., 2008 | 19 | 159 | 14 | 93 | | 0.77 | [0.36; 1.61] | 3.4% | 3.4% |
| Park et al., 2008 | 32 | 133 | 33 | 99 | | 0.63 | [0.36; 1.13] | 5.6% | 5.6% |
| Prez et al., 2008 | 15 | 64 | 11 | 57 | | 1.28 | [0.53; 3.07] | 2.4% | 2.4% |
| Unsal et al., 2009 | 10 | 44 | 6 | 50 | | 2.16 | [0.71; 6.52] | 1.5% | 1.5% |
| Pusalkar et al., 2009 | 14 | 100 | 8 | 100 | | 1.87 | [0.75; 4.68] | 2.2% | 2.2% |
| Liu et al., 2011 | 13 | 55 | 11 | 50 | | 1.10 | [0.44; 2.74] | 2.2% | 2.2% |
| Zaho et al., 2011 | 59 | 177 | 46 | 159 | | 1.23 | [0.77; 1.95] | 8.7% | 8.7% |
| Cirilo et al., 2012 | 18 | 117 | 8 | 105 | | 2.20 | [0.92; 5.31] | 2.4% | 2.4% |
| Dasgupta et al., 2014 | 19 | 60 | 14 | 54 | | 1.32 | [0.59; 3.00] | 2.8% | 2.8% |
| Li et al., 2015 | 21 | 318 | 28 | 306 | | 0.70 | [0.39; 1.27] | 5.4% | 5.4% |
| Banerjee et al., 2016 | 22 | 75 | 20 | 73 | | 1.10 | [0.54; 2.25] | 3.6% | 3.6% |
| Wu et al., 2017 | 61 | 260 | 52 | 237 | | 1.09 | [0.72; 1.66] | 10.5% | 10.5% |
| Kaur et al., 2018 | 25 | 250 | 10 | 250 | | 2.67 | [1.25; 5.68] | 3.3% | 3.3% |
| Rahimi et al., 2019 | 0 | 50 | 0 | 109 | | | | 0.0% | 0.0% |
| Ashraf et al., 2020 | 70 | 394 | 42 | 306 | | 1.36 | [0.90; 2.06] | 10.8% | 10.8% |
| Munawar et al., 2020 | 4 | 204 | 2 | 100 | | 0.98 | [0.18; 5.44] | 0.6% | 0.6% |
| **Fixed effect model** | | 3462 | | 2898 | | 1.22 | [1.07; 1.40] | 100.0% | -- |
| **Random effects model** | | | | | | 1.22 | [1.07; 1.40] | -- | 100.0% |

Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0$, $p = 0.47$

0.01   0.1   1   10   100

**Figure 3.** Forest plot of meta-analysis in the recessive model (CC *vs*. CT+TT).

**Figure 4**

| Study | Experimental Events | Total | Control Events | Total | OR | 95%-CI | Weight (fixed) | Weight (random) |
|---|---|---|---|---|---|---|---|---|
| Diamanti-Kandarakis et al., 1999 | 33 | 50 | 28 | 50 | 1.53 | [0.68; 3.42] | 1.9% | 3.4% |
| Cao et al., 1999 | 39 | 56 | 22 | 30 | 0.83 | [0.31; 2.24] | 1.3% | 2.8% |
| Marszalek et al., 2001 | 38 | 55 | 36 | 56 | 1.24 | [0.56; 2.74] | 2.0% | 3.5% |
| Kahsar-Miller et al., 2004 | 180 | 259 | 111 | 161 | 1.03 | [0.67; 1.57] | 6.8% | 5.2% |
| Tan et al., 2005 | 106 | 118 | 85 | 106 | 2.18 | [1.02; 4.69] | 2.1% | 3.6% |
| Ding et al., 2007 | 274 | 329 | 245 | 275 | 0.61 | [0.38; 0.98] | 5.4% | 5.0% |
| Luo et al., 2007 | 36 | 74 | 11 | 27 | 1.38 | [0.56; 3.37] | 1.5% | 3.1% |
| Li et al., 2008 | 50 | 61 | 31 | 45 | 2.05 | [0.83; 5.09] | 1.5% | 3.1% |
| Echiburu et al., 2008 | 100 | 159 | 50 | 93 | 1.46 | [0.87; 2.45] | 4.6% | 4.8% |
| Park et al., 2008 | 93 | 133 | 74 | 99 | 0.79 | [0.44; 1.41] | 3.6% | 4.4% |
| Prez et al., 2008 | 41 | 64 | 41 | 57 | 0.70 | [0.32; 1.50] | 2.1% | 3.6% |
| Unsal et al., 2009 | 29 | 44 | 30 | 50 | 1.29 | [0.56; 2.99] | 1.7% | 3.3% |
| Pusalkar et al., 2009 | 56 | 100 | 38 | 100 | 2.08 | [1.18; 3.65] | 3.9% | 4.5% |
| Liu et al., 2011 | 36 | 55 | 33 | 50 | 0.98 | [0.44; 2.19] | 1.9% | 3.4% |
| Zaho et al., 2011 | 159 | 177 | 127 | 159 | 2.23 | [1.19; 4.15] | 3.2% | 4.3% |
| Cirilo et al., 2012 | 64 | 117 | 40 | 105 | 1.96 | [1.15; 3.35] | 4.3% | 4.7% |
| Dasgupta et al., 2014 | 45 | 60 | 36 | 54 | 1.50 | [0.67; 3.38] | 1.9% | 3.4% |
| Li et al., 2015 | 160 | 318 | 169 | 306 | 0.82 | [0.60; 1.12] | 12.4% | 5.7% |
| Banerjee et al., 2016 | 55 | 75 | 55 | 73 | 0.90 | [0.43; 1.88] | 2.3% | 3.7% |
| Wu et al., 2017 | 170 | 260 | 156 | 237 | 0.98 | [0.68; 1.42] | 9.0% | 5.5% |
| Kaur et al., 2018 | 143 | 250 | 104 | 250 | 1.88 | [1.32; 2.68] | 9.8% | 5.6% |
| Rahimi et al., 2019 | 15 | 50 | 17 | 109 | 2.32 | [1.05; 5.14] | 1.9% | 3.5% |
| Ashraf et al., 2020 | 279 | 394 | 198 | 306 | 1.32 | [0.96; 1.82] | 12.1% | 5.7% |
| Munawar et al., 2020 | 116 | 204 | 14 | 100 | 8.10 | [4.32; 15.19] | 3.1% | 4.2% |
| **Fixed effect model** | | 3462 | | 2898 | **1.30** | **[1.16; 1.45]** | 100.0% | -- |
| **Random effects model** | | | | | **1.38** | **[1.11; 1.71]** | -- | 100.0% |

Heterogeneity: $I^2 = 71\%$, $\tau^2 = 0.1906$, $p < 0.01$

0.1  0.5  1  2  10

**Figure 4.** Forest plot of meta-analysis in the dominant model (CC+CT *vs*. TT).

**Figure 5**

| Study | Experimental Events | Total | Control Events | Total | OR | 95%-CI | Weight (fixed) | Weight (random) |
|---|---|---|---|---|---|---|---|---|
| Diamanti-Kandarakis et al., 1999 | 29 | 50 | 28 | 50 | 1.09 | [0.49; 2.40] | 1.7% | 3.2% |
| Cao et al., 1999 | 17 | 56 | 14 | 30 | 0.50 | [0.20; 1.24] | 1.2% | 2.7% |
| Marszalek et al., 2001 | 27 | 55 | 29 | 56 | 0.90 | [0.43; 1.89] | 1.9% | 3.4% |
| Kahsar-Miller et al., 2004 | 142 | 259 | 94 | 161 | 0.87 | [0.58; 1.29] | 6.6% | 5.3% |
| Tan et al., 2005 | 66 | 118 | 55 | 106 | 1.18 | [0.70; 1.99] | 3.8% | 4.5% |
| Ding et al., 2007 | 145 | 329 | 151 | 275 | 0.65 | [0.47; 0.89] | 10.0% | 5.7% |
| Luo et al., 2007 | 33 | 74 | 10 | 27 | 1.37 | [0.55; 3.38] | 1.3% | 2.7% |
| Li et al., 2008 | 32 | 61 | 18 | 45 | 1.66 | [0.76; 3.61] | 1.7% | 3.2% |
| Echiburu et al., 2008 | 81 | 159 | 36 | 93 | 1.64 | [0.98; 2.77] | 3.8% | 4.5% |
| Park et al., 2008 | 61 | 133 | 41 | 99 | 1.20 | [0.71; 2.03] | 3.8% | 4.5% |
| Prez et al., 2008 | 26 | 64 | 30 | 57 | 0.62 | [0.30; 1.27] | 2.0% | 3.5% |
| Unsal et al., 2009 | 19 | 44 | 24 | 50 | 0.82 | [0.36; 1.86] | 1.6% | 3.1% |
| Pusalkar et al., 2009 | 42 | 100 | 30 | 100 | 1.69 | [0.94; 3.03] | 3.1% | 4.2% |
| Liu et al., 2011 | 23 | 55 | 22 | 50 | 0.91 | [0.42; 1.98] | 1.7% | 3.3% |
| Zaho et al., 2011 | 100 | 177 | 81 | 159 | 1.25 | [0.81; 1.92] | 5.6% | 5.1% |
| Cirilo et al., 2012 | 46 | 117 | 32 | 105 | 1.48 | [0.85; 2.58] | 3.4% | 4.3% |
| Dasgupta et al., 2014 | 26 | 60 | 22 | 54 | 1.11 | [0.53; 2.34] | 1.9% | 3.4% |
| Li et al., 2015 | 139 | 318 | 141 | 306 | 0.91 | [0.66; 1.25] | 10.5% | 5.7% |
| Banerjee et al., 2016 | 33 | 75 | 35 | 73 | 0.85 | [0.45; 1.63] | 2.5% | 3.9% |
| Wu et al., 2017 | 109 | 260 | 104 | 237 | 0.92 | [0.65; 1.32] | 8.2% | 5.5% |
| Kaur et al., 2018 | 118 | 250 | 94 | 250 | 1.48 | [1.04; 2.12] | 8.2% | 5.5% |
| Rahimi et al., 2019 | 15 | 50 | 17 | 109 | 2.32 | [1.05; 5.14] | 1.6% | 3.2% |
| Ashraf et al., 2020 | 209 | 394 | 156 | 306 | 1.09 | [0.81; 1.46] | 11.6% | 5.8% |
| Munawar et al., 2020 | 112 | 204 | 12 | 100 | 8.93 | [4.60; 17.33] | 2.4% | 3.8% |
| **Fixed effect model** | | 3462 | | 2898 | **1.10** | **[1.00; 1.22]** | 100.0% | -- |
| **Random effects model** | | | | | **1.17** | **[0.97; 1.42]** | -- | 100.0% |

Heterogeneity: $I^2 = 68\%$, $\tau^2 = 0.1443$, $p < 0.01$

0.1  0.5  1  2  10

**Figure 5.** Forest plot of meta-analysis in the over-dominant model (CT *vs*. CC+TT).

| Study | Experimental Events | Total | Control Events | Total | Odds Ratio | OR | 95%-CI | Weight (fixed) | Weight (random) |
|---|---|---|---|---|---|---|---|---|---|
| Diamanti-Kandarakis et al., 1999 | 4 | 21 | 0 | 22 | | 11.57 | [0.58; 229.60] | 0.3% | 0.5% |
| Cao et al., 1999 | 22 | 39 | 8 | 16 | | 1.29 | [0.40; 4.16] | 2.0% | 2.9% |
| Marszalek et al., 2001 | 11 | 28 | 7 | 27 | | 1.85 | [0.59; 5.82] | 2.1% | 2.9% |
| Kahsar-Miller et al., 2004 | 38 | 117 | 17 | 67 | | 1.41 | [0.72; 2.77] | 6.0% | 5.9% |
| Tan et al., 2005 | 40 | 52 | 30 | 51 | | 2.33 | [0.99; 5.47] | 3.7% | 4.5% |
| Ding et al., 2007 | 129 | 184 | 94 | 124 | | 0.75 | [0.45; 1.26] | 10.1% | 7.6% |
| Luo et al., 2007 | 3 | 41 | 1 | 17 | | 1.26 | [0.12; 13.08] | 0.5% | 0.9% |
| Li et al., 2008 | 18 | 29 | 13 | 27 | | 1.76 | [0.61; 5.11] | 2.4% | 3.3% |
| Echiburu et al., 2008 | 19 | 78 | 14 | 57 | | 0.99 | [0.45; 2.19] | 4.3% | 4.9% |
| Park et al., 2008 | 32 | 72 | 33 | 58 | | 0.61 | [0.30; 1.22] | 5.6% | 5.7% |
| Prez et al., 2008 | 15 | 38 | 11 | 27 | | 0.95 | [0.35; 2.59] | 2.7% | 3.6% |
| Unsal et al., 2009 | 10 | 25 | 6 | 26 | | 2.22 | [0.66; 7.48] | 1.9% | 2.7% |
| Pusalkar et al., 2009 | 14 | 58 | 8 | 70 | | 2.47 | [0.95; 6.38] | 3.0% | 3.9% |
| Liu et al., 2011 | 13 | 32 | 11 | 28 | | 1.06 | [0.38; 2.98] | 2.5% | 3.4% |
| Zaho et al., 2011 | 59 | 77 | 46 | 78 | | 2.28 | [1.14; 4.57] | 5.7% | 5.7% |
| Cirilo et al., 2012 | 18 | 71 | 8 | 73 | | 2.76 | [1.11; 6.84] | 3.3% | 4.1% |
| Dasgupta et al., 2014 | 19 | 34 | 14 | 32 | | 1.63 | [0.62; 4.31] | 2.9% | 3.7% |
| Li et al., 2015 | 21 | 179 | 28 | 165 | | 0.65 | [0.35; 1.20] | 7.3% | 6.6% |
| Banerjee et al., 2016 | 22 | 42 | 20 | 38 | | 0.99 | [0.41; 2.38] | 3.5% | 4.3% |
| Wu et al., 2017 | 61 | 151 | 52 | 133 | | 1.06 | [0.66; 1.70] | 12.0% | 8.1% |
| Kaur et al., 2018 | 25 | 132 | 10 | 156 | | 3.41 | [1.57; 7.40] | 4.5% | 5.1% |
| Rahimi et al., 2019 | 0 | 35 | 0 | 92 | | | | 0.0% | 0.0% |
| Ashraf et al., 2020 | 70 | 185 | 42 | 150 | | 1.57 | [0.98; 2.49] | 12.7% | 8.3% |
| Munawar et al., 2020 | 4 | 92 | 2 | 88 | | 1.95 | [0.35; 10.95] | 0.9% | 1.5% |
| Fixed effect model | | 1812 | | 1622 | | 1.30 | [1.10; 1.54] | 100.0% | |
| Random effects model | | | | | | 1.37 | [1.10; 1.71] | -- | 100.0% |

Heterogeneity: $I^2 = 38\%$, $\tau^2 = 0.1008$, $p = 0.04$

0.01 0.1 1 10 100

**Figure 6.** Forest plot of meta-analysis in the model CC *vs*. TT.

| Study | Experimental Events | Total | Control Events | Total | Odds Ratio | OR | 95%-CI | Weight (fixed) | Weight (random) |
|---|---|---|---|---|---|---|---|---|---|
| Diamanti-Kandarakis et al., 1999 | 4 | 33 | 0 | 28 | | 8.69 | [0.45; 168.93] | 0.2% | 0.2% |
| Cao et al., 1999 | 22 | 39 | 8 | 22 | | 2.26 | [0.77; 6.63] | 1.8% | 1.8% |
| Marszalek et al., 2001 | 11 | 38 | 7 | 36 | | 1.69 | [0.57; 4.98] | 1.8% | 1.8% |
| Kahsar-Miller et al., 2004 | 38 | 180 | 17 | 111 | | 1.48 | [0.79; 2.77] | 5.3% | 5.3% |
| Tan et al., 2005 | 40 | 106 | 30 | 85 | | 1.11 | [0.61; 2.01] | 5.9% | 5.9% |
| Ding et al., 2007 | 129 | 274 | 94 | 245 | | 1.43 | [1.01; 2.03] | 17.1% | 17.1% |
| Luo et al., 2007 | 3 | 36 | 1 | 11 | | 0.91 | [0.08; 9.74] | 0.4% | 0.4% |
| Li et al., 2008 | 18 | 50 | 13 | 31 | | 0.78 | [0.31; 1.95] | 2.5% | 2.5% |
| Echiburu et al., 2008 | 19 | 100 | 14 | 50 | | 0.60 | [0.27; 1.33] | 3.3% | 3.3% |
| Park et al., 2008 | 32 | 93 | 33 | 74 | | 0.65 | [0.35; 1.22] | 5.3% | 5.3% |
| Prez et al., 2008 | 15 | 41 | 11 | 41 | | 1.57 | [0.62; 4.02] | 2.4% | 2.4% |
| Unsal et al., 2009 | 10 | 29 | 6 | 30 | | 2.11 | [0.65; 6.83] | 1.5% | 1.5% |
| Pusalkar et al., 2009 | 14 | 56 | 8 | 38 | | 1.25 | [0.47; 3.35] | 2.2% | 2.2% |
| Liu et al., 2011 | 13 | 36 | 11 | 33 | | 1.13 | [0.42; 3.05] | 2.1% | 2.1% |
| Zaho et al., 2011 | 59 | 159 | 46 | 127 | | 1.04 | [0.64; 1.69] | 8.9% | 8.9% |
| Cirilo et al., 2012 | 18 | 64 | 8 | 40 | | 1.57 | [0.61; 4.04] | 2.3% | 2.3% |
| Dasgupta et al., 2014 | 19 | 45 | 14 | 36 | | 1.15 | [0.47; 2.81] | 2.6% | 2.6% |
| Li et al., 2015 | 21 | 160 | 28 | 169 | | 0.76 | [0.41; 1.40] | 5.6% | 5.6% |
| Banerjee et al., 2016 | 22 | 55 | 20 | 55 | | 1.17 | [0.54; 2.52] | 3.5% | 3.5% |
| Wu et al., 2017 | 61 | 170 | 52 | 156 | | 1.12 | [0.71; 1.77] | 10.0% | 10.0% |
| Kaur et al., 2018 | 25 | 143 | 10 | 104 | | 1.99 | [0.91; 4.35] | 3.4% | 3.4% |
| Rahimi et al., 2019 | 0 | 15 | 0 | 17 | | | | 0.0% | 0.0% |
| Ashraf et al., 2020 | 70 | 279 | 42 | 198 | | 1.24 | [0.81; 1.92] | 11.1% | 11.1% |
| Munawar et al., 2020 | 4 | 116 | 2 | 14 | | 0.21 | [0.04; 1.29] | 0.6% | 0.6% |
| Fixed effect model | | 2317 | | 1751 | | 1.17 | [1.01; 1.35] | 100.0% | |
| Random effects model | | | | | | 1.17 | [1.01; 1.35] | -- | 100.0% |

Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0$, $p = 0.49$

0.01 0.1 1 10 100

**Figure 7.** Forest plot of meta-analysis in the model CC *vs*. CT.

| Study | Experimental Events | Total | Control Events | Total | Odds Ratio | OR | 95%-CI | Weight (fixed) | Weight (random) |
|---|---|---|---|---|---|---|---|---|---|
| Diamanti-Kandarakis et al., 1999 | 29 | 46 | 28 | 50 | | 1.34 | [0.59; 3.04] | 2.0% | 3.6% |
| Cao et al., 1999 | 17 | 34 | 14 | 22 | | 0.57 | [0.19; 1.71] | 1.1% | 2.6% |
| Marszalek et al., 2001 | 27 | 44 | 29 | 49 | | 1.10 | [0.48; 2.52] | 2.0% | 3.5% |
| Kahsar-Miller et al., 2004 | 142 | 221 | 94 | 144 | | 0.96 | [0.62; 1.48] | 7.1% | 5.3% |
| Tan et al., 2005 | 66 | 78 | 55 | 76 | | 2.10 | [0.95; 4.65] | 2.2% | 3.7% |
| Ding et al., 2007 | 145 | 200 | 151 | 181 | | 0.52 | [0.32; 0.86] | 5.5% | 5.0% |
| Luo et al., 2007 | 33 | 71 | 10 | 26 | | 1.39 | [0.56; 3.48] | 1.6% | 3.2% |
| Li et al., 2008 | 32 | 43 | 18 | 32 | | 2.26 | [0.85; 6.02] | 1.4% | 3.0% |
| Echiburu et al., 2008 | 81 | 140 | 36 | 79 | | 1.64 | [0.94; 2.86] | 4.4% | 4.7% |
| Park et al., 2008 | 61 | 101 | 41 | 66 | | 0.93 | [0.49; 1.76] | 3.4% | 4.4% |
| Prez et al., 2008 | 26 | 49 | 30 | 46 | | 0.60 | [0.26; 1.38] | 2.0% | 3.5% |
| Unsal et al., 2009 | 19 | 34 | 24 | 44 | | 1.06 | [0.43; 2.60] | 1.7% | 3.3% |
| Pusalkar et al., 2009 | 42 | 86 | 30 | 92 | | 1.97 | [1.07; 3.62] | 3.7% | 4.5% |
| Liu et al., 2011 | 23 | 42 | 22 | 39 | | 0.94 | [0.39; 2.25] | 1.8% | 3.3% |
| Zaho et al., 2011 | 100 | 118 | 81 | 113 | | 2.19 | [1.15; 4.19] | 3.3% | 4.3% |
| Cirilo et al., 2012 | 46 | 99 | 32 | 97 | | 1.76 | [0.99; 3.15] | 4.1% | 4.6% |
| Dasgupta et al., 2014 | 26 | 41 | 22 | 40 | | 1.42 | [0.58; 3.45] | 1.7% | 3.3% |
| Li et al., 2015 | 139 | 297 | 141 | 278 | | 0.85 | [0.62; 1.19] | 12.8% | 5.8% |
| Banerjee et al., 2016 | 33 | 53 | 35 | 53 | | 0.85 | [0.38; 1.88] | 2.2% | 3.7% |
| Wu et al., 2017 | 109 | 199 | 104 | 185 | | 0.94 | [0.63; 1.41] | 8.4% | 5.5% |
| Kaur et al., 2018 | 118 | 225 | 94 | 240 | | 1.71 | [1.19; 2.48] | 10.1% | 5.6% |
| Rahimi et al., 2019 | 15 | 50 | 17 | 109 | | 2.32 | [1.05; 5.14] | 2.2% | 3.7% |
| Ashraf et al., 2020 | 209 | 324 | 156 | 264 | | 1.26 | [0.90; 1.76] | 12.2% | 5.8% |
| Munawar et al., 2020 | 112 | 200 | 12 | 98 | | 9.12 | [4.69; 17.74] | 3.1% | 4.2% |
| **Fixed effect model** | | **2795** | | **2423** | | **1.25** | **[1.11; 1.41]** | **100.0%** | **--** |
| **Random effects model** | | | | | | **1.32** | **[1.05; 1.66]** | **--** | **100.0%** |

Heterogeneity: $I^2 = 70\%$, $\tau^2 = 0.2083$, $p < 0.01$

0.1　0.5　1　2　　10

**Figure 8.** Forest plot of meta-analysis in the model CT *vs*. TT.

**Table 5.** Subgroup analyses were performed by ethnicity.

| Model | Ethnicity | Number of studies | Test of association | | | Test of heterogeneity | | | Publication bias |
|---|---|---|---|---|---|---|---|---|---|
| | | | OR | 95% CI | *p*-value | Model | *p*-value | I^2 | *p*-value (Egger's test) |
| **Allele contrast (C *vs*. T)** | Overall | 24 | 1,2658 | [1.1083; 1.4458] | 0,00051 | Random | 0 | 0,6355 | 0,067 |
| | Asian | 17 | 1,2728 | [1.0722; 1.5109] | 0,005829 | Random | 0 | 0,7187 | 0,126 |
| | Caucasian | 7 | 1,2421 | [1.0566; 1.4602] | 0,008597 | Fixed | 0,4256 | 0 | 0,4428 |
| **Recessive model (CC *vs*. CT+TT)** | Overall | 23 | 1,2214 | [1.0655; 1.4001] | 0,004101 | Fixed | 0,4696 | 0 | 0,1669 |
| | Asian | 16 | 1,1811 | [1.0169; 1.3718] | 0,029266 | Fixed | 0,4753 | 0 | 0,6845 |
| | Caucasian | 7 | 1,4432 | [1.0336; 2.0151] | 0,031244 | Fixed | 0,4216 | 0,0024 | 0,118 |
| **Dominant model (CC+CT *vs*. TT)** | Overall | 24 | 1.3780 | [1.1078; 1.7141] | 0,003989 | Random | 0 | 0,7068 | 0,3003 |
| | Asian | 17 | 1,4304 | [1.0725; 1.9079] | 0,014846 | Random | 0 | 0,7782 | 0,2942 |
| | Caucasian | 7 | 1,2768 | [1.0146; 1.6069] | 0,037221 | Fixed | 0,3905 | 0,0476 | 0,9054 |
| **Over-dominant model (CT *vs*. CC+TT)** | Overall | 24 | 1,1718 | [0.9654; 1.4223] | 0,108724 | Random | 0 | 0,6837 | 0,2432 |
| | Asian | 17 | 1,2366 | [0.9660; 1.5829] | 0,091886 | Random | 0 | 0,7523 | 0,1642 |
| | Caucasian | 7 | 1,0452 | [0.8361; 1.3066] | 0,697852 | Fixed | 0,2503 | 0,2344 | 0,7128 |
| **CC *vs*. TT** | Overall | 23 | 1,3699 | [1.0958; 1.7127] | 0,005735 | Random | 0,0366 | 0,3756 | 0,0748 |
| | Asian | 16 | 1,3177 | [1.0019; 1.7330] | 0,048454 | Random | 0,0194 | 0,4711 | 0,2753 |
| | Caucasian | 7 | 1,5304 | [1.0634; 2.2025] | 0,021963 | Fixed | 0,4315 | 0 | 0,1434 |
| **CC *vs*. CT** | Overall | 23 | 1,1704 | [1.0127; 1.3527] | 0,033067 | Fixed | 0,4932 | 0 | 0,9185 |
| | Asian | 16 | 1,1366 | [0.9697; 1.3321] | 0,113977 | Fixed | 0,5094 | 0 | 0,3357 |
| | Caucasian | 7 | 1,3527 | [0.9510; 1.9241] | 0,092878 | Fixed | 0,3741 | 0,0706 | 0,1833 |
| **CT *vs*. TT** | Overall | 24 | 1.3230 | [1.0520; 1.6637] | 0,016698 | Random | 0 | 0,7028 | 0,3992 |
| | Asian | 17 | 1,3954 | [1.0325; 1.8856] | 0,030125 | Random | 0 | 0,7726 | 0,3357 |
| | Caucasian | 7 | 1,1896 | [0.9336; 1.5158] | 0,160228 | Fixed | 0,3407 | 0,1164 | 0,7274 |
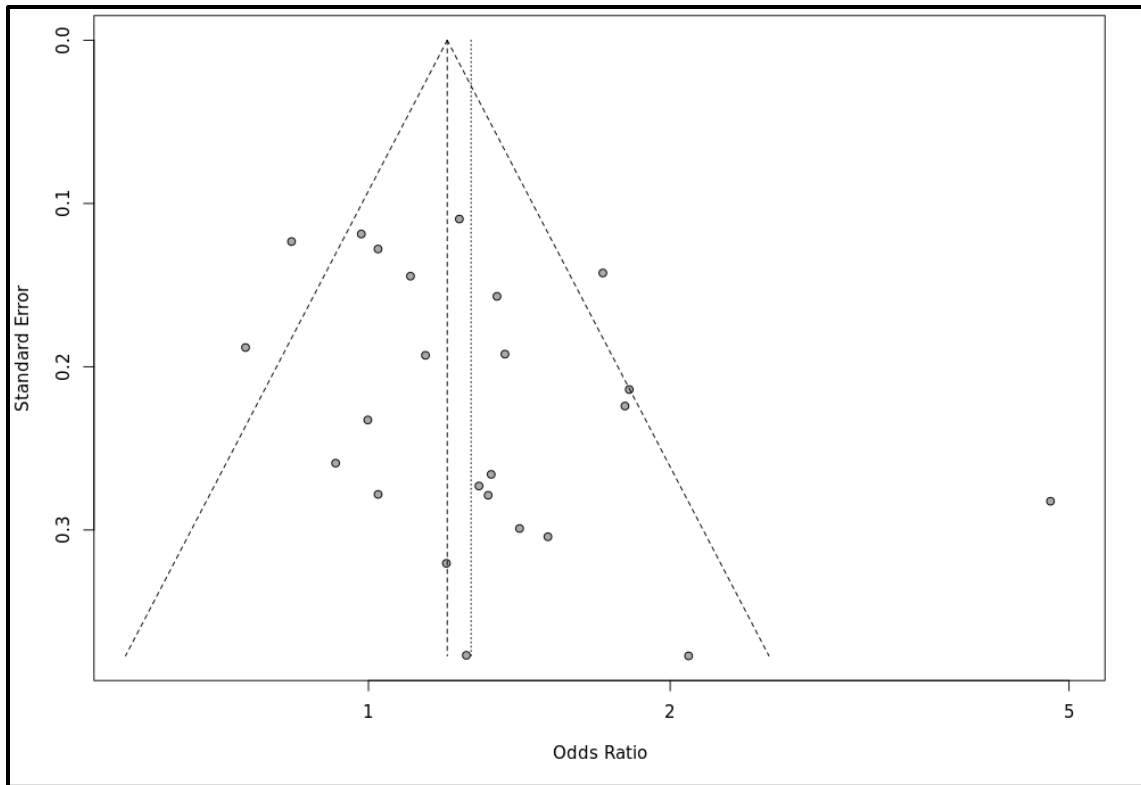
**Figure 9.** Funnel plot of meta-analysis in the allele contrast (C *vs*. T).
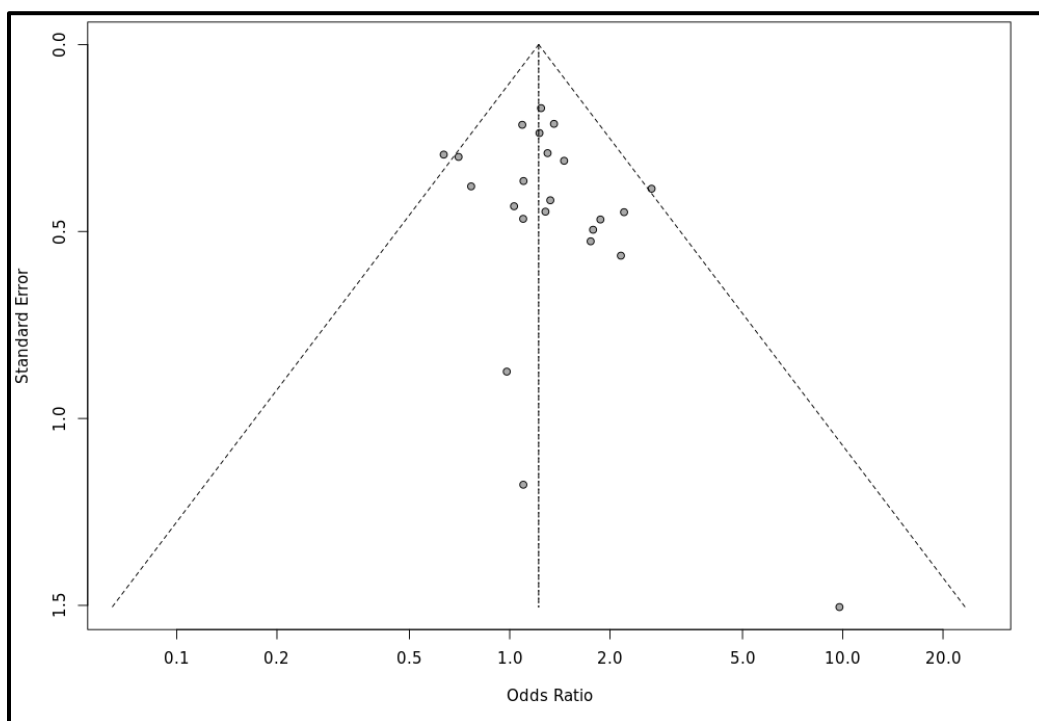


**Figure 10.** Funnel plot of meta-analysis in the recessive model (CC *vs*. CT+TT).
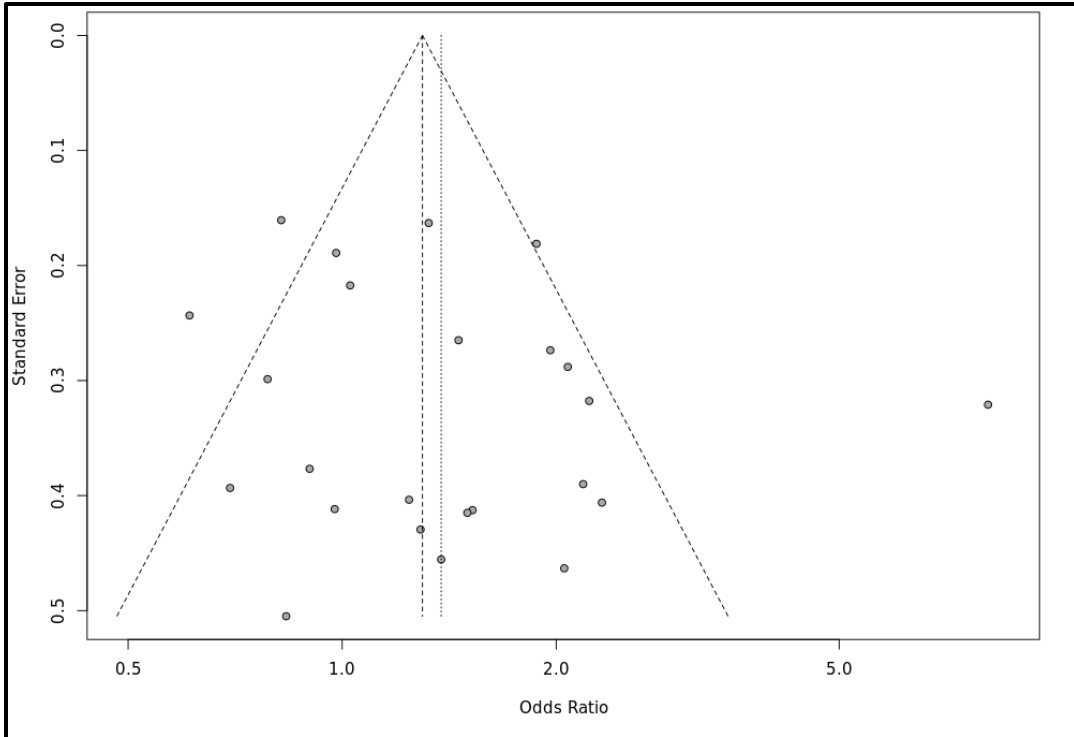
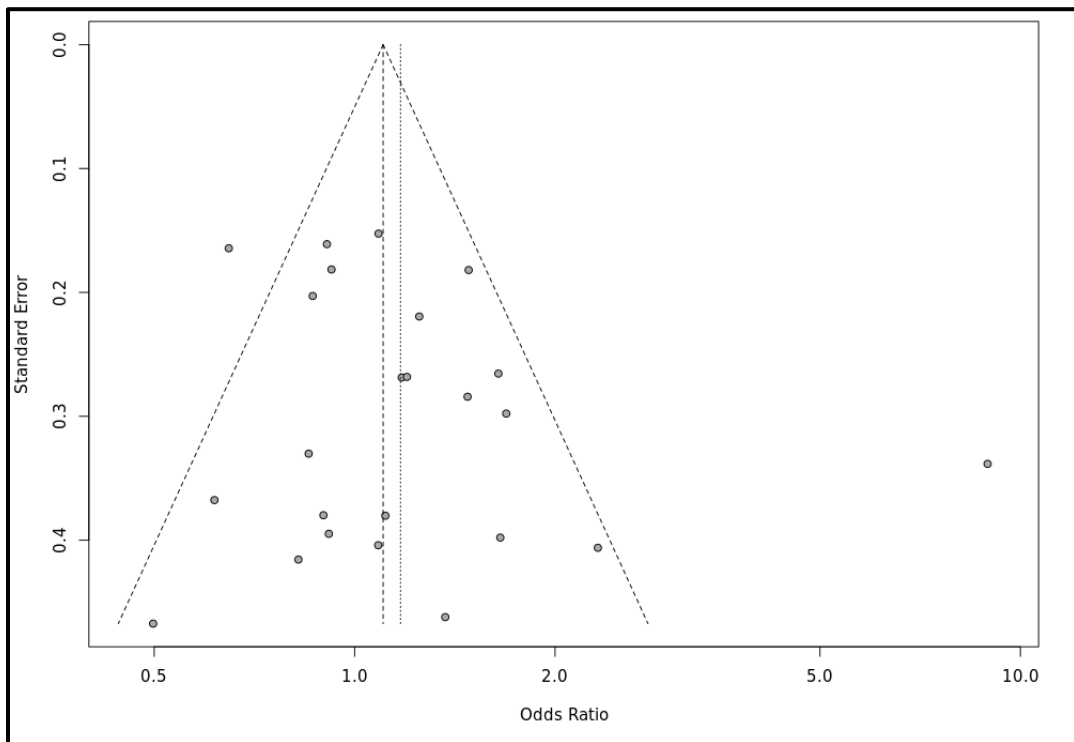**Figure 11.** Funnel plot of meta-analysis in the dominant model (CC+CT *vs*. TT).



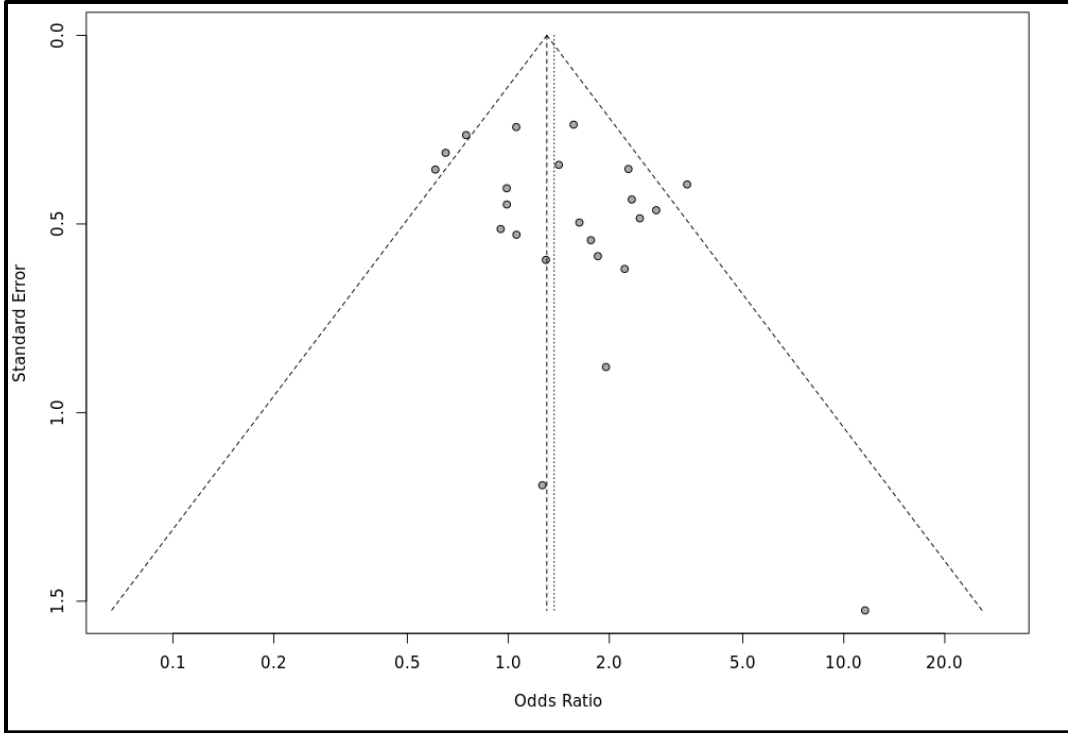**Figure 12.** Funnel plot of meta-analysis in the over-dominant model (CT *vs*. CC+TT).

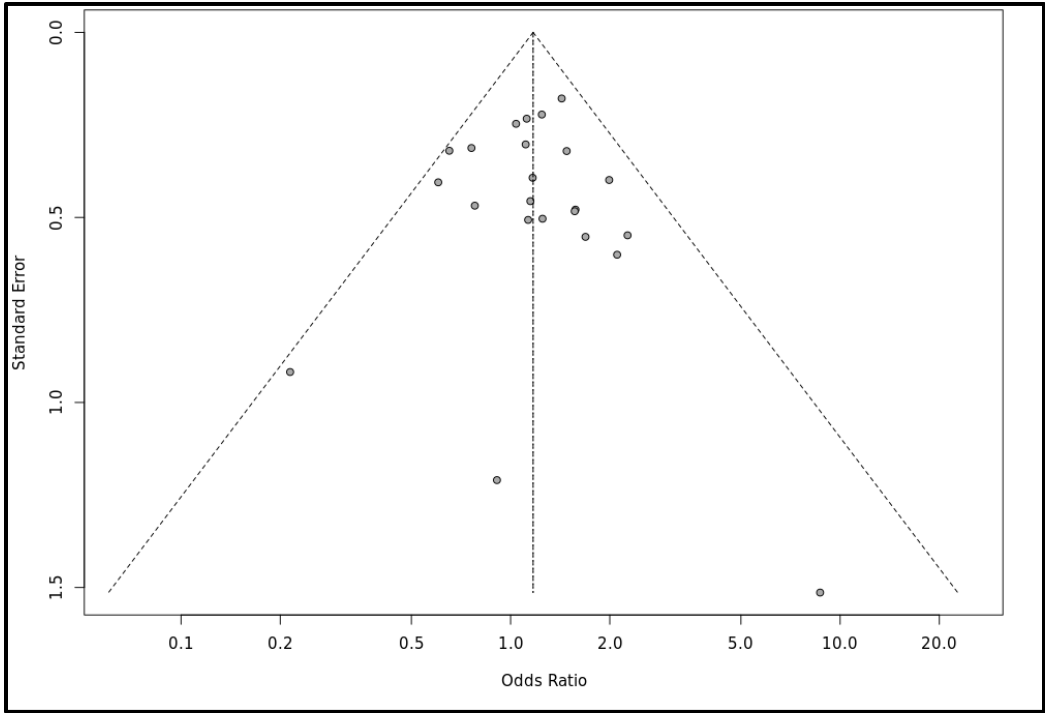**Figure 13.** Funnel plot of meta-analysis in the model CC *vs*. TT.
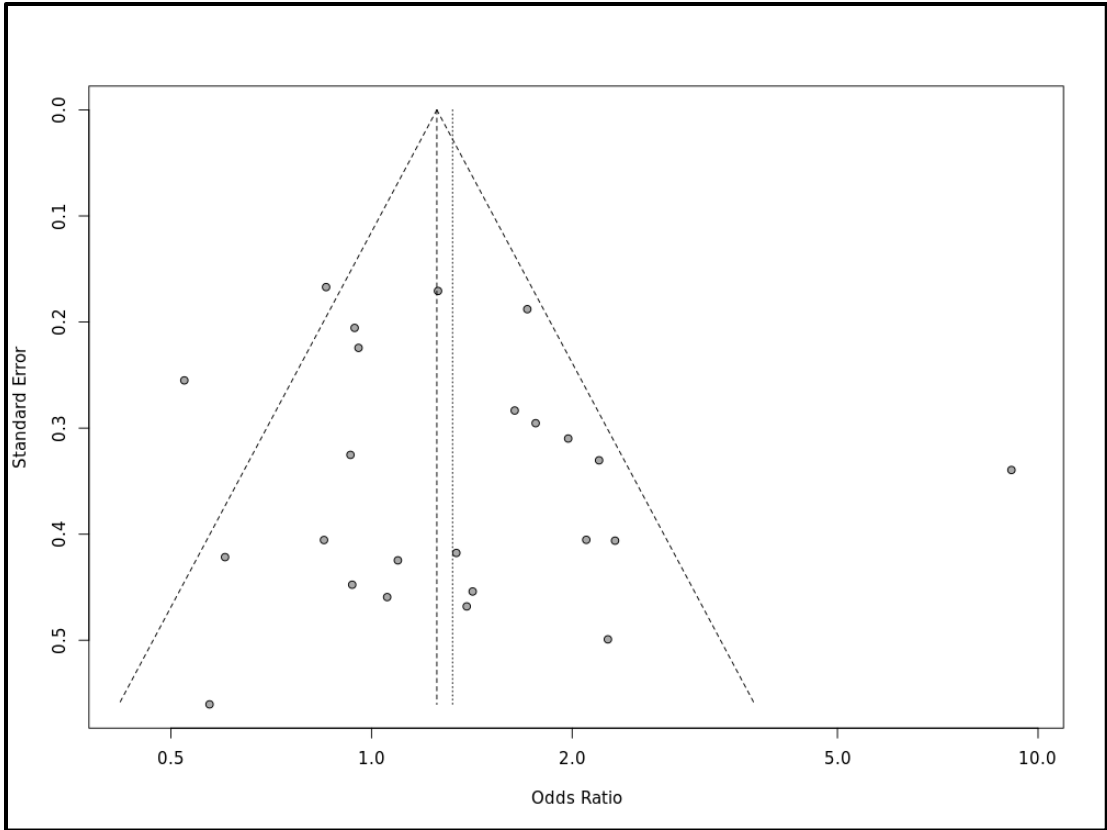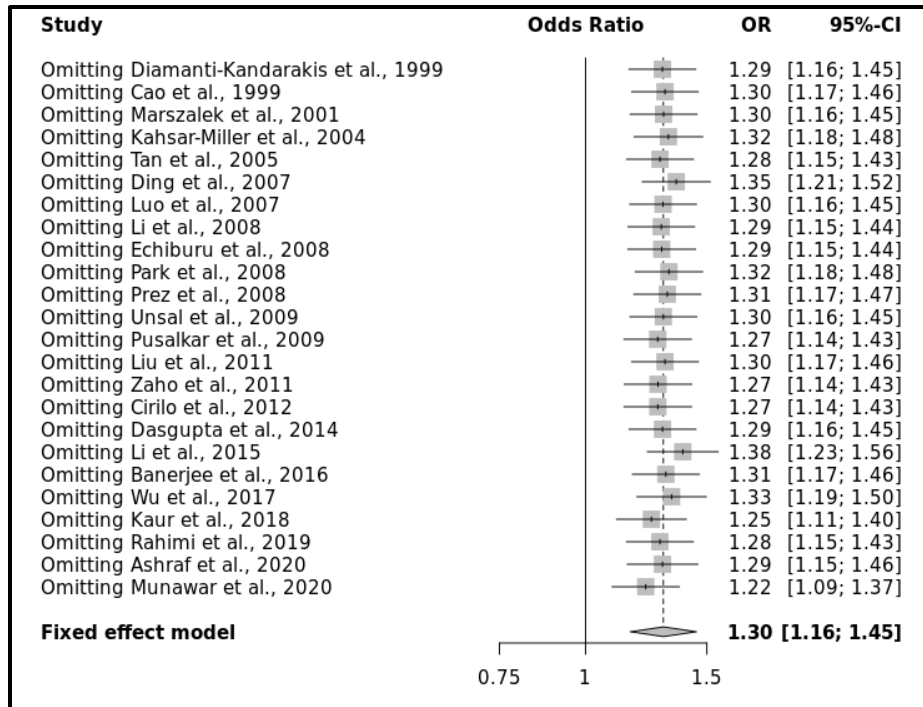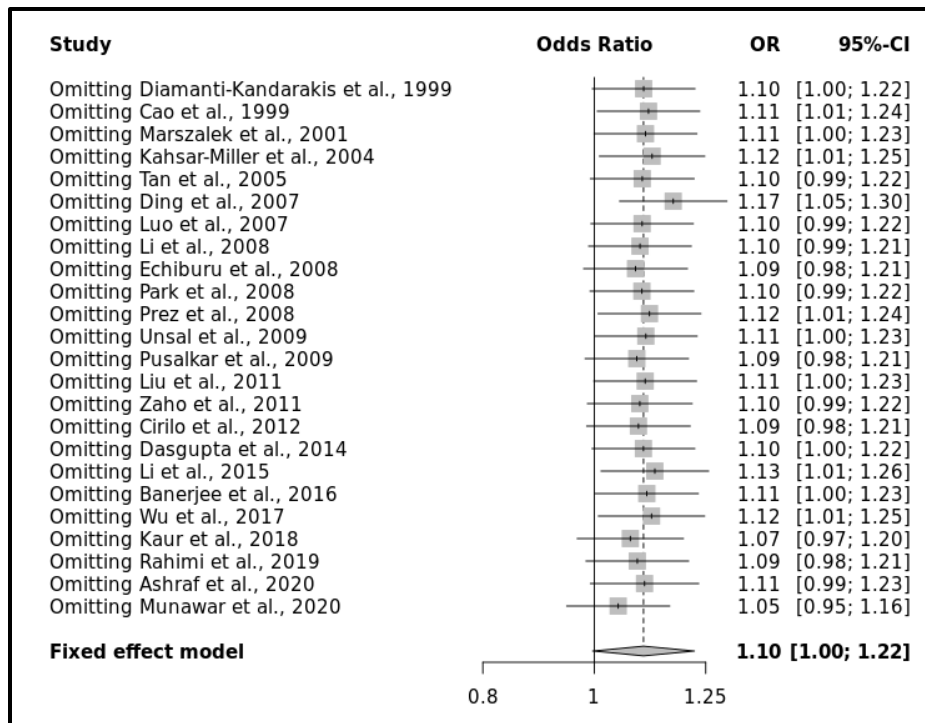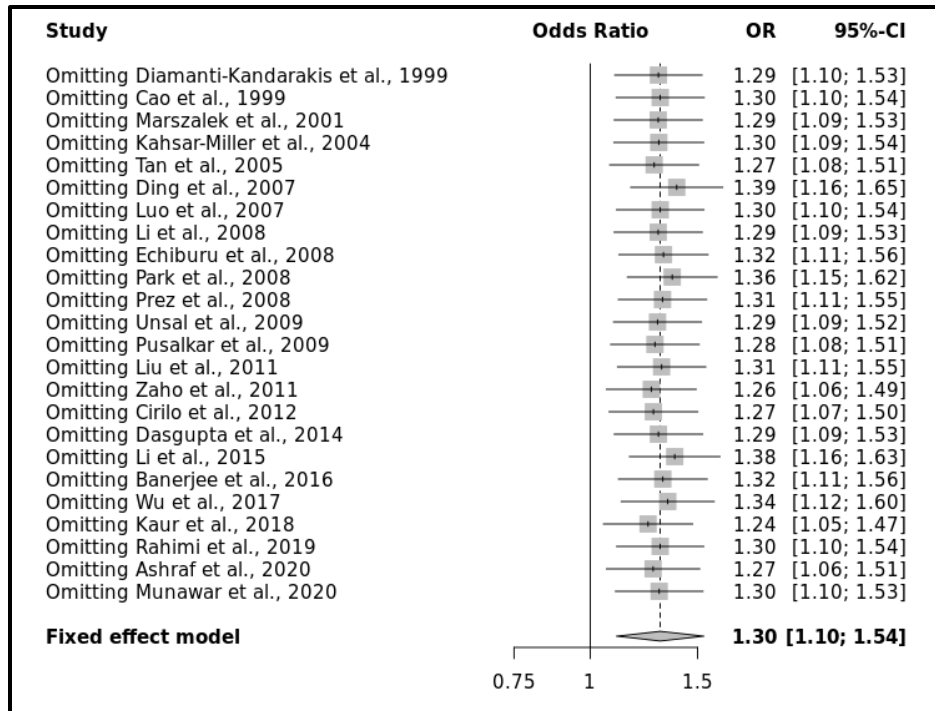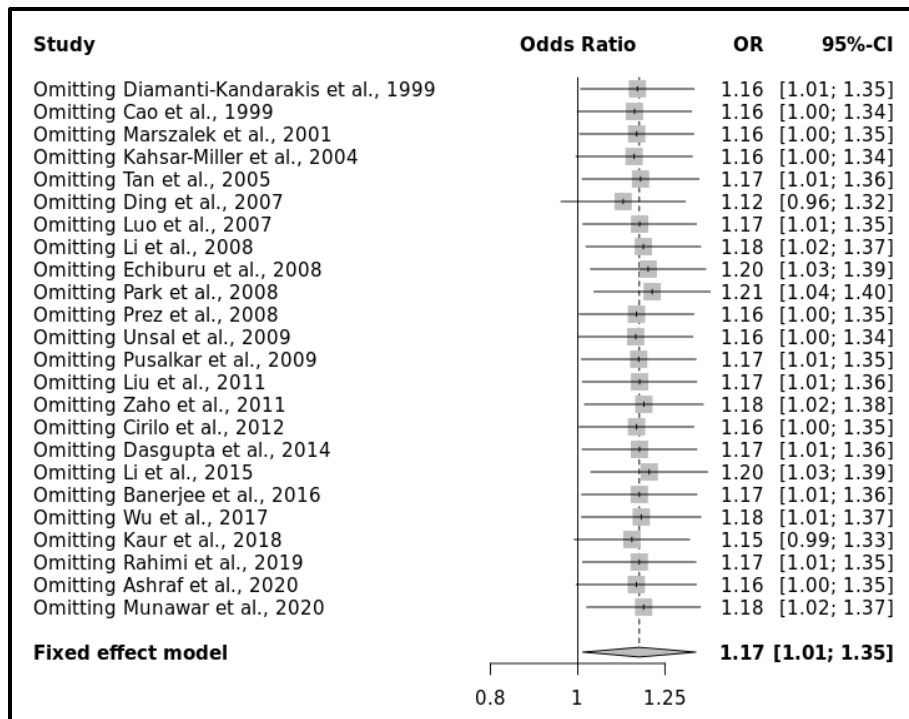


**Figure 14.** Funnel plot of meta-analysis in the model CC *vs*. CT.

**Figure 15.** Funnel plot of meta-analysis in the model CT *vs*. TT.
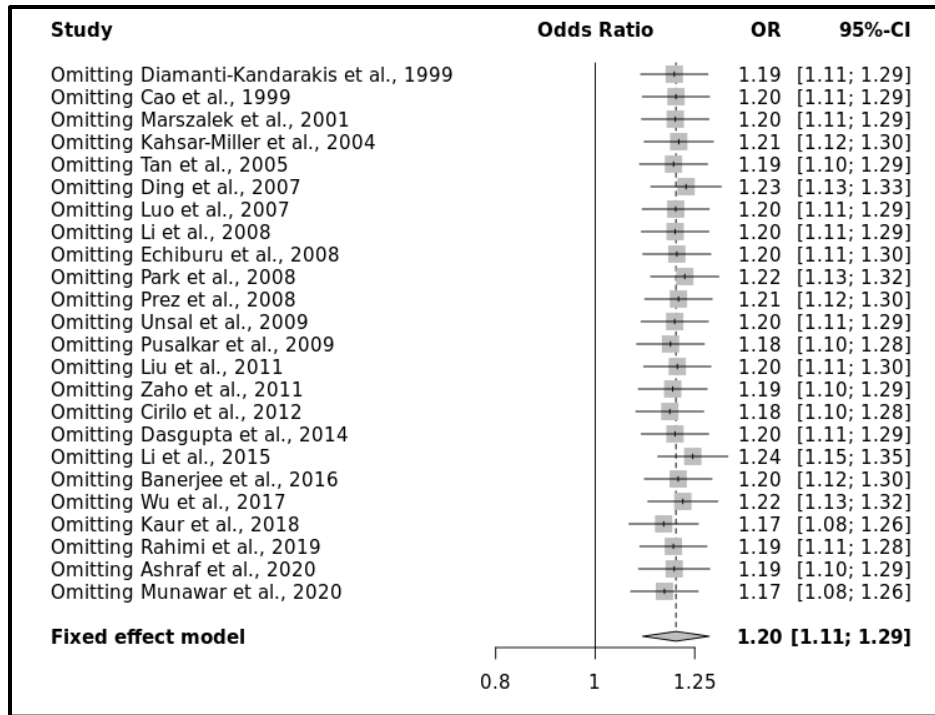
**Figure 16.** Leave-1-out Forest plot of meta-analysis in the allele contrast (C *vs*. T).
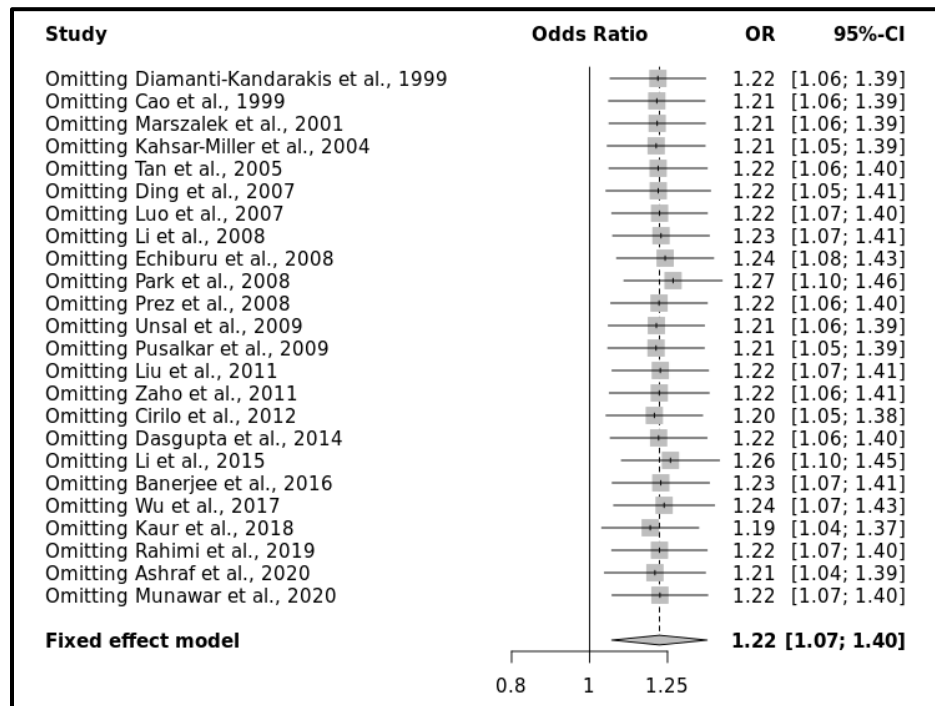


**Figure 17.** Leave-1-out Forest plot of meta-analysis in the recessive model (CC *vs*. CT+TT).

**Figure 18.** Leave-1-out Forest plot of meta-analysis in the dominant model (CC+CT *vs*. TT).



**Figure 19.** Leave-1-out Forest plot of meta-analysis in the over-dominant model
(CT *vs*. CC+TT).

**Figure 20.** Leave-1-out Forest plot of meta-analysis in the model CC *vs*. TT.



**Figure 21.** Leave-1-out Forest plot of meta-analysis in the model CC *vs*. CT.

| Study | Odds Ratio | OR | 95%-CI |
|---|---|---|---|
| Omitting Diamanti-Kandarakis et al., 1999 | | 1.25 | [1.11; 1.41] |
| Omitting Cao et al., 1999 | | 1.26 | [1.12; 1.42] |
| Omitting Marszalek et al., 2001 | | 1.26 | [1.12; 1.41] |
| Omitting Kahsar-Miller et al., 2004 | | 1.28 | [1.13; 1.44] |
| Omitting Tan et al., 2005 | | 1.24 | [1.10; 1.39] |
| Omitting Ding et al., 2007 | | 1.32 | [1.17; 1.49] |
| Omitting Luo et al., 2007 | | 1.25 | [1.11; 1.41] |
| Omitting Li et al., 2008 | | 1.24 | [1.10; 1.40] |
| Omitting Echiburu et al., 2008 | | 1.24 | [1.10; 1.39] |
| Omitting Park et al., 2008 | | 1.27 | [1.12; 1.43] |
| Omitting Prez et al., 2008 | | 1.27 | [1.13; 1.43] |
| Omitting Unsal et al., 2009 | | 1.26 | [1.12; 1.41] |
| Omitting Pusalkar et al., 2009 | | 1.23 | [1.09; 1.39] |
| Omitting Liu et al., 2011 | | 1.26 | [1.12; 1.42] |
| Omitting Zaho et al., 2011 | | 1.23 | [1.09; 1.39] |
| Omitting Cirilo et al., 2012 | | 1.24 | [1.10; 1.39] |
| Omitting Dasgupta et al., 2014 | | 1.25 | [1.11; 1.41] |
| Omitting Li et al., 2015 | | 1.33 | [1.17; 1.50] |
| Omitting Banerjee et al., 2016 | | 1.26 | [1.12; 1.42] |
| Omitting Wu et al., 2017 | | 1.29 | [1.14; 1.45] |
| Omitting Kaur et al., 2018 | | 1.21 | [1.07; 1.37] |
| Omitting Rahimi et al., 2019 | | 1.24 | [1.10; 1.39] |
| Omitting Ashraf et al., 2020 | | 1.25 | [1.11; 1.42] |
| Omitting Munawar et al., 2020 | | 1.18 | [1.04; 1.32] |
| **Fixed effect model** | | **1.25** | **[1.11; 1.41]** |

0.75    1    1.5

**Figure 22.** Leave-1-out Forest plot of meta-analysis in the model CT *vs*. TT.

# Conclusion and perspectives

PCOS is the most common endocrine disorder in women and it has a significant impact on the quality of life. Its multiple aspects: reproductive, metabolic, oncological, and cardiovascular, have a significant impact on the general, reproductive, as well as psychosocial health of women. The main symptoms of this syndrome are hyperandrogenism that leads to infertility, menstrual disorders, hirsutism, insulin resistance that leads to type 2 diabetes, and abnormalities in lipids that may lead to the development of cardiovascular disease. Yet understanding it remains a challenge, which is more complex because it is the subject of many discussions. Although PCOS very common and has serious consequences, its main cause has not been determined and a complete cure for it has not yet been discovered.

Patients and clinicians alike have viewed PCOS as largely a "reproductive" condition that settles in the ovaries because it is so widespread and sadly often neglected. This isn't just an ovarian illness; it's a systemic disorder that causes a slew of health and metabolic issues. To emphasize the complexity of PCOS, the researchers propose renaming it "reproductive metabolic syndrome." As a result, this research is being conducted, with the goal of better understanding the physiological mechanism of this condition, uncovering its secrets, and identifying effective and effective treatment techniques.

Both genes and the environment contribute to PCOS. Obesity, exacerbated by poor dietary choices and physical inactivity, worsens PCOS in susceptible individuals. The role of other environmental modifiers such as infectious agents or toxins are speculative. Phenotype confusion has characterized genetic studies of PCOS. Although several loci have been proposed as PCOS genes including CYPs genes, the insulin gene, the follistatin gene, and a region near the insulin receptor, the evidence supporting linkage is not overwhelming. But, to date, no gene has been identified that causes or contributes substantially to the development of a PCOS phenotype.

In the study of many causes of this syndrome, it is considered the genetic cause or is the first candidate for the existence of this syndrome, and many genes that may be involved in the presence of PCOS were explained in the study, but the focus was mainly on the *CYP17* gene polymorphism and the *ACE* gene for which the I/D polymorphism is reported.

Our case-control study suggests the relevance of the *ACE* gene's I/D polymorphism in the incidence of PCOS. It is thought to play a substantial impact in the development of PCOS, with carriers of the homozygous DD genotype having a significantly higher risk than those with the heterozygous ID and homozygous II genotypes. The vast majority of studies published in the literature provide evidence that this polymorphism plays a more or less important role in the development of PCOS, both in Asians and Caucasians, independently of other risk factors. Even if the exact mechanisms are not yet clearly identified, its involvement in the pathophysiology of this dysfunction is increasingly argued.

On the other hand, the current findings in our meta-analysis result suggest that *CYP17* T/C (*rs*74357) gene polymorphism plays an important role in increasing the susceptibility of PCOS when carrying the C allele (genotype TC and CC). Despite the undoubted connection of *CYP17* gene polymorphism to PCOS, the range to which *CYP17* gene polymorphism contributes to metabolic dysfunction in PCOS is unidentified and needs further study Meanwhile, due to the strong correlation between PCOS and *CYP17A1 rs*7435742 polymorphism, it could be used as a genetic marker for PCOS, and might supply another tool for assessing women's susceptibility. Likewise, the *CYP17A1* could be applied to the treatment of PCOS as a potentially feasible target.

Following the completion of this study, we have identified three future opportunities that we think are essential and can be proposed:

-   To estimate the true prevalence of this disease in Algeria, multicenter national epidemiological studies are being conducted. It will be required to take into account the fact that the vast majority of PCOS cases are treated at the level of private gynecological practices, which do not, for the most part, record clinical and biological data from their patients.
-   PCOS is plagued by a late diagnosis and a lack of knowledge. To address this issue, Algerian women must be informed and educated about the serious health ramifications of this dysfunction if it is not addressed promptly, which extends beyond the scope of a reproductive problem or an aesthetic problem (hirsutism, acne).
-   To investigate the genetics of PCOS development by conducting family and molecular genetic investigations that can reveal some genetic risk factors specific to the Algerian population. These genetic changes can operate as particular markers for this disease, allowing for early detection starting in adolescence.

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# Appendix

# **Annex I:** consent for the use of biological data.

**BENDAOUD Hiba and EL KHOUR Jihan**
Student(s) Master 2 Genetics
Department of Animal Biology - SNV Faculty
Mentouri Brothers University - Constantine I


I, the undersigned: ……………………….. born on ..…./…../……… in
…………

Certify that I have received from BENDAOUD Hiba and EL KHOUR Jihan exhaustive and understandable information concerning the possible causes of my health problem. I had the opportunity to ask all the questions I wanted.

I understand that a genetic analysis is offered to me from a blood sample from which my DNA will be extracted. The purpose of this analysis is to determine if my genome has an anomaly or a variation related to my health problem. I fully understood the possible implications of this study and I could obtain, if I wished, any additional information.

The results of these analyzes will be sent to me if I wish. They will remain confidential and can only be communicated with my exclusive authorization.

I can at any time decide not to pursue this process. The genetic data and biological material concerning me may be destroyed at my request.

I agree that my biological samples will be kept and used for medical and/or biological research purposes without restriction under cover of anonymity.


Made in  ………………….… on .…./…../………


**Applicant signature**

**Signature of the researcher**

-

# Appendix I: questionnaire

Last name : …………………….. 	 First name : …………………….. 	 Age : ……

Address : ………………….… 	 Family situation : …………….

Socio-professional level: …………. ……………. .. 	 Profession: …………. ……………. …...

Attending doctor: …………. …… .. 	 Age of diagnosis:. ….

Reason for consultation : …………

Anthropometric parameters of women

Cut : …..    Current weight:…    BMI:….   Minimum weight: ……   Kg,    at what age; ……

Maximum weight: …… Kg , at what age; ……      Waist size : …. Cm      Hip circumference:…. Cm

RTH (waist to hip ratio): ……

Are you having trouble maintaining your ideal weight?      ☐ Yes ☐ No

## Parameters related to women's reproductive health

Nature of cycles: Regular ☐  Irregular ☐  Duration ☐

days Length of menstrual cycle (days):… .. ………

Annual number of periods:… …...

Problems associated with menstruation (menstrual bleeding problems): …………. ……………. …………
……………………………………………………………………………………………….

Age of first period: ……………………. Age of marriage: ………… 	 Number of children: …….

Problem of infertility? ☐ Yes ☐ No

if yes, specify the duration: ……………. .. ☐ If infertility; ☐ primary secondary

Age at first pregnancy :………… Number of pregnancies including this ☐

in the long term ☐ Number of miscarriages ☐ Date of last ☐ Number of living children ☐

Cause of miscarriage: ………….. ……………….. ……………………………………………

Number of children who are: Death Stillborns premature births Use of contraception? ☐ Yes ☐ No
If yes which one : ……………..

# Parameters related to polycystic ovary syndrome

Hirsutism: ……………………… Location: ……………………………………………… Acne: ……….. …………………… .

Other skin problems (Nigerian acanthosis)……………………………………………

……………………………………………………………………………………………………..

Sleep problems: ☐ Yes ☐ No  /  Mood swings: ☐ Yes ☐ No  /  Headache: ☐ Yes ☐ No

Appearance of the ovaries on ultrasound: ………… … ……………………………………… … ……………

FSH:…..                LH: ……                    LH / FSH:…….

Testosterone: …….                Prolactin: …….                    Delta 4 androstenedione: …………………

Estradiol, 17 hydroxyprogesterone: …………….

Other : ……………………………………………………………………………………….

Fasting blood sugar: ……                        Post-meal blood sugar: ……

Glycated hemoglobin (HbA1c):…

LDL: ……            HDL: ……                        Triglycerides:….

Blood pressure : …../….          Other : ………………………………………………………………………………………….

Treatment prescribed by the attending physician: ………………………………………………………………………

Do you have other pathologies? ☐ Yes ☐ No        If yes, which ones: …………………………..…………..

Age of diagnosis: ………………………………

Treatment followed: ………………………………………………………………………………………….

………………………………………………………………………………………….

Do you have any women in the family with OPK? ☐ Yes ☐ No

If yes, specify the family relationship: ……………………………………………………………………………………………

Do you have any problems in the family (related to the first degree)? ☐ ☐ Yes ☐ No

If yes, specify which ones as well as the family relationship: …………………………………………………….

……………………………………………………………………………………………………………………………………………………

Further information:

…………………………………………………………………………………………………………………………

…………………………………………………………………………………………………………………………

…………………………………………………………………………………………………………………………

# PRISMA 2020 Checklist

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review. | |
| **ABSTRACT** | | | |
| Abstract | 2 | See the PRISMA 2020 for Abstracts checklist. | |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of existing knowledge. | |
| Objectives | 4 | Provide an explicit statement of the objective(s) or question(s) the review addresses. | |
| **METHODS** | | | |
| Eligibility criteria | 5 | Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses. | |
| Information sources | 6 | Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted. | |
| Search strategy | 7 | Present the full search strategies for all databases, registers and websites, including any filters and limits used. | |
| Selection process | 8 | Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process. | |
| Data collection process | 9 | Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process. | |
| Data items | 10a | List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect. | |
| | 10b | List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information. | |
| Study risk of bias assessment | 11 | Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process. | |
| Effect measures | 12 | Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results. | |
| Synthesis methods | 13a | Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)). | |
| | 13b | Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions. | |
| | 13c | Describe any methods used to tabulate or visually display results of individual studies and syntheses. | |
| | 13d | Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used. | |
| | 13e | Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression). | |
| | 13f | Describe any sensitivity analyses conducted to assess robustness of the synthesized results. | |
| Reporting bias assessment | 14 | Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases). | |
| Certainty assessment | 15 | Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome. | |

# PRISMA 2020 Checklist

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| **RESULTS** | | | |
| Study selection | 16a | Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram. | |
| | 16b | Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded. | |
| Study characteristics | 17 | Cite each included study and present its characteristics. | |
| Risk of bias in studies | 18 | Present assessments of risk of bias for each included study. | |
| Results of individual studies | 19 | For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots. | |
| Results of syntheses | 20a | For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies. | |
| | 20b | Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect. | |
| | 20c | Present results of all investigations of possible causes of heterogeneity among study results. | |
| | 20d | Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results. | |
| Reporting biases | 21 | Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed. | |
| Certainty of evidence | 22 | Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed. | |
| **DISCUSSION** | | | |
| Discussion | 23a | Provide a general interpretation of the results in the context of other evidence. | |
| | 23b | Discuss any limitations of the evidence included in the review. | |
| | 23c | Discuss any limitations of the review processes used. | |
| | 23d | Discuss implications of the results for practice, policy, and future research. | |
| **OTHER INFORMATION** | | | |
| Registration and protocol | 24a | Provide registration information for the review, including register name and registration number, or state that the review was not registered. | |
| | 24b | Indicate where the review protocol can be accessed, or state that a protocol was not prepared. | |
| | 24c | Describe and explain any amendments to information provided at registration or in the protocol. | |
| Support | 25 | Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review. | |
| Competing interests | 26 | Declare any competing interests of review authors. | |
| Availability of data, code and other materials | 27 | Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review. | |

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# Abstracts

# Susceptibilité génétique au syndrome des ovaires polykystiques (SOPK) : étude moléculaire du polymorphisme I/D du gène *ACE* et méta-analyse sur l'implication du polymorphisme *rs*74357 du gène *CYP17A1*

**Résumé :**

Le syndrome des ovaires polykystiques (SOPK) est l'un des troubles endocriniens les plus courants chez les femmes en âge de procréer et c'est la cause la plus fréquente d'anovulation chronique et d'infertilité. Traditionnellement, il était considéré comme un trouble de la reproduction montrant une hyperandrogénie, une anovulation chronique et une infertilité. Cependant, des aspects métaboliques importants associés à des séquelles de santé à plus long terme du SOPK ont été reconnus. Bien que l'étiologie du SOPK reste indéterminée, il est considéré comme une maladie multifactorielle, avec plusieurs altérations métaboliques, endocriniennes, environnementales et génétiques. À ce jour, les variations des gènes impliqués dans de multiples mécanismes et voies moléculaires tels que la sécrétion et l'action de l'insuline, le métabolisme énergétique, la biosynthèse des hormones stéroïdes et l'action des gonadotrophines ont été largement étudiées en tant que polymorphismes potentiels prédisposés au SOPK.

Notre étude comporte deux volets : le premier consiste à investiguer la contribution du polymorphisme I/D (Insertion/Délétion) (*rs*1799752) du gène de l'enzyme de conversion de l'angiotensine (ACE) dans l'étiologie du SOPK dans la région de Constantinois. La seconde consiste à déterminer si le polymorphisme du gène *CYP17* T/C (*rs*74357) est un risque d'exposition au SOPK, en effectuant une méta-analyse complète résumant toutes les études cas-témoins publiées antérieurement sur ce sujet.

L'étude cas-témoin moléculaire a impliqué 9 patients atteints du SOPK et 31 témoins sains. Après extraction de l'ADN, génotypage du polymorphisme I/D du gène *ACE* par PCR, suivi de la comparaison des fréquences génotypiques et alléliques entre patients et témoins, nous avons trouvé des différences de distribution statistiquement significatives selon le modèle de contraste allélique ($p = 0,036$) . D'après ces observations, les femmes porteuses de l'allèle D (génotype DD et à un moindre degré DI) sont plus à risque de développer un SOPK que les femmes homozygotes pour l'allèle I, ayant une activité réduite par rapport à l'allèle D. Ces résultats sont en accord avec ceux rapportés par d'autres études antérieures (cas-témoins et méta-analyses) qui concluaient que la présence de l'allèle D constitue un facteur de risque probable dans la genèse de ce dysfonctionnement.

Pour la méta-analyse, les résultats globaux obtenus ont validé que le polymorphisme du gène *CYP17*T/C était significativement associé au risque de SOPK dans 5 modèles génétiques : modèle récessif (effet fixe et aléatoire), modèle dominant (effet aléatoire), CC *vs* TT (effet fixe), CT *vs* TT (effet fixe) et contraste allélique (effet aléatoire). Des analyses stratifiées par origine ethnique/pays ont également détecté une association significative entre les Asiatiques et les Caucasiens dans les modèles récessif, dominant, CC *vs* TT, CC *vs* CT et les modèles de contraste allélique. Ces résultats suggèrent que ce polymorphisme joue un rôle crucial dans l'augmentation de la susceptibilité au SOPK lorsqu'il est porteur de l'allèle récessif C, qui peut être proposé comme facteur prédictif du risque de SOPK ou une voie importante dans la dérégulation métabolique et hormonale associée au SOPK, en particulier la résistance à l'insuline. .

Pour clarifier l'impact de ces deux polymorphismes, des recherches génétiques supplémentaires doivent être menées pour évaluer l'impact des relations gène-gène et gène-environnement qui pourraient conduire à une meilleure compréhension des fondements génétiques du SOPK.

**Mots-clefs :** syndrome des ovaires polykystiques, étude cas-témoins, méta-analyse, gène *ACE*, gène *CYP17A1*.

# القابلية الوراثية لمتلازمة تكيس المبايض (PCOS): دراسة جزيئية لتعدد الأشكال الجيني ACE I/D والتحليل البعدي لتورط تعدد الأشكال CYP17A1 (rs74357)

**الملخص:**

تعد متلازمة تكيس المبايض (PCOS) من أكثر اضطرابات الغدد الصماء شيوعًا في سن الإنجاب عند النساء، وهي السبب الأكثر شيوعًا للإباضة المزمنة والعقم. تقليديا كان يعتبر اضطراب تناسلي يظهر فرط الأندروجين، الإباضة المزمنة والعقم. ومع ذلك، فقد تم التعرف على جوانب التمثيل الغذائي الهامة جنبًا إلى جنب مع العواقب الصحية طويلة المدى لمتلازمة تكيس المبايض. على الرغم من أن مسببات متلازمة تكيس المبايض لا تزال غير محددة، إلا أنها تعتبر مرضًا متعدد العوامل، مع العديد من التغيرات الأيضية والغدد الصماء والبيئية والجينية. حتى الآن، تمت دراسة الاختلافات في الجينات المشاركة في آليات متعددة والمسارات الجزيئية مثل إفراز الأنسولين وعمله، واستقلاب الطاقة، والتخليق الحيوي لهرمون الستيرويد وعمل الجونادوتروبين على نطاق واسع على أنها تعدد أشكال محتمل مهيأ لمتلازمة تكيس المبايض.

دراستنا لها جانبان: الأول هو التحقيق في مساهمة تعدد الأشكال I/D (الإدراج / الحذف) (rs1799752) من الإنزيم المحول للأنجيوتنسين (OMIM: 106180) (ACE) في مسببات متلازمة تكيس المبايض في منطقة قسنطينة. والثاني هو تحديد ما إذا كان تعدد الأشكال الجيني T/C (rs74357) CYP17 (OMIM: 609300) يمثل خطر التعرض لمتلازمة تكيس المبايض، عن طريق إجراء تحليل بعدي شامل يلخص جميع دراسات الحالة المنشورة السابقة حول هذا الموضوع.

اشتملت دراسة الحالات الجزيئية على 9 مرضى مصابين بمتلازمة تكيس المبايض و31 عنصر تحكم سليم. بعد استخراج الحمض النووي، التنميط الجيني لتعدد الأشكال I/D لجين ACE بواسطة PCR، متبوعًا بمقارنة الترددات الوراثية والأليلية بين المرضى والضوابط، وجدنا فروق ذات دلالة إحصائية في التوزيع وفقًا لنموذج التباين الأليلي ($p = 0.036$). وفقًا لهذه الملاحظات، فإن النساء ذوات الأليل D (النمط الوراثي DD وبدرجة أقل DI) أكثر عرضة للإصابة بمتلازمة تكيس المبايض من النساء المتماثلات للأليل I، ولديهن نشاط أقل مقارنة بالأليل D. تتوافق هذه النتائج مع تلك التي أبلغت عنها دراسات سابقة أخرى (ضوابط الحالة والتحليلات البعدية) والتي خلصت إلى أن وجود الأليل D يشكل عامل خطر محتمل في نشأة هذا الخلل الوظيفي. لكن، بالنسبة للتحليل البعدي، تم التحقق من صحة النتائج الإجمالية التي تم الحصول عليها من أن تعدد الأشكال الجيني CYP17T/C كان مرتبطًا بشكل كبير بمخاطر متلازمة تكيس المبايض في 5 نماذج وراثية: النموذج المتنحي (التأثير الثابت والعشوائي)، النموذج السائد (التأثير العشوائي)، CC مقابل TT (تأثير ثابت)، CT مقابل TT (تأثير ثابت)، وتباين الأليل (تأثير عشوائي). كشفت التحليلات الطبقية حسب العرق / الدولة أيضًا عن ارتباط كبير بين الآسيويين والقوقازيين تحت نماذج التباين المتنحية، السائدة، CC مقابل TT، CC مقابل CT، ونماذج التباين الأليل. تشير هذه النتائج إلى أن تعدد الأشكال لعب دورًا حاسمًا في زيادة قابلية الإصابة بمتلازمة تكيس المبايض عند حمل الأليل المتنحي C، والذي يمكن اقتراحه كعامل تنبؤي لخطر الإصابة بمتلازمة تكيس المبايض أو مسار مهم في اضطراب التمثيل الغذائي والهرموني المصاحب لمتلازمة تكيس المبايض وخاصة مقاومة الأنسولين.

لتوضيح تأثير هذين تعدد الأشكال، يجب إجراء المزيد من الأبحاث الجينية لتقييم تأثير الجينات والعلاقات بين الجينات والبيئة التي يمكن أن تؤدي إلى فهم أفضل للأسس الجينية لمتلازمة تكيس المبايض.

**الكلمات المفتاحية:** متلازمة تكيس المبايض، دراسة الحالات والشواهد، التحليل البعدي، مورثه ACE ، مورثه CYP17A1 .

| **Academic year:** 2021-2022 | **Presented by:** BENDAOUD Hiba and EL KHOUR Jihan |
| --- | --- |

Genetic susceptibility to polycystic ovary syndrome (PCOS):
Molecular study of I/D *ACE* gene polymorphism and meta-analysis on the *CYP17A1*
(*rs*74357) polymorphism involvement

**Thesis Presented for the Graduation of a Master of Genetic**

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in women's reproductive age and it is the most frequent cause of chronic anovulation and infertility. Traditionally it was considered as a reproductive disorder showing hyperandrogenism, chronic anovulation and infertility. However, significant metabolic aspects in conjunction with longer-term health sequelae of PCOS have been recognized. Although the etiology of PCOS remains undetermined, it is considered a multifactorial disease, with several metabolic, endocrine, environmental and genetic alterations. To date, variations in genes involved in multiple mechanisms and molecular pathways such as insulin secretion and action, energy metabolism, steroid hormone biosynthesis and gonadotropin action have been widely studied as potential polymorphisms predisposed to PCOS.

Our study has two aspects: the first is to investigate the contribution of the I/D (Insertion/Deletion) polymorphism (*rs*1799752) of the angiotensin-converting enzyme (*ACE*) (OMIM : 106180) gene in the etiology of PCOS in the Constantine region. The second is to determine whether the *CYP17* (OMIM: 609300) T/C (*rs*74357) gene polymorphism is an exposure risk for PCOS, by performing a comprehensive meta-analysis summarizing all previous published case-control studies on this topic.

The molecular case-control study involved 9 patients with PCOS and 31 healthy controls. After DNA extraction, genotyping for the I/D polymorphism of the *ACE* gene by PCR, followed by the comparison of genotypic and allelic frequencies between patients and controls, we found statistically significant differences in distribution according to the allelic contrast model ($p = 0.036$). According to these observations, women with the D allele (DD genotype and to a lesser degree DI) are more at risk of developing PCOS than women homozygous for the I allele, having a reduced activity in comparison with the D allele. These results are in line with those reported by other previous studies (case-controls and meta-analyses) which concluded that the presence of the D allele constitutes a probable risk factor in the genesis of this dysfunction. However, these results remain preliminary and should be verified on a larger sample.

For the meta-analysis, the overall obtained results validated that the *CYP17*T/C gene polymorphism was significantly associated with PCOS risk in 5 genetic models: recessive model (fixed and random effect), dominant model (random effect), CC *vs*. TT (fixed effect), CT *vs*. TT (fixed effect), and allele contrast (random effect). Stratified analyses by ethnicity/country also detected significant association between Asian and Caucasian under the recessive, dominant, CC *vs*. TT, CC *vs*. CT, and the allele contrast models. These results suggested that this polymorphism played a crucial role in increasing the susceptibility of PCOS when carrying the recessive C allele, which can be proposed as a predictive factor for the risk of PCOS or an important pathway in PCOS associated metabolic and hormonal dysregulation especially insulin resistance.

To clarify the impact of these two polymorphisms more genetic research needs to be conducted to assess the impact of gene-gene and gene-environment relationships that could lead to a better understanding of the genetic underpinnings of PCOS.

**Keywords:** polycystic ovary syndrome, case-control study, meta-analysis, *ACE* gene, *CYP17A1* gene.

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