T.C.

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INVESTIGATION OF SINGLE NUCLEOTIDE POLYMORPHISMS rs1801157 IN THE X-C-X CHEMOKINE LIGAND12 (CXCL12) GENE IN OVARIAN CANCER.

DOCTOR OF PHILOSOPHY THESIS

MB ChB. EMAN AHMED BOUNI

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APPROVAL
This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

Prof. Dr. Bayram YILMAZ
Director of Institute of Health Sciences

DECLARATION

I declare that this thesis is my own work from its planning to the conclusion, and I have cited pieces of information from previous research and studies while retaining the property rights of these sources, which I list in the list of references. I further declare that this thesis has not been submitted for any previous degree. The work was done under the guidance of Professor Dr. Seda Güleç Yılmaz.

20.07.2022

Eman Ahmed Bouni

DEDICATION

To my beloved parents, my father Prof Ahmed who has been my source of inspiration and role model in the academic field, and my mother Salmin who always supports me and gives me the strength to keep going.

A special feeling of gratitude to my family, my husband Mohamed, my daughters, the flowers Bahan, Bayan, and Jumana, and to a little prince my son Yahya.

To my sisters, brothers, relatives, and friends who have encouraged me throughout the process.

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Finally, I would like to give my warmest thanks to my parents, my husband, my children, and all my family for their continuous support and patience during my journey. Thank you all. Your prayers and advice were the best support for me to complete this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

ACKRs	is atypical chemokine receptors
BMI	Body Mass Index
BOT	borderline tumor
CA125	cancer antigen 125
CCC	clear-cell carcinoma
CXCL12	C-X-C chmokin ligand 12
CXCR4	CXC chemokine receptor 4
CXCR7	CXC chemokine receptor7
DG	dysgerminoma
EC	endometrioid cancer
EDTA	Ethylenediaminetetraacetic acid
EOC	epithelial ovarian cancer.
FIGO	International Federation of Gynecology and Obstetrics
HBOC	Hereditary Brest and Ovarian cancer syndrome
HGSOC	high grade serous ovarian carcinoma
HNPCC	hereditary nonpolyposis colorectal cancer
HRT	hormone replacement therapy
LGSOC	low grade serous ovarian carcinoma
MOC	mucinous ovarian cancer
NHL	Non-Hodgkin's lymphomas
OC	ovarian cancer.
SCST	sex cord stromal tumor.
SDF1	stromal derived factor 1
SNP	single nucleotide polymorphism
SPSS	Statistical Package for Social Science
STIC	serous tubal intraepithelial carcinoma
WHO	World Health Organization

ABSTRACT

Eman, B. (2022). Investigation of Chemokine LIGAND12 (CXCL12) Gene Polymorphism in Ovarian Cancer Among the Turkish Population. Yeditepe University, Institute of Health Science, Department of Molecular Medicine, Ph.D. thesis, İstanbul.

Ovarian cancer is considered the eighth killer cancer in women globally. In 2021, about 4059 women were identified as ovarian cancer cases, and 2730 of them were reported as cancer deaths in Turkey. It is relatively uncommon, but it has a high death rate among women due to its being hard to detect at early stages when there are no reliable screening methods. Therefore, most of the patients presented at advanced stages with metastasis. Ovarian cancer is a heterogeneous polygenic disease in which there are numerous morphological and molecular types of ovarian cancer. Several gene mutations are involved in the molecular pathogenesis of ovarian cancer.Chemokine ligand 12 (CXCL12) is a chemokine that acts as a ligand for chemokine (C-X-C motif) receptor 4 (CXCR4). The CXCL12 gene is expressed in normal and ovarian cell cancer. Both CXCL12 and CXCR4 are involved in ovarian carcinogenesis through enhanced tumor cell growth, angiogenesis, and metastasis. This thesis study aims to investigate the possible association between SNP rs1801157 in CXCL12gene and susceptibility to ovarian cancer among the Turkish population. The two groups were investigated (n = 40) ovarian cancer patients and the (n = 47) control group, using real-time PCR and statistical analysis of data was performed by the SPSS program. We focused on the genotypic and allelic distribution of this SNP. According to our findings, homozygote wild type (CC), heterozygote (CT), and homozygote mutant type (TT) rates are calculated respectively as 44.7%, 46.8%, and 8.5% in the control group. However, homozygote wild type (CC), heterozygote (CT), and homozygote mutant type (TT) rates are determined respectively as 42.5%, 47.5%, and 10% in the patients' groups. There was no significant relationship between genotypes in patients and the control group subjects under study. We obtained a p-value of (0.962)

Keywords: Ovarian Cancer Chemokine ligand 12, CXCL12,SNP, Genotypes

ÖZET

EMAN, B. (2022). Türk popülasyonunda over kanseri hastalarında Kemokin ligant 12 (CXCL12) Gen Polimorfizminin Etkisinin Araştırılması.Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Tıp Anabilim Dalı, Doktora Tezi, İstanbul

Yumurtalık cancer, dünya çapında dünyaya yönelik öldürücü kanser olarak kabul edilir. 2021 yılındaki yaklaşık 4059 yumurtalık kanserden vaka olarak ve bunlardan 2730'u Türkiye'de kanserden ölüm olarak rapor edilmiş.Bu evrende nadirdir, ancak erken yaştakilerde gördüklerinin zor günlerinde kadınlardan yüksek ölüm üzerinde var. ilerlemiş tarama ve yumurtalık kanserinin spesifik belirtileri veya yoktur. bu artık günümüzde çoğu zaman üst düzeylerde metastaz ve yüksek düzeyde nüks ile başvurudu. Yumurtalık kanserleri, yumurtalık kanserlerinin çok sayıda morfolojik ve ölüm tipinin bulunduğu heterojen poligenik bir patlama. Yumurtalık felaketlerinin patogenezinde çeşitli gen mutasyonları yer almaktadır. Kemokin ligandı 12 (CXCL12), kemokin (C-X-C motifi) reseptörü 4 (CXCR4) için bir ligand görevi gören bir kemokindir. CXCL12 geni, normal ve yumurtalık kanserlerinde eksprese edilir. Hem CXCL12 hem de CXCR4, gelişmiş olgunluk için, üniversitegenez ve metastaz üzerinden yumurtalık karsinogenezinde rol oynar. Bu tez, Türk cihazında12 CXCL SNP ile ilgili olasılık konusundaki şüphelerinizi araştırmayı düşünüyorum. Tow grubu (=40) kanserli hasta ve (n= 47) üzerinde kontrol grubu, real-time PCR incelendi ve analiz analizi yapıldı. Bu SNP'nin genotipik ve alelik kurslarına odaklandık. homozigot tip (CT) ve homozigot mutant tip (TT) %46,7, %8 ve %8.5 olarak hesaplanmıştır. Hastada homozigot vahşi tip (CC), heterozigot, (CT) ve homozigot tip (TT) gösterici %42,5 ve %47,5 ve %10 olarak belirlenir.) hasta ve kontrol edici genotipler arasında önemli ifadeler arasında yer alır. . P değeri ((p = 0.962) CXCL12 genindeki SNP rs 1801157 ile çalışma deneklerde yumurtalık kanserleri arasında riskler arasında bir ilişki içindeyken vardık.

Anahtar kelimeler: yumurtalık kanserleri. Kemokin ligandı 12, CXCL12 geni, SNP. genotipler

1. INTRODUCTION AND PURPOSE

Ovarian cancer is considered the eighth killer cancer in women globally. According to GLOBACAN 2020, there were 313,000 new cases of ovarian cancer, with close to 207,252 deaths globally [1]. In the United States, it is expected to diagnose over 19,000 ovarian cancer cases in 2022, leading to over 12,000 deaths, making ovarian cancer the fifth cause of cancer mortality in women [2]. In 2021, about 4059 women were identified as ovarian cancer cases, and 2730 of them were reported as cancer deaths in Turkey [1]. The 5-year survival rate for ovarian cancer is only 49% [2]. Ovarian cancer is relatively uncommon, but the high cancer-related death rate makes ovarian cancer a significant global health issue and suggests that there is a growing need to develop screening, prevention, and treatment methods to reduce cancer mortality rates. The high death rate from ovarian cancer is often because of its late presentation, which reflects late-stage detection of these lesions and the heterogeneity of cancer histology and underlying molecular pathogenesis.

Ovarian Cancer OC includes many cancers that are believed to arise from the ovary and fallopian tube. This diversity in cancer types is based on the diversity in the types of normal cells that consist of the ovarian tissue (the surface epithelium covering the ovary and fallopian tube, the germ cells, and the sex cells/steroid-producing cells/stromal cells). Each of these cell types gives rise to three major groups of OCS: 1-Epithelial ovarian cancer [EOC]. 2-Germ cell tumor. 3-and specialized stromal cell cancer. Ovarian cancer [EOC] accounts for almost 90% of ovarian cancer. EOCs are classified into different histologic subtypes. [5]

Its incidence rises with the presence of several risk factors such as advanced age, early menarche, late menopause, nulliparity, use of hormone replacement therapy, presence of endometriosis, and infertility. [9], and a family history of ovarian and breast cancer (Hereditary Breast and Ovarian Cancer (HBOC) syndrome). Furthermore, individual factors may contribute to ovarian cancer development, such as genetic variation, which is present in 22% to 25% of cases. In addition to mutations in BRCA genes, which are the main genes in familial OC, many other mutations in different genes in different signaling pathways were

identified, for instance, mutations in the TP53 gene (in about 50% of ovarian cancers), KRAS gene (in up to 30% of tumors), BRAF gene, and PIK3CA gene. Recent evidence reported by many genome-wide association studies proposes that single nucleotide polymorphism (SNP) identification is thought to elucidate an additional 6.4% of the familial risk of ovarian cancer, with 34 susceptibility loci identified. [5.8].

EOC is often called a silent killer as most cases are diagnosed in the late stages of the disease and there are no specific symptoms in the early stages of the disease. Women at high risk are screened with ultrasound imaging and for serum levels of serum cancer antigen 125 (CA-125). However, these screening methods have sensitivity only in the late stages of cancer. Therefore, the majority of cancer cases are diagnosed at late stages with metastasis. The treatment of ovarian cancer is based on the clinical stage and includes debulking surgery and chemotherapy. Despite that, the recurrence rate is high in this type of malignancy [7].

CXC motif chemokine ligand 12(CXCL12) also referred to as stromal cell-derived factor 1 is a hemostatic chemotactic chemokine that binds to the CXCR4 receptor and triggers intracellular transduction signals which have a fundamental role in normal and diseased cells, including cancer cells. Evidence suggests that CXCL12 andCXCR4, are expressed at high levels in several cancer types such as breast, lung, colorectal prostatic cancers as well as ovarian cancer tissue and have been involved in ovarian cancer development through enhanced tumor cell growth, angiogenesis, and metastasis. [16]. Furthermore, more recently, it has been demonstrated that many single nucleotide polymorphisms (SNPs) are reported on the CXCL12 gene. rs1801157 SNP is a genetic variant located on the CXCL12 gene and is suggested to be associated with many cancers type. Breast cancer and Non-Hodgiın Lymphomas are one of these cancers that have been proposed to associate with rs 1801157 SNP of the CXCL12 gene. [17]

Aim and purpose:

Considering ovarian cancer is commonly detected at late stages and has a consequential high death rate, identifying susceptibility SNP loci for ovarian cancer is a critical and urgent clinical demand, which may lead to the development of an effective screening method to assess an individual's susceptibility to ovarian cancer and to detect new diagnostic tools and target treatment, therefore improving risk reduction strategies.

Recent promising evidence suggests that SNPs can be considered in clinical screening methods. Furthermore, it is a challenge to investigate the association of related SNPs with ovarian cancer.

This research aims to investigate the association of single nucleotide polymorphisms (SNP) rs1801157 in the C-X-C chemokine ligand 12 (CXCL12) gene with the susceptibility of females to ovarian cancer in a population of Turkey.

2. LITERATURE REVIEW

2.1. Ovarian Anatomy and Histology

In a female pelvis, there are two pairs of ovaries. They are almond-shaped and measure about 3 cm in length, 1.5 cm in width, and 1 cm in thickness. They lie on the lateral wall of the pelvic cavity and are kept in their position by a mesentery (the mesovarium) that is a posterior extension of the broad ligament.[4] Fig 2.1.

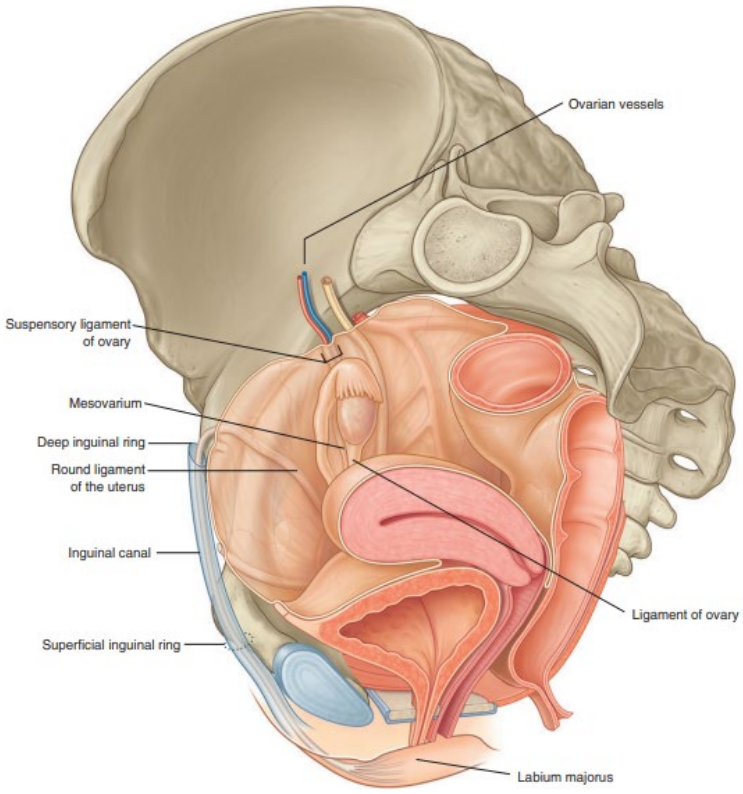

Figure 2.1: Diagram shows the anatomical position of the ovary in relation to the adjacent structures.[4]

The ovary is composed of three layers: covering coelomic epithelium, the outer cortex, and an inner medulla. The epithelial layer of germinal epithelium, also known as primordial germ cells, consists of a single layer of cuboidal and squamous cells. The cortex surrounds the medulla and extends to the peripheral part of the ovary. The cortex consists of the specialized gonadal stroma, non-specialized gonadal stroma, and ovarian follicles in different stages. The single ovarian follicle consists of a specialized gonadal stroma surrounding the central germ cell ovum. The specialized gonadal stroma has two components: an avascular granulosa cell component (encircling the ovum); and a theca cell component. The theca cell portion has two parts: theca interna and theca externa. The unspecialized gonadal stroma consists of spindle-shaped connective tissue cells and smooth muscle fibers. The medulla consists of loose connective tissue with blood vesicles, lymphatics, and nerves and is located in the central part of the ovary. Between the surface epithelium and the cortex, there is a dense connective tissue layer known as the Tunica albuginea. fig2.2

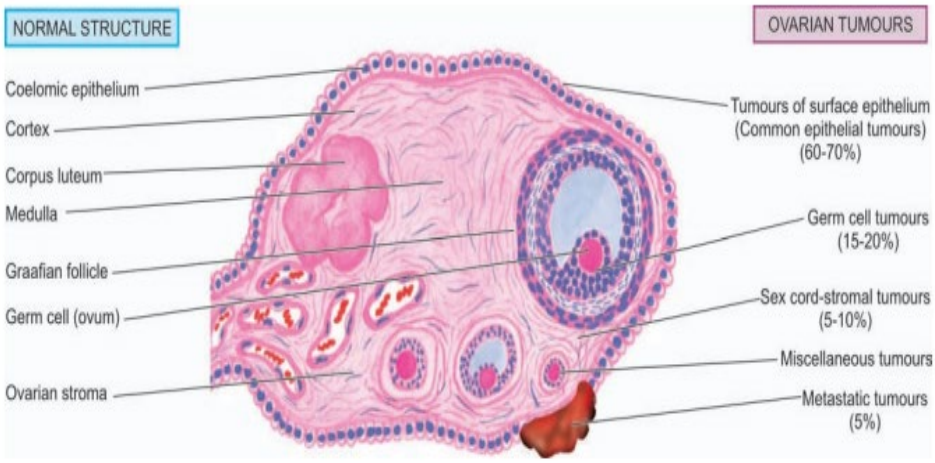

Figure 2.2: Schematic drawing of histological structure of the cortex of the ovary and associated ovarian tumors.[3]

The ovary is responsible for the production of eggs (oogenesis) and steroid hormones (steroidogenesis). Estrogen is secreted by granulosa cells and theca interna cells. On the other hand, progesterone is secreted by the corpus luteum, which is formed after bursting the fully mature ovarian follicle and releasing the ovum. Both hormones have a major role in the preparation of the uterus for the implantation of a fertilized ovum. [3,4,5]

2.2. Ovarian Tumors

Benign, malignant, and low-malignant potential (borderline) tumors have been identified in the ovaries. Benign tumors are more common and occur in females less than 40 years old. Malignant tumors can be primary or secondary (metastatic). Primary malignant ovarian tumors (ovarian cancer, OC) are more common in females older than 40 years old. Secondary ovarian tumors affect women at any age, depending on the primary site of metastasis. [3]

According to the WHO classification of ovarian tumors, there are five major groups of ovarian cancer; the first group is surface epithelium (common epithelial tumors). The second group is germ cell tumors. The third group is sex cord-stromal tumors. The fourth group is miscellaneous tumors. And the fifth one is metastatic tumors.[44]

Semi-malignant tumors with different cell types (serous, mucinous, endometroid, clear cell, transitional, and mixed epithelial cells) are defined as low malignant potential (borderline tumors, BOTs) tumors that have the ability to progress to low-grade serous carcinoma. [3.6]

Table 2.1: WHO classification of ovarian cancer.[44]

I. TUMOURS OF SURFACE EPITHELIUM (COMMON EPITHELIAL TUMORS) (60-70%)	II. GERM CELL TUMORS (15-20%)	III. SEX CORD-STROMAL TUMORS (5-10%)	IV. MISCELLANEOUS TUMORS	V. METASTATIC TUMOURS (%5)
A. Serous tumors 1. Serous cystadenoma 2. Borderline serous tumor 3. Serous cytadenocarnioma B. Mucinous tumors 1. Mucinous cystadenoma 2. Borderline mucinous tumor 3. Mucinous cystadenocarcinoma C. Endometrioid tumors D. Clear cell (mesonephroid) tuors E. Brenner tumors	A. Teratomas 1. Benign (mature, adult) • Benign cystic teratoma (dermoid cyst) • Benign solid teratoma 2. Malignant (immature) teratoma 3. Monodermal or specialized teratoma • Struma ovaririi • Carcinoid tumor D. Choriocarcinoma E. Others (embryonal carcinoma, polyembryoma. mixed germ cell tumors)	A. Granulosatheca cell tumours 1. Granulosa cell tumour 2. Thecoma 3. Fibroma B. Sertoli-Leydig cell tumours (Androblastoma. Arrhenoblastoma) C. Gynandroblastoma	A. Lipid cell tumours B. Gonadblastoma	A. Krukenberg tumour B. Others

2.2.1. Primary malignant ovarian tumors

2.2.1.1. Epithelial ovarian cancer (EOC)

About 90% of malignant ovarian tumors are epithelial in type. EOCs can be classified according to the histological morphology of the cancer cells into different types. The most common major types are I-serous carcinoma (about 40% of malignant ovarian tumors in patients aged between 45 and 65 years old). Two types of serous carcinoma are described:

high grade serous ovarian carcinoma (HGSOC), which represents 75% of EOCs; and low grade serous ovarian carcinoma (LGSOC), which represents 2% of all OCs. II-mucinous ovarian cancer (MOC), which accounts for 10% of all ovarian cancers; III-endometrioid cancer (EC), which accounts for 20% of all ovarian cancers; and IV-clear-cell carcinoma (CCC). In addition, other less common types of surface epithelial tumors may differentiate along with a mesonephros pattern, forming a clear cell (mesonephroid) adenocarcinoma (approximately 5% of all ovarian cancers). [3.7]

2.2.1.2. Ovarian germ cell tumors

This type of tumor arises from germ cells that are responsible for the production of female gametes. It is commonly aggressive and occurs in women at a younger age. There are several types of germ cell tumors, including dysgerminoma (DG), Yolk sac tumor, embryonal carcinoma, polyembryony, choriocarcinoma, immature teratoma, and mixed germ cell tumors. [6]

2.2.1.3. Sex cord-stromal tumors (SCSTs).

SCSTs arise from specialized stromal cells. They occur in a wide age group and have a variable prognosis. However, they are usually non-aggressive and affect the ovary unilaterally. Moreover, most of these tumors produce steroid hormones, and they are associated with hormone-related disorders. Gragranulosal tumors, theca cell tumors, and Sertoli-leyding cell tumors are uncommon. [6.7]

2.2.2. Secondary malignant ovarian tumors

Approximately 5–10% of ovarian tumors are secondary tumors. Both ovaries are frequently affected by the local spread of the adjacent primary tumor sites or by distant metastasis via blood or lymph. The primary sites can be gynecologic primary sites, e.g., uterine body, uterine cervix, and fallopian tube, or non-gynecologic primary sites, e.g., breast, stomach, colon, pancreas, appendix, biliary tract, and hematopoietic malignancies. Trans-coelomic spread allows cancers of the stomach, colon, appendix, and breast to spread

bilaterally to the ovaries, resulting in the Krukenberg tumor, a secondary malignant tumor. The majority (75%) of Krukenberg tumors arise from gastric cancer, and the remainder (25%) often arise from metastatic colon cancer. [3,6]

2.3.1. Epidemiology and pathogenesis of ovarian cancer

Ovarian cancer is considered the eighth killer cancer in women globally. Annual death numbers among women because of OC and its related morbidity are more than 200,000. [1]. In the United States, it is expected to diagnose over 19,000 ovarian cancer cases in 2022, leading to over 12,000 deaths, making ovarian cancer the fifth cause of cancer mortality in women [2]. In 2021, about 4059 women were identified as ovarian cancer cases, and 2730 of them were reported as cancer deaths in Turkey [1]. The 5-year survival rate for ovarian cancer is only 49%. 1 in 72 women is susceptible to OC during her life. and 1 in 96 women with OC is expected to die from it. OC incidence is variable among races and ethical groups. Several studies done in the United States show that it is lower in African Americans than in Caucasian Americans. [20]

The exact etiology of ovarian cancer is still unknown. However, reproductive and hormonal as well as hereditary predisposition and genetic alterations have been identified as risk factors for epithelial ovarian cancer.[8]

Many studies hypothesize that ovulation plays a major role in the carcinogenesis of ovarian cancer, where it was found that the rate of ovarian cancer is higher in females who have early menarche, late menopause, nulliparity, infertility, and use of fertility drugs and hormone replacement therapy (HRT). In contrast, high parity, breastfeeding, and the use of oral contraceptive pills are assumed to be protective against OC. Additional risk factors for OC include advanced age, smoking, increased weight, height, and body mass index (BMI). [8]

The most significant risk factor for ovarian cancer development is a family history of hereditary cancers such as ovarian, breast, or colorectal cancer. The risk increases with an increase in the number of first-degree relatives affected. Familial cases of ovarian cancer

account for 5% to 10% of all cases. The presence of mutations in the BRCA1/2 genes is the major cause of familial (hereditary) OCs. However, such mutations are seen in only 8% to 10% of sporadic cases. The average lifetime risk for OC is approximately 30% in BRCA1 carriers and is lower in BRCA2 carriers. Several molecular pathways and gene alterations have been identified in the pathogenesis of EOC, such as p53 tumor suppressor gene mutation and ERBB-2 and K-RAS overexpression. [5.]

Ovarian cancer is also seen as a part of a spectrum of familial cancer syndromes caused by several hereditary gene mutations.

The pathogenesis of germ cell cancers is not well understood. However, a mutation in the c-KIT gene and the presence of Y-chromosome material have been reported in the DG [6]. Furthermore, dysgerminomas are associated with high risk in patients who have certain genetic diseases like Turner's syndrome, Triple X syndrome, and Swyer syndrome [7].

Sex cord-stromal tumors (SCSTs**)** There are no specific risk factors for SCSTs. However, some evidence supports that the presence of certain hereditary cancer syndromes predisposes patients toward SCST [7].

2.3.2. The molecular pathogenesis of ovarian cancer

OCs are heterogeneous at the genomic level, and each type has its own underlying molecular genetic alterations. Numerous molecular studies have identified several gene mutations.[6]

The origin of EOC has been controversial. Studies hypothesize that high-grade serous carcinomas and possibly endometrioid carcinomas are believed to be derived from surface epithelial inclusion glands, from the fallopian tube epithelium, and from deposits of endometriosis, whereas most low-grade serous carcinomas are thought to arise from cystadenoma and endometriosis. However, recent evidence suggests that serous tubal intraepithelial carcinoma (STIC) may be a precursor of high-grade serous tumors in high-risk patients with BRCA1/2 mutations[6.9.]

2.3.3. Hereditary ovarian cancer

Ovarian cancer is considered heritable cancer. It accounts for 5%–10% of ovarian cancers in females who have a family history of this disease. It is proposed that up to 25% of all EOCs have a heritable component.

Hereditary breast-ovarian cancer syndrome (HBOC)

Approximately 25% of HBOC cases are combined with the presence of mutations in BRCA tumor suppressor genes. The BRCA1 gene is located on chromosome 17q and the BRCA2 gene is located on chromosome 13q. Moreover, mutation of the p53 tumor suppressor gene and overexpression of ERBB-2 and K-RAS have been identified as molecular abnormalities associated with ovarian cancer development. However, recent advances in molecular genetics have revealed substantial locus heterogeneity among affected families without *BRCA1 or BRCA2* mutations. [11.13]

Lynch syndrome hereditary nonpolyposis colorectal cancer (HNPCC).

Lynch syndrome is an inherited disorder caused by mutations in the mismatch repair genes MLH1, MSH2, MSH6, PMS2, and EPCAM. It accounts for 10–15% of hereditary OC. It has been linked to an increased risk of certain types of tumors in patients, including colorectal cancer, ovarian cancer, endometrial cancer, gastric cancer, brain, upper urinary tract, and skin cancer. [13]

Ovarian cancer in conjunction with other genetic syndromes

There are other rarer syndromes that are associated with hereditary ovarian cancer. They include Li-Fraumani syndrome (linked to penetrant PALB2, TP53 genes), Cowden syndrome (linked to penetrant PTEN gene), and Peutz-Jeghers syndromes (linked to penetrant STKII gene). [7]

2.3.4. The molecular changes and pathways that affect each EOC variant

Each subtype of ovarian cancer is characterized by the presence of distinct deregulation in specific signaling pathways caused by various genetic mutations. Ovarian cancer can progress in two ways; type I (low-grade pathway), which progresses through a step-wise mutation process; Type II (high-grade pathway) progresses through a separate pathway with high genetic instability, resulting in rapid metastasis. [10]

Both the TP53 mutations and the dysfunction of BRCA1/2 are associated with high-grade serous carcinomas. On the other hand, mutations in KRAS and BRAF lead to activation of the RAS-RAF signaling pathway and are associated with low-grade serous carcinomas; mutations in KRAS and mutations in PIK3CA/PTEN (regulator of PI3K-PTEN-AKT pathway); BRAF mutation; and CTNNB1/APC mutations (regulator of Wnt-signaling pathway) are combined with mucinous carcinoma. Mutations in *PIK3CA* which regulate the PI3K-PTEN-AKT pathway, are the most common genomic alteration identified in clear cell carcinoma CCCs. [7]

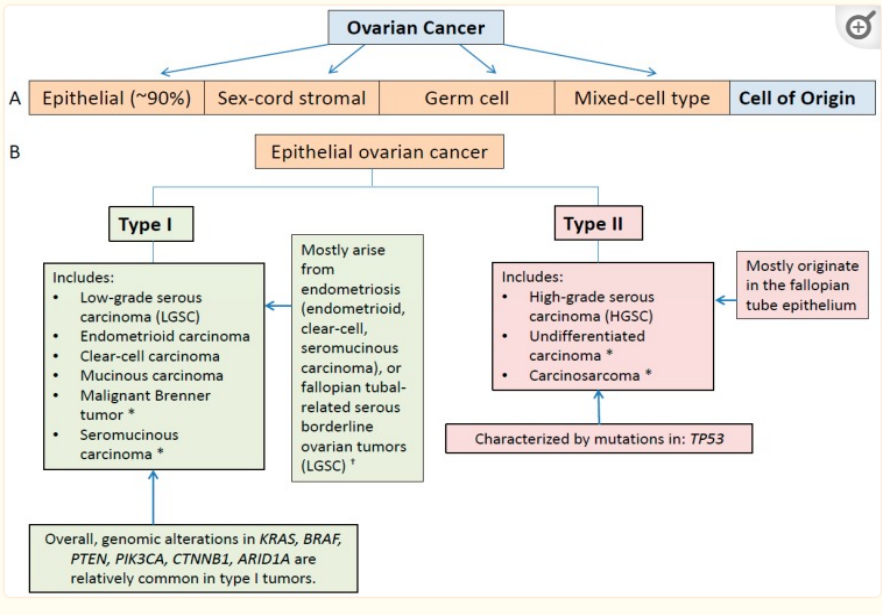

Figure 2.3: (**A**) ovarian cancer is classified based on histological structures, and (**B**) epithelial ovarian cancer is classified based on the grade of tumor [12].

Table 2.2: Histological epithelial ovarian tumor types and associated molecular alterations [7]

EOC sub- type	Chromosomal aberrations	Major pathway affected
(HGOC)	TP53 mutations 90%, BRCA mutations <20%	HR pathway
(LGOC)	Mutations in BRAF/KARS/NRAS, ERBB2, PI3KCA, FFAR1, USP9X, EIF1AX.	MAPK pathway, PI3K pathway, mTOR pathway.
Endometrioid carcinomas (EC)	ARID1A (30%), CTNNB1 (25-60%), KRAS/BRAF (20%), PIK3CA (12%), and TP53 (25%)	MAPK pathway, β-catenin signaling, PI3K/ PTEN pathway.
Mucinous ovarian carcinomas (MOC)	KRAS, TP53, PI3KCA/PTEN, ARIDA1, BRAF, CTNNB1/APC, andHER2	MAPK pathway, Wnt signaling pathway PI3K/ PTEN/AKT pathway
Clear-cell carcinomas	SMARCA4, PIK3CA (50%), PIK3R1, AKT2, PTEN, , ERBB2 KRAS, PPP2R1A, TP53, TERT ARID1A	MAPK pathway, PI3K-PTEN-AKT pathway.

2.4. Management of ovarian cancer

2.4.1. Ovarian cancer screening

Despite the importance of screening women to detect and treat silent cases of ovarian cancer before it spreads, there is currently no accurate and cost-effective screening method available. However, annual pelvic examination, vaginal ultrasound screening, and detection of tumor biomarker CA 125 for high-risk group (females with a family history of breast or ovarian cancer or who have developed persistent nonspecific symptoms for less than one

year) is recommended in some countries as a national screening program, although they don't have high sensitivity and specificity. [18]

2.4.2. Ovarian cancer patients' clinical histories and examinations

In the early stages, when the disease is still confined to the ovary, the patient is usually asymptomatic or has nonspecific complaints. When cancer directly extends to nearby organs in the pelvis and abdomen, many patients may have epigastric pain and fullness or generalized fatigability and abdominal heaviness. At the late stage, when distant metastasis has occurred, the patients start to have serious symptoms such as significant weight loss, loss of appetite, and intestinal or urethral obstructions may develop. On bimanual pelvic examination, an adnexal mass may be detected. Vaginal ultrasound and serum CA125 measurement (which is elevated in approximately 80% of patients in the late stages of EO) are important in determining the degree of suspicion. In the case of a high index of suspicion of ovarian cancer, further investigation such as radiological imaging, laparoscopic surgery, and biopsy is recommended. A biopsy is a gold standard to confirm the diagnosis. [18]

2.4.3. Ovarian cancer staging and treatment

Because OC can spread to the peritoneal cavity easily, initiated debulking surgery with cytoreduction by removing macroscopically visible tumor is the key for staging, treatment and improving the outcome of OC patients. Based on the International Federation of Gynecology and Obstetrics (FIGO) staging system, there are four stages of OC (FIGO stage I, II (early stages, about 25% of patients have a good prognosis) and III, VI (late stages, about 75% of cases, their prognosis is variable and depends on clinical circumstances and debulking surgery). Chemotherapy may be administered before or after surgery or both. There is a considerable prevalence of recurrence in patients with OC in spite of treatment. [18]

Table 2.3: FIGO staging and progression of Ovarian cancer[18]

FIGO STAGE	CHARACTERISTICS	STAGE DISTRIBUTION	10-YEAR SURVIVAL RATE
I	Disease confined to the ovaries	20%	73%
IA	One ovary, capsule intact, no ascites		
IB	Both ovaries, capsule intact, no ascites		
IC	Stage IA plus ascites or washing, capsule ruptures,		
II	Disease spread confined to the pelvis		
III	Disease confined to the abdominal cavity, including surface of the liver; pelvic, inguinal, or para-aortic lymph nodes; omentum or bowel	5%	45%
IIIA	Negative lymph nodes, plus microscopic seeding of peritoneal surface	58%	21%
IIIB	Negative lymph nodes, peritoneal implants < 2 cm		
IIIC	Positive lymph nodes and/or abdominal pmplants > 2 cm		
IV	Spread to liver parenchyma, lung, pleura, or other exra-abdominal sites	17%	<5%

2.5.1. CXC-Motif ligand (CXCL) structure and receptor

Chemokines are a type of cytokine that has chemotactic activity. They are a super family of small proteins, approximately 8–10 kilodalton in mass. These proteins act as ligands that bind to the G protein-coupled receptor GPCR known as CXCR. There have been 20 distinct chemokine receptors discovered in humans. [15] Chemokines are grouped based on their structure into four chemokine families: C Chemokines (2 types), CC Chemokines (25 types), CXC (17 types) Chemokines, and Chemokines CX3C (one type). This classification comes from the number of cysteine residues attached to the N-terminal region

of chemokine. fig. Furthermore, they are classified according to their function into three functional groups: hemostatic, inflammatory, and angiogenic chemokines. Hemostatic chemokines have a major role in embryogenesis and tissue formation during wound healing. While inflammatory and angiogenic chemokines are highly secreted in response to inflammation and tissue damage, they have a key role in the induction of migration of immune cells toward targeting tissue, leukocyte trafficking, and vascular formation, which occur in many diseases such as viral infection and cancer.[14.15.20]

Two types of chemokine receptors are expressed. The first group is conventional chemokine receptors (CCKRs), For example, CXCR4 binds with the CXCL12 ligand and activates G proteins, causing conformational changes and inducing intracellular signaling transduction. The second group is atypical chemokine receptors (ACKRs), for instance, CXCR7 which binds with CXCL12 and signals through induction of beta-arrestin, not through activation of G-protein activating signal transduction.[22]

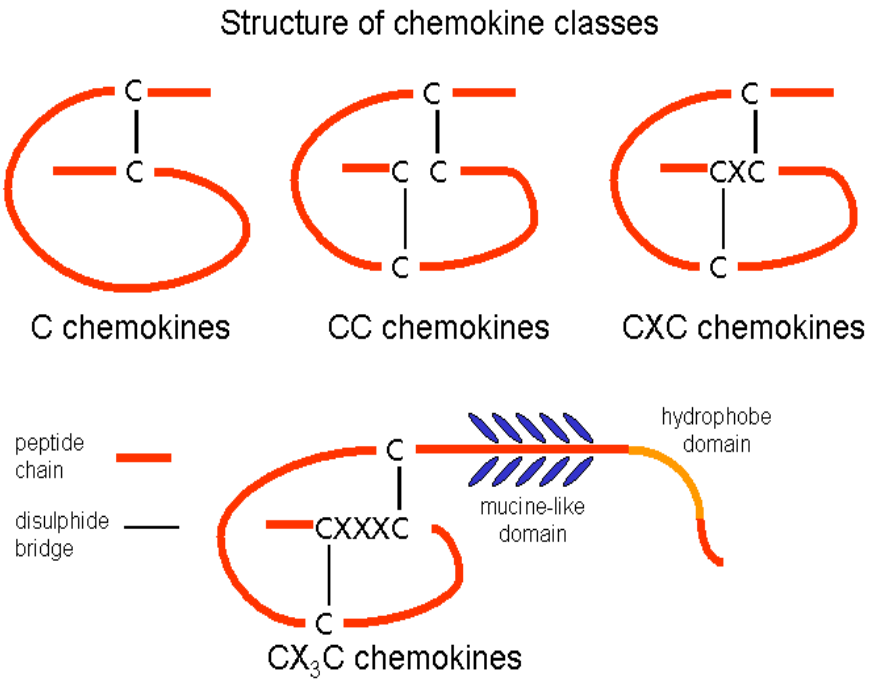

Figure 2.4: Structural classification of chemokines (C=cysteine)[45]

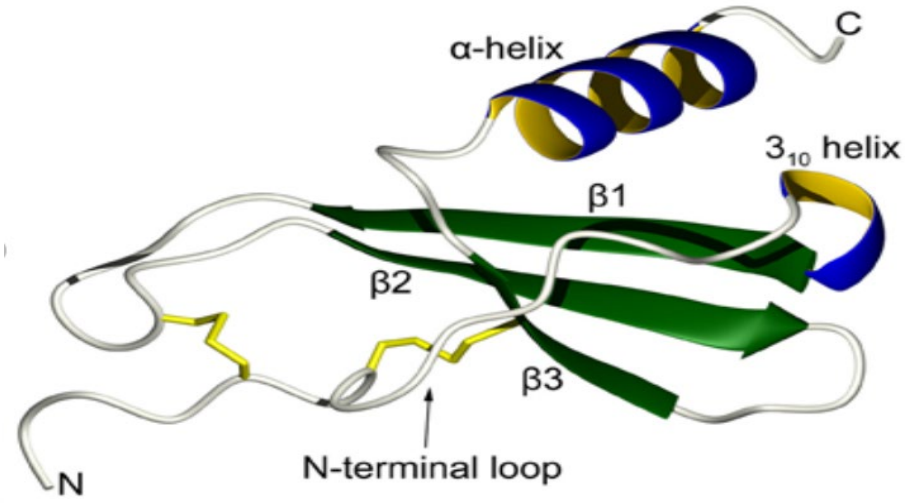

Figure 2.5: Structure of typical chemokine ligand [46]

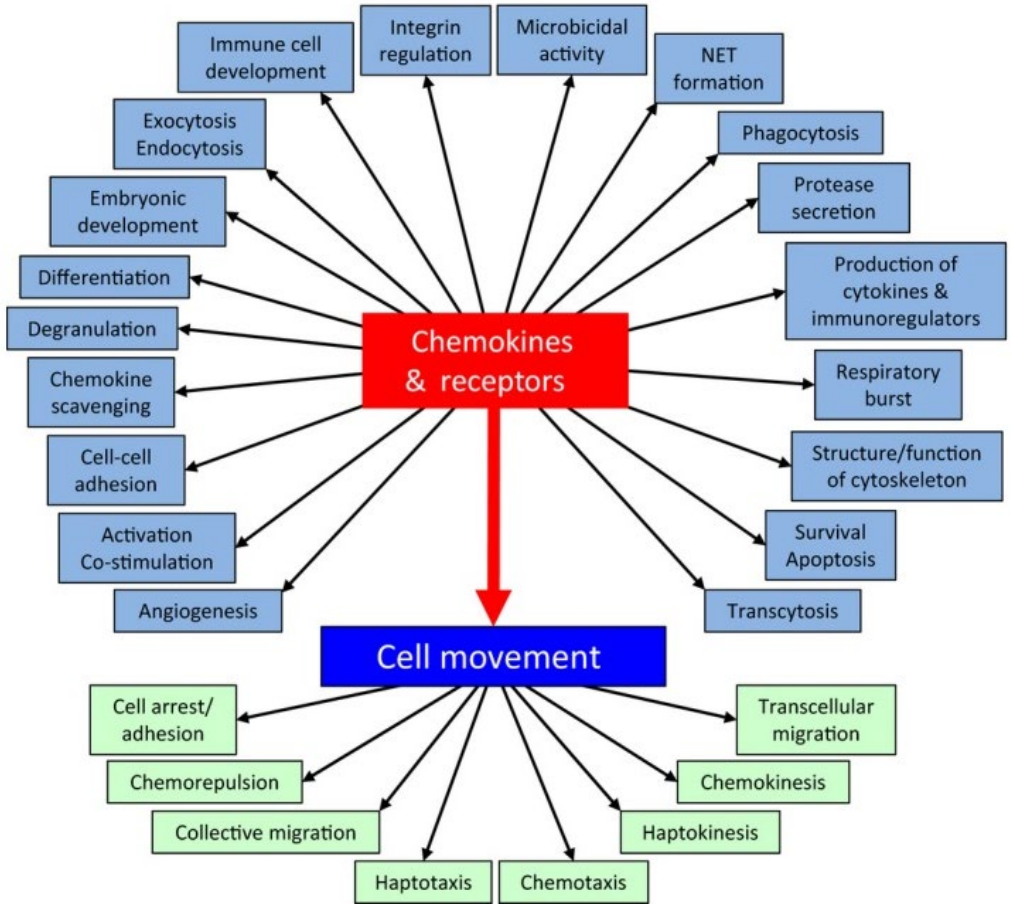

Figure 2.6: The function of chemokine ligands and their receptors.[15]

2.5.2. C-X-C motif chemokine ligand 12 gene(CXCL12)

According to the Atlas of Genetics and Cytogenetics in Oncology and Hematology, the CXCL12 gene, also referred to as stromal-derived factor SDF, is located on chromosome 10 at band q11.21. It has four exons spanning 14.94 kb. There are eight transcriptional variants of this gene resulting from alternative splicing events, leading to the production of six protein isoforms: Alpha, Beta, Gamma, Delta, Epsilon, and Theta. [21]

Other names for the CXCL12 gene include TPAR1, SCYB12, PBSF, IRH, and SDF1-a, SDF1-b.

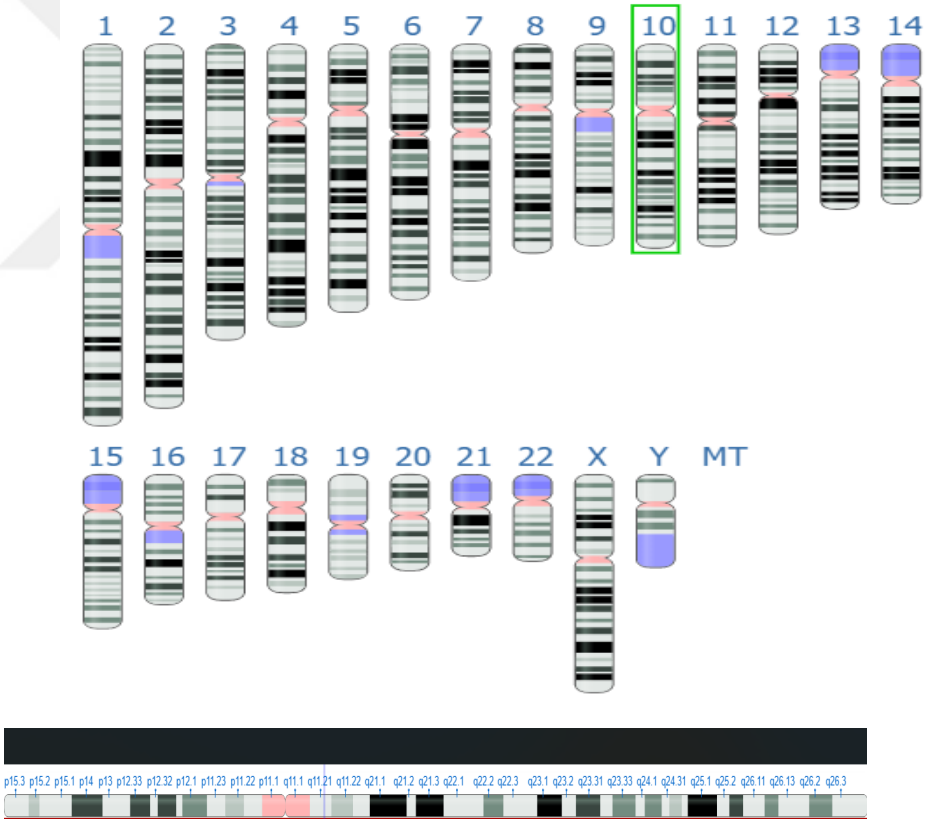

Figure 2.7: The diagram shows the structure and location of CXCL12 gene.[48]

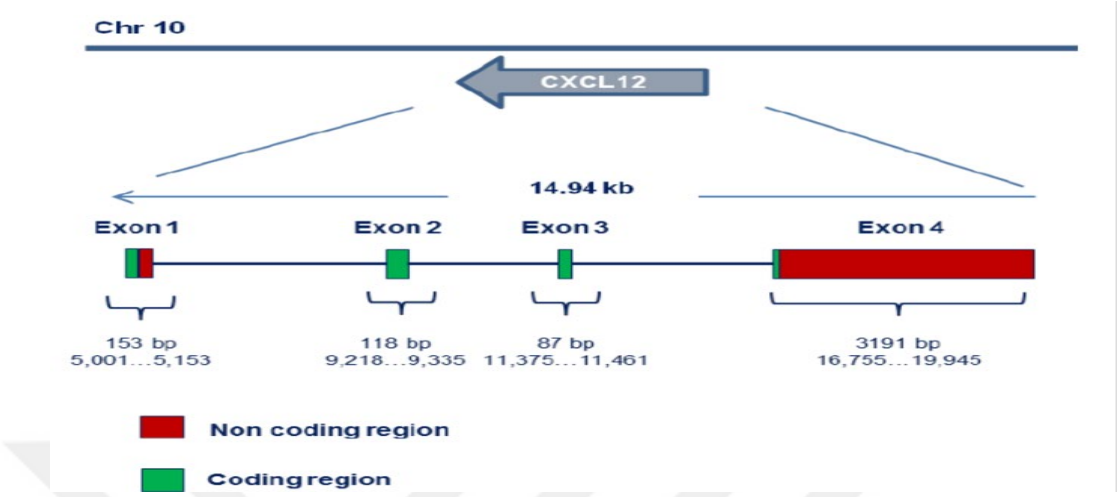

Figure 2.8: CXCL12gene.three coding exons (in green color) and one non-coding exon (in red)[47]

Table 2.4: Protein isoforms of CXCL12.[21]

Isoform name	Beta(full-length)	Alpha (full-length	Gamma	Delta	Epsilon	Theta
Length of amino acid sequence	93	89	119	140	90	100
Site of expression	liver spleen pancreas	Liver spleen pancreas	heart	pancreas during development	pancreas during development	pancreas during development
post-translational modification	become SDF-1-alpha	become SDF-1-beta	Not occur	Not occur	Not occur	Not occur

2.5.3. CXCL12 (chemokine (C-X-C motif) ligand 12)

is a hemostatic chemokine that belongs to the CXC subgroup. It acts as a ligand for chemokine (C-X-C motif) receptor 4 (CXCR4) and chemokine (C-X-C motif) receptor 7 (CXCR7. It is secreted by fibroblasts, osteoblasts, and endothelial cells lining blood vessels. It has a key role in homeostasis, angiogenesis, apoptosis, immune response, and embryogenesis. CXCL12 is normally abundant in many organ tissues, including the

endometrium, spleen, lymph nodes, urinary bladder, gall bladder, liver, kidney, brain, skeletal muscles, and colon. addition, it is reported that CXCL12 is over-expressed in tissues during pathological conditions such as inflammations, cancers, and autoimmune diseases, where it promotes cell proliferation and migration as well as tumor growth and metastasis. [20.21.22]

2.5.4. The role of C-X-C motif chemokine ligand 12 in cancer CXCL12.

Many cancer cells express high levels of CXCL12 (stromal cell-derived factor-1 (SDF-1), including breast cancer, urinary bladder cancer, hepatocellular carcinoma, prostatic cancer, lung cancer, and ovarian cancer cells. CXCL12 and its receptors, CXCR4 and CXCR7, have an essential role in cancer evolution and spread. Furthermore, the CXCL12/CXCR4/CXCR7 axis is involved in tumor development, growth, angiogenesis, distant metastasis, tumor microenvironment, and resistance to chemotherapy. Recent studies demonstrated that CXCL12, which is highly expressed in ovarian epithelial cancer cells, binds to the CXCR4 receptor and regulates transduction of various signaling pathways, e.g., AKT and ERK pathways, that stimulate cancer cell growth, survival, migration and metastasis. *In addition,* CXCL 12 ligand binds with the CXCR7 receptor, which is suggested to be highly expressed in endothelial cells of cancer cells, and its function is obvious in tumor progression and invasion. [22]

There are different principal signaling pathways that are induced by the CXCL12/CXCR4/CXCR7 axis depending on cell and tissue type. These transduction pathways control cell migration toward cancer tissue, gene transcription, cell survival, and proliferation. CXCL12/CXCR4 activates G protein-coupled receptors and consequentially triggers MAPK, ERK 1/2, and AKT signaling pathways, which are responsible for cell survival and proliferation. On the other hand, CXCL12/CXCR7 triggers the MAPK signaling pathway either independently by induction of Beta-arrestin or by the formation of a CXCR4/CXCR7 heterodimer.[22]

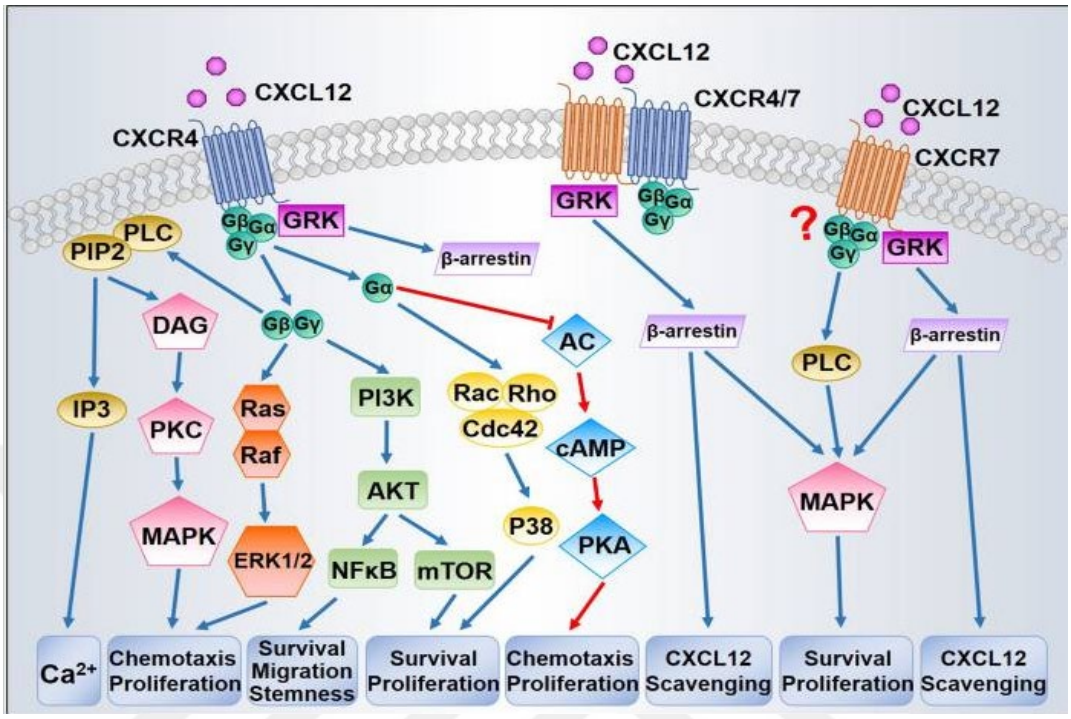

Figure 2.9: CXCL12/CXCR4/CXCR7 signalling transduction pathways.[22]

2.5.5. The role of C-X-C motif chemokine ligand 12 in ovarian cancer

A recent study suggested that CXCL12 may increase malignant progression by stimulating DNA synthesis in papillary serous carcinoma cell types. In addition, it was proposed that CXCL12 could be involved in the induction, production, and migration of inflammatory cells as well as tumor growth through activation of ERK1/2 and MAPK signaling pathways. [23]

2.6. SNP rs1801157 in the CXCL12 Gene

A SNP refers to the presence of a genetic variant in which there is a substitution of a single nucleotide base at a specific position in a specific gene in more than 1% of the population. The presence of certain SNPs is associated with increasing the risk and susceptibility of an individual to different diseases. SNP also has an impact on an individual's ability to respond to drugs and stress.

Many SNPs have been identified in the CXCL12 gene. CXCL gene shows a G801A transition at position 801 in the 3′-untranslated region of the transcript, known as SDF1-3′A (rs 1801157). It proposed that single nucleotide polymorphism in this position may increase the synthesis of the SDF1[CXCL12] protein[49]

Hence, several recent studies have proved that there is an association between the CXCL12 rs1801157 polymorphism and the risk of many cancer types, such as breast cancer and Non-Hodgkin's lymphomas NHL [17.24.25]. However, this association with ovarian cancer risk has as yet not been confirmed [26]. Furthermore, evidence shows that CXCL12 is highly expressed in ovarian cancer cells. Therefore, this study aims to assess the association of SNP rs1801157 in the CXCL12 gene with the risk of ovarian cancer among the Turkish population.

3. MATERIALS AND METHODS

3.1. Sample Selection and Definition

The study was conducted on a total of 87 subjects.40 female patients with ovarian cancer and 47 apparently healthy were also included in a control group. Ethical approval was obtained from Ethics Committee of Yeditepe University for the study.

Patient Group: This group consists of n=40 females' patients, aged, between 18-85 years with histologically-determined primary ovarian cancer OC patients at the Department of Obstetrics and Gynecology in the yeditepe university hospital.

Control Group: This group consisted of females(n=47), aged between 18-85 years, and clinically proven free of ovarian cancer.

NOTE: since this research was done during the covid19 pandemic,it was difficult to obtaion more abundant cases.

3.2. Materials and Devices Used in the Experiment

3.2.1. Materials used in the DNA isolation from the peripheral blood

The peripheral venous blood samples were set in tubes at 4 °C. The tubes contained Ethylenediaminetetraacetic acid (EDTA), which prevents blood clotting. DNA İsolation Robot (IPrep pure link, Invitrogen and the Thermo Fisher Scientific Inc) system was used for DNA isolation.

The mixture used in the DNA isolation was composed of 1.12mg/ml Proteinase K, 8M Guanidine hydrochloride, 10.5 nM EDTA, 10.5 nM NaCl and 10.5 nM Tris-Cl of pH 8,8.

3.2.2. The Equipment used in the Experiment

DNA Isolation Robot (Iprep Purelink, Invitrogen, Thermo Fisher Scientific Inc), Nanodrop 2000 (Thermofisher Scientific Inc), Real-Time PCR (LIGHT CYCLE 480 II

Instrument, Roche Diagnostics, Fast Real Time 7500, Applied Biosystems), 7500 Fast Real-Time PCR Instrument, Plate Centrifuge (Hettich), Centrifuge (Centrifuge 22R-Beckman Coulter), +4 C° Refrigerator (Haier), -20 C° Refrigerator (Haier), Ultra

Pure Water (Pure Lab Option Q,Elga), Vortex (V.I. Plus Biosan) and a Pipette Kit (Thermo Fisher Scientific Inc.) were used in this experiment.

3.3. Methods

3.3.1. Genomic DNA isolation from blood

All venous blood samples of the patient and control groups were taken into the tubes with EDTA in a volume of 5 ml. Blood samples were stored in a refrigerator at + 4 ° C until DNA isolation. DNA isolation was performed by using a robot of iPrep DNA extraction (Invitrogen), and from the blood genomic DNA isolation Kit with iPrep. DNA can be isolated from 350 µl peripheric blood by this system, so 13 blood samples could be studied at the same time. One cartridge is used for each of samples, the cartridges are agitated for a period of time to bond the magnetic beads to the DNA efficiently before putting the samples to own cartridge.

The robot of iPrep works according to ChargeSwitch® technology (CST®), as an automated extraction method. A high amount of DNA can be prepared from samples with this method. In this method, amounts of genomic DNA are prepared from samples purely by using paramagnetic particles. These particles are surrounded by a DNA binding surface. CST® (Charge Switch® Technology) extraction method has a unique principle when compared to the silica-based DNA extraction method. The charge of beads can be changed by the pH of their surrounding buffer. In the event of low pH conditions, the backbone of the DNA is negatively charged, then it binds to the positively charged beads. These charged beads are neutralized by using a low salt buffer that has a higher pH in order to allow for the elution of DNA. Purified nucleic acids pass into the wash buffer, then DNA samples are ready to use).

At the end of the experiment aqueous DNA samples were obtained and stored at +4 ° C in the refrigerator.

3.3.2. Measurement of DNA purity

UV absorbance of nucleic acids is measured at 260 nm by UV spectroscopy that called NanoDrop. In this spectrophotometer cuvettes or capillaries are not required. DNA concentrations of both OD260/OD280 and OD260/OD230 proportions are determined by NanoDrop. Thanks to the NanoDrop device, the purity as well as the concentration of nucleic acid molecules such as DNA were able to observed. On the other hand, it has not been shown to distinguish several molecules like RNA, nucleotides, double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA) because this device quantifies the absorbance of nucleic acids .

In this experiment, we quantified DNA using the NanoDrop 2000 (Thermofisher Scientific Inc). 1,5 µl of DNA samples were used. The DNA samples before measurement were diluted in the ratio of 1/100. The sample was put into place for measurement by opening the arm then the device's arm was turned off. After each measurement, the surface was cleaned by distilled water, and thus it could be safe for the next measurement.

50 µg / ml of double-stranded DNA at a wavelength of 260 nm is equal to one Optical Density (OD) Unit. The purity of DNA samples was measured by analyzing the OD260 / OD280 ratio. The suitable ratio of OD260 / OD280 is between 1.7-1.9 when performing genotyping .

The following formula was used to calculate the concentration of DNA at 260 nm.

dsDNA concentration = 50 µg/mL × OD260 × dilution factor

3.3.3. Real-Time PCR Conditions for CXCL12Polymorphism

Genotyping analysis was performed by using the 7500 Fast-Real-Time Polymerase chain reaction (Applied Biosystems) device with Real-Time PCR.

By Real-Time PCR, fluorescence dyes of probes are utilized to determine the single nucleotide polymorphisms (SNPs). It is a system that allows genotyping by reading the fluorescence radiations. There are two TaqMan probes, one prob is labeled a FAM dye and the other prob is labeled a VIC dye. The fluorescent dye-bound DNA probes bind to the amplified region. The probes are hydrolyzed by Taq polymerase. Fluorescent signals can be easily detected. The probes with fluorescence dye used in the Real-Time PCR have two different wavelengths for allele-specific, which are wildtype and mutant alleles, detection.

The primer sequence of CXCL12 reported by Lundström et al. in 1991 is indicated below. This primer was determined according to the sequence of the CXCL12 gene in human cells and it was used in this experiment is used (64). In this method, the region containing this polymorphism was increased by using 5-GCTGCCCTCCCAGAAGAGGCAGACC-3 forward and 5-GGCTCCCATGTGGATGGAGAAGGGG 3(revers) prime. A region of the gene was generated by genotyping and CXCL12 (rs1801157) polymorphism was analyzed. The focused gene region of genotyping was rs1801157 (C>T) for the CXCL12 gene. Region-specific primer and probe sets are used 'TaqMan Genotyping Assays' and fluorescence dyes of probes are given below. Allelic discrimination has been shown using the software of the 7500 Fast Real-Time PCR tool.

Forward **5'-** GCTGCCCTCCCAGAAGAGGCAGACC**3'**

Revers **5'**=GGCTCCCATGTGGATGGAGAAGGG3**5'**

3.3.3.1. Real time protocol

Real-time PCR reagents and the mixture of reaction have been recorded in Table 3.1. Total volume for each sample was determined by the protocol.

Table 3.1: The reaction mixture for the Real-Time PCR

The Material Used	Quantity
Master Mix	5 µl
TaqMan Genotyping Assay	0.5 µl
DNAse, RNAse Free water	3.5 µl
Template DNA	1 µl

The conditions for Real-Time PCR were arranged by waiting for 10 minutes at 95° C, accomplishing denaturation for 15 seconds at 92° C for each cycle and also connecting/elongation for 1 minute at 60° C for each cycle. As illustrated in Table 3.2., denaturation and connecting/elongation were completed for 40 cycles.

Table 3.2: The Real-Time PCR conditions

		40 Cycles	
	Waiting	**Denaturation**	**Connecting/Elongation**
Temperature	95° C	92° C	60° C
Duration	10 minutes	15 seconds	1 minutes

3.3.4. Statistical analysis

Student's t-test was used to analyze numeric values. The data obtained from genotyping was evaluated using Chi-square and Fisher's Exact Tests via the SPSS 25.0 Program for statistical analysis. Chi-square and Fisher's Exact Tests were used for evaluating the distributions of genotypes and alleles among groups. p-values less than 0,05 were considered statistically significant.

4. RESULTS

4.1. Statistical Evaluation of Real-Time PCR Results

Allelic discriminations were analyzed automatically by the software of the 7500 Fast-Real Time PCR instrument. The readings and interpretations of the fluorescence irradiation are performed by dyes found in the probes. However, some samples could not be discriminated.

FAM dye shows blue color, while VIC dye shows green color. ROX is a reference color for comparing FAM and VIC dyes. Allelic discrimination was analyzed by examining and interpreting the radiance curves (Figure 4.1.)

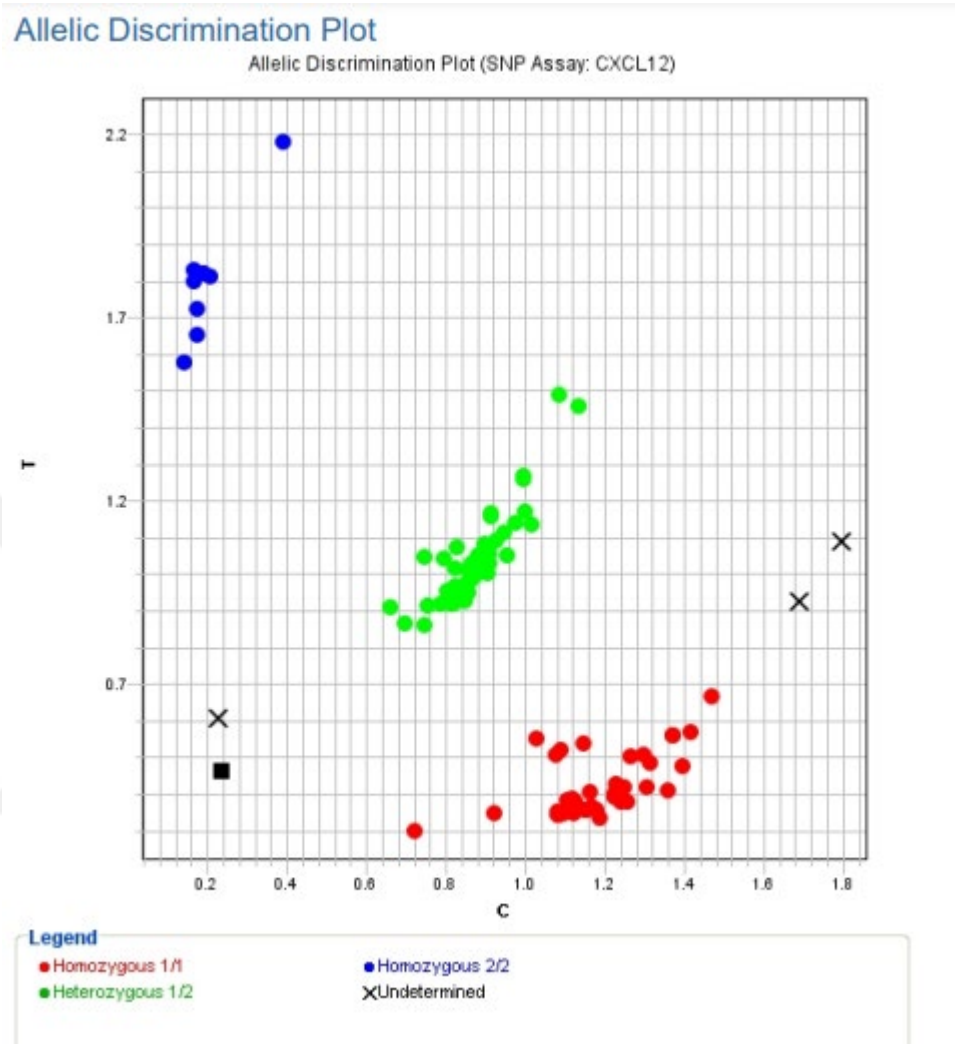

Figure 4.1: Allelic Discrimination Analysis of CXCL12 genotype

CC : Homozygote Wild Type **CT :** Heterozygote **TT:** Homozygote Mutant Type

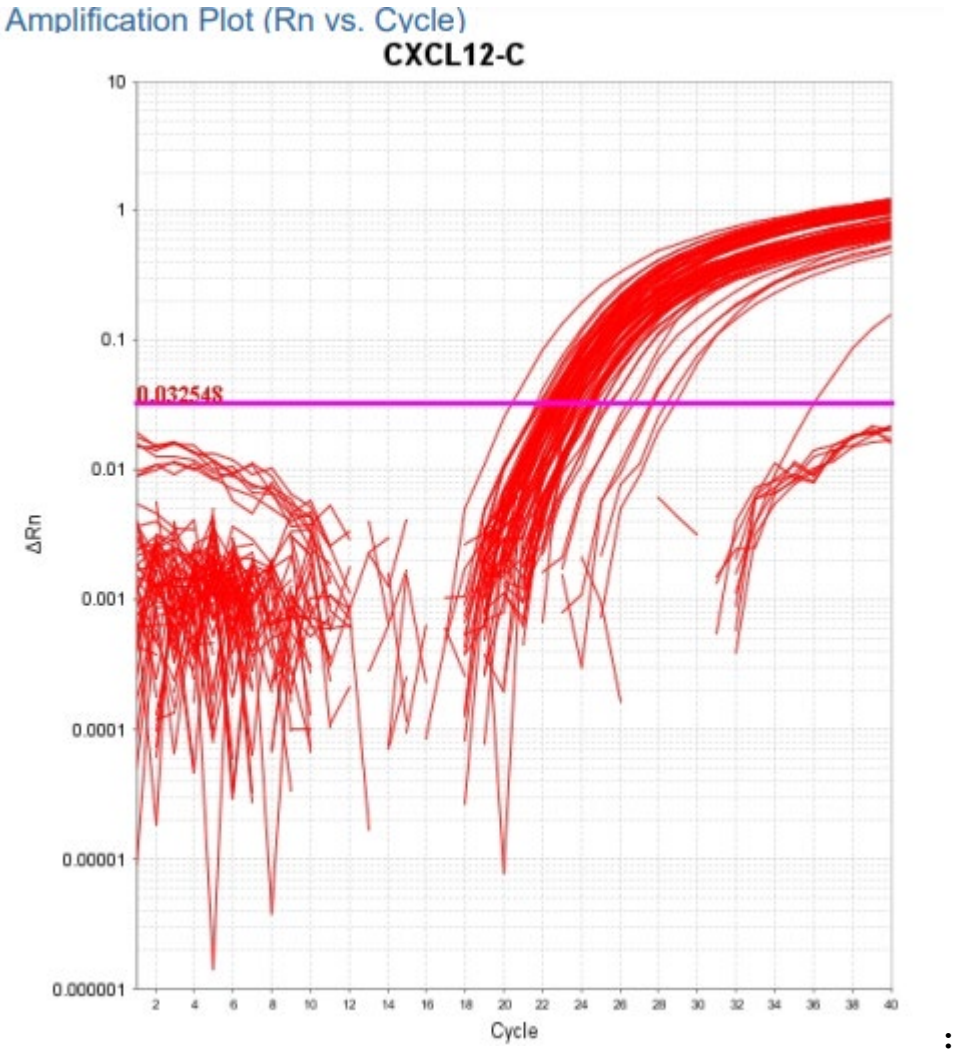

Figure 4.2: Amplification plot display of Allele C

Figure 4.2. shows amplification plots of Allele C. Threshold value (0.032548) is shown as a pink line.

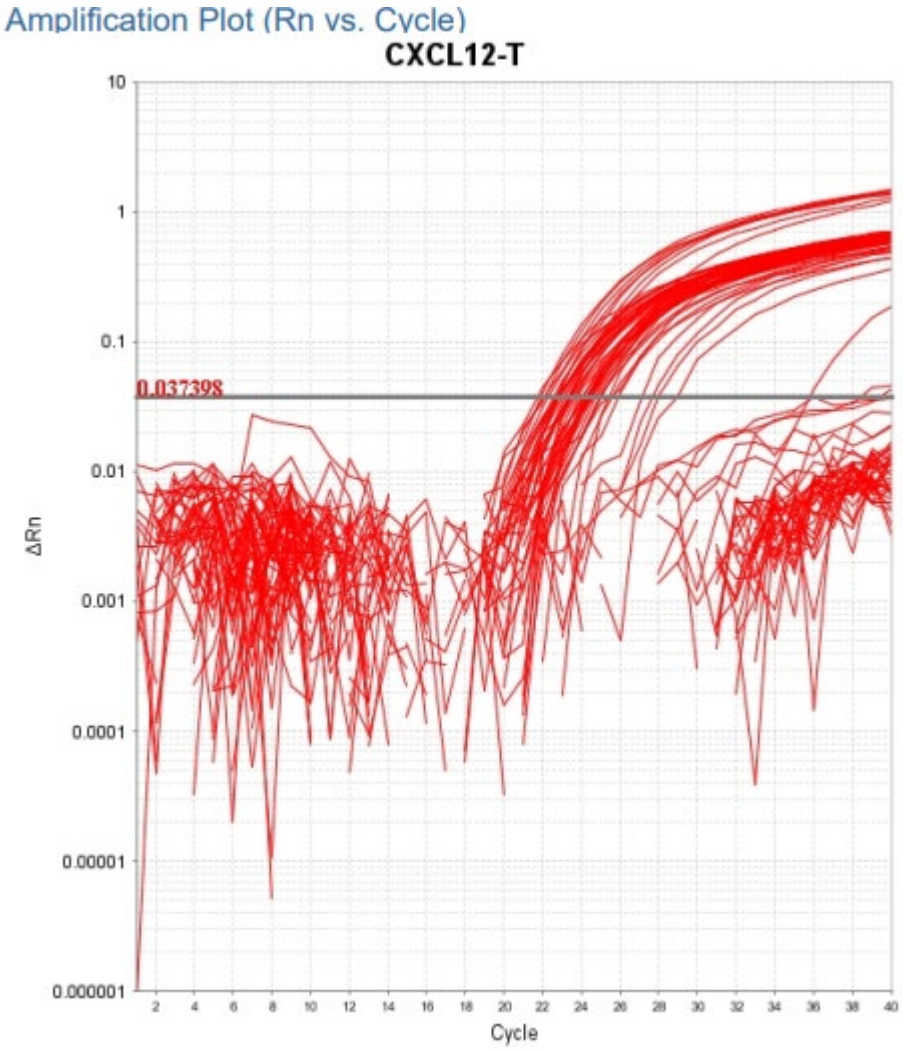

Figure 4.3: Amplification plot display of Allele T

Figure 4.3. shows amplification plots of Allele T. The fluorescent signal passes over the threshold to obtain any data. The threshold value (0.037398) is shown as a gray line.

4.2. Analysis of Genotype and Allele Related to the Patient and Control Groups

In this experiment CXCL12, rs 1801157 polymorphism in patients with ovarian cancer and healthy control groups was investigated. This study found no Significant differences in allelic and genotypic distribution between OC patients and healthy individuals (p = 0.962) were observed.

Table 4.1, shows that there is no significant relationship between the patient and control groups. The value of the homozygote mutant genotype is (P=0.811), that of the heterozygote genotype is 0.658, while the homozygote wild type TT genotype's value is 0.547.

According to our findings, homozygote wild type (CC), heterozygote (CT), and homozygote mutant type (TT) rates are calculated respectively as 44.7%, 46.8%, and 8.5% in the control group. On the other hand, homozygote wild type (CC), heterozygote, (CT) and homozygote mutant type (TT) rates are determined respectively as 42.5%, 47.5%, and 10 % in the patients' groups (Table 4.1.).

Table 4.1 shows that the value of the C allele 48.3% in the control group and (41%) in patient group was found to be higher in the control group. the value of T allele (27.8% in the control group and (24.1%) in patients' group. Accordingly, the value of both alleles is also not statistically significant distinctly among groups (p=0.922)) for C allele and (0.886) for T allele. Table 4.1

Table 4.2 .shows allelic distribution of C and T carrier and noncarrier , the values also found not statistically significant .

Table 4.1: The genotype and allelic distributions for the CXCL12 Gene between the patient and control groups

CXCL12 Genotypes	Control group n=47	Patient group n=40	p-value	Odds ratio
CC	(44.7%) n=21	(42.5%) n=17	0.838 NS	0.915
CT	(46.8%) n=22	(47.5%) n=19	0.949 NS	1.028
TT	(8.5%) n=4	(10 %) n=4	0.811 NS	1.194
Allelic distributions				
C	n=42 (48.3%)	n=36 (41.4%)	(0.922)	1.071
T	n=22 (27.8%)	n=19 (24.1%)	(0.886)	1.067

n: number of sample, S)= significantly different ($p< 0.05$), NS= non significant ($p>0.05$).

Table 4.2: The distributions of allelic C and T carrier and noncarrier for the CXCL12 Gene between the patient and control groups

Alleles distribution	Control group n=47	Patient group n=40	P-value	Odds ratio
C-carrier	n=42 (89.4%)	n=36 (46.2%)	(o.922) NS	1.071
C-noncarrier	n=5 (10.6%)	n=4 (10.0%)		
T-carrier	n=22 (51.2%)	n=19 (52.8%)	(0.886) NS	1.067
T-noncarrier	n=21 (48.8%)	n=17 (47.2%)		

4.3. Analysis of Genotypes of Patients Related to Other Variables

Tables 4.3, and 4.4 shows the distribution of stage according to genotypes. no statical significance is detected.

Table 4.3: The distributions of stage at presentation according to genotypes of CXCL12 gene polymorphism in the patient group

Genotypes	CC	CT	TT	P-value
stages				
Stage I	n=3 (37.5%)	n=4 (50.0%)	n=1 (12.5%)	(0.813) NS
Stage II	n=2 (40.0%)	n=2 (40.0%)	n=1 (20.0%)	
Stage III	n=7 (50.0%)	n=7 (50.0%)	n=0 (0.0%)	
Stage IV	n=3 (50.0%)	n=2 (33.3%)	n=1 (16.7%)	

The table 4.4 and 4.5 shows the frequency distribution of different ovarian cancer types according to genotypes. The table 4.5 shows that ovarian cancer cases are less among TT carrier patients P(0.027)

Table 4.4: The distributions of ovarian cancer types according to genotypes of CXCL12 gene polymorphism in the patient group.

Genotypes / Tumor type	CC	CT	TT
Mixed Epithelial tumors	**n=2** (50.0%)	**n=2 (50.0%)**	**n=0 (0%)**
Serous tumors	**n=7 (36.8%)**	**n=11 (57.9%)**	**n=1 (5.3%)**
Mucinous tumors	**n=2 (100%)**	**n=0 (o%)**	**n=0 (0%)**
Endometrioid tumors	**n=0 (0%)**	**n=o (0%)**	**n=1 (100%)**
Clear cell tumors	**n=1 (100%)**	**n=0 (0%)**	**n=0 (0%)**
epithelial tumors	**n=12 (44.4%)**	**n=13 (48.1%)**	**n=2 (7.4%)**
Germ cell tumors	**n=1 (100%)**	**n=0 (0%)**	**n=0 (0%)**
Sex-cord stromal tumors	**n=1 (100%)**	**n=0 (0%)**	**n=0 (0%)**

Table 4.5: The distributions of ovarian cancers types according to genotypes TT genotype carries and noncarrier of CXCL12 gene polymorphism in the patient group

TT Genotype / Cancer type	TT-carrier	TT noncarrier	P-value
Mixed Epithelial tumors	**n=0** (0%)	**n=4** (100%)	(0.027%) S
Serous tumors	**n=1** (5.3%)	**n=18** (94.7%)	
Mucinous tumors	**n=0** (0%)	**n=2** (100%)	
Endometrioid tumors	**n=1** (100%)	**n=0**	
Clear cell tumors	**n=0 (0%)**	**n=1** (100%)	
Germ cell tumors	**n=0 (0%)**	**n=1** (100%)	
Sex-cord stromal tumors	**n=0 (0%)**	**n=1** (100%)	

5. DISCUSSION AND CONCLUSION

Ovarian cancer deaths accounted for a high percentage of deaths among gynecological cancer deaths worldwide.[26] Ovarian cancer develops more commonly in older women who have a family history of hereditary cancer susceptibility disorders such as a history of breast cancer, colorectal carcinoma, and prostatic cancer. In addition, decrease the number of pregnancies, menopause, use of hormone replacement therapy, and infertility. Modified risk factors such as smoking(increase the risk of MOC). Endometriosis(increase the risk of clear cell and endometroid ovarian cancer) and high BMI are also implicated in the carcinogenesis of ovarian cancer.[27]. these factors interact with other immunogenetic factors and are involved in the activation of pathogenic molecular pathways eventually leading to the development of ovarian cancer.[28]

On the other hand, several factors are considered significant risk reduction factors, such as pregnancy, oral contraceptive usage, and breastfeeding. They found that the incidence of ovarian cancers is significantly lower among females with multiparty, they reported a 10-16% decrease in the development of ovarian cancer in each pregnancy furthermore this frequency is increased twice in females who get pregnant above 35yesrs old. breastfeeding for more than 18 months also proved to have a protective effect against ovarian cancer.in addition, it has been reported that regular usage of oral contraceptive pills reduces the risk by 30-50% [32.34].

Although the current study did not find a significant relationship between different types of ovarian cancer and some risk factors under search, because this kind of variable is supposed to be conducted on a large group of subjects to be relevant, however, it has been proved by many other studies.

based on the foregoing, health education of people about risk factors and protective factors against these diseases, especially in high-risk group females is necessary and critical to improve screening, early diagnosis, and treatment. Moreover, giving more attention to conducting studies that give more determination and clear definitions to the high-risk group will be helpful to put them under search, therefore, more knowledge will be available to

examine the early stages of these types of cancer .molecular studies are fundamental importance among all study types because they can solve the mystery of the origin and early stages during tumorigenesis of this disease, and people with high risk is considered an appropriate sample for molecular research, so accurate identification of risk factors will be beneficial, and they can aid in developing risk reduction strategy.

Ovarian cancers are a diverse group of cancers. Each type is unique at the level of risk factors, tissue of origin, mode of metastasis, and molecular and genetic basis. Also, they are variable in clinical presentation, resistance to chemotherapy, and prognosis.[31]

For instance, epithelial tumors are the most common type(90%), and clear cell carcinoma has a lower rate of response to chemotherapy. Furthermore, HGSC high-grade serous carcinoma in most cases is associated with a mutation in P53and BRCA genes, however, LGSC is found to have a mutation in KRAS and BRAF genes and is not related to P53 and BRCA mutations. Moreover, a study found that ovarian cancer patients with Lynch syndrome (HNPCC) are different from others where they are presented in early stages with epithelial invasive type.[29.30.31] knowing the molecular events and mutations causing tumors to develop and progress in individual cancer types is necessary to create a database from which effective screening, diagnostic, and treatment programs can be developed.

The heterogenicity of ovarian tumors is disparate, it is clear between ovarian cancer types, between the same type of cancer, and between cancer cells within the tumor itself referred intratumor heterogeneity(ITH). İn addition, the heterogenicity is proven to influence the response of cancer to chemotherapy such as breast cancer. the heterogenicity is obvious also at the genetic level, where there are morphologically similar tumors that are genetically heterogeneous.[34]

Another study examined tumor tissue genetically of the primary tumor and it's secondary in comparison to normal tissues and shows that besides the clear genetic heterogeneity they found that the secondary sites show genetically distinct metastatic deposits each deposit is different from others [35]

Due to the presence of these differences and disparities between the types of ovarian cancer,understanding the molecular alteration involved in various types is critical and can improve the present classifiecation, this was very helpful in some types of gynecological cancers,such as epithelial uterine cancers , which recentlty are classified based on new molecular criteria into four categories;1. POLE mutated cancer3%–10% (in which there are Somatic mutations in the DNA polymerase epsilon (POLE) gene-encoded POLE polymerase enzyme which is important to correct errors after DNA replication.2. MMR abnormal(in which there is a defect in the Mismatch repair system (MMR) 8%–19% which is responsible for maintaining genome stability. 3. p53 abnormal, 11%–24% (in which there are mutations in P53 a type of tumor suppressor gene), and 4. Non-special type NST: 58%–61% (other types except the previously mentioned types. types).[36,37.38.39]. this classification is important in treatment and prognosis. For instance, fewer deaths rate were observed in *POLE* mutated tumors.[38]

In this study, the variation and diversity between different ovarian cancer types were clear, since, in this limited sample, all types of ovarian cancer are present. And because each type has its own characteristics, so each type must be studied and treated as an incipient disease to get more accurate results that will improve the long-term overall survival. the current study suggested that the ovarian cancer frequency is less among females who have TT genotype of rs1801157 SNP of CXCL12 gene, therefore it is possible to adopt this proposition and conduct further studies to prove or deny this proposition. Moreover, the development of approved molecular classifications is vital because it will make the target points of treatment or research clearer and make the result more accurate.

Molecular diversity is obvious in ovarian cancer types, numerous genes mutation and genetic alterations are reported in ovarian cancers they are different according to the type of tumor. In general, BRCA1/2, TP53, PIK3CA, *BRAF, PTEN*, and KRAS genes are more frequent. however, *KRAS, TP53*, and *CDKN2A* gene mutations are more commonly associated with Ovarian carcinoma*, ARID1A, PTEN, CTNNB1*, and *PI3KCA* gene mutations are frequent with Endometrioid Ovarian Cancers, and *KRAS, TP53*, and *CDKN2A* gene mutations are characteristic of Mucinous Ovarian Carcinomas. [40.41]

The presence of these polygenic mutations and consequential regulation of intracellular signaling pathways such as the PIK3/Akt pathway and MAPK pathway will eventually lead to the development of ovarian cancer by induction of cell migration. enhance tumor growth angiogenesis as well as metastasis. [42]

CXCL12 is a chemokine that binds with CXCR4, or CXCR7 receptors, and contributes to a normal cellular function where their expression increases during inflammation, wound healing, and viral infection. However, CXL12 has a role in tumor progression via CXCL/CXCR4 axis activation which is reported in breast, colon, ovarian, pancreatic, melanoma, prostate, lung, and gastric cancers.

CXCL12 gene is documented to be overexpressed in many cancer types including ovarian cancer and CXCL12 protein is found in high concentration in the ovarian cancer cells, furthermore, several studies proved the association of SNP rs1801157 in this gene with the development of other cancer types .for instance, a study conducted among Pakistani population and reported an association between SNP rs1801157 and breast cancer (BC) [17] furthermore another study suggests that CXCL12 SNP rs1801157 may have an important role in the pathogenesis of non-Hodgkin lymphoma.,[24]Moreover, the study confirmed that the presence of SNP rs 1801157 in Asian people increases their susceptibility to lung and urological cancers. [43] therefore it is logical to expect that variation in this gene may also associate with the risk development of ovarian cancer patients.

Thus, SNP rs 1801157 in CXCL12 genes was investigated for ovarian cancer patients and the control group, using real-time PCR. We focused on the genotypic and allelic distribution of this SNP,(C/T) Finally, we concluded that no association between SNP rs1801157 in CXCL12 and ovarian cancer among Turkish patients under study. Because of limitations, further studies are recommended to examine this hypothesis in a large homogenous group of patients.

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7. APPENDECIES

APPENDIX-1: ROW DATA

CXCL12 rs1801157 * patient control group

Crosstab

			hasta_kontrol_grubu		
			control	over ca	Total
rs1801157	CC	Count	21	17	38
		% within **rs1801157**_rs	55.3%	44.7%	100.0%
		% within patient control	44.7%	42.5%	43.7%
		% of Total	24.1%	19.5%	43.7%
	CT	Count	22	19	41
		% within **rs1801157**	53.7%	46.3%	100.0%
		% within patient control	46.8%	47.5%	47.1%
		% of Total	25.3%	21.8%	47.1%
	TT	Count	4	4	8
		% within **rs1801157**	50.0%	50.0%	100.0%
		% within hasta_kontrol_grubu	8.5%	10.0%	9.2%
		% of Total	4.6%	4.6%	9.2%
Total		Count	47	40	87
		% within **rs1801157**_rs	54.0%	46.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.078[a]	2	.962
Likelihood Ratio	.078	2	.962
Linear-by-Linear Association	.070	1	.791
McNemar-Bowker Test	.	.	.[b]
N of Valid Cases	87		

CC * hasta_kontrol_grubu

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
CC	NO	Count	26	23	49
		% within CC	53.1%	46.9%	100.0%
		% within hasta_kontrol_grubu	55.3%	57.5%	56.3%
		% of Total	29.9%	26.4%	56.3%
	YES	Count	21	17	38
		% within CC	55.3%	44.7%	100.0%
		% within hasta_kontrol_grubu	44.7%	42.5%	43.7%
		% of Total	24.1%	19.5%	43.7%
Total		Count	47	40	87
		% within CC	54.0%	46.0%	100.0%
		% within hasta_kontrol_grubu	100.0%	100.0%	100.0%
		% of Total	54.0%	46.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.042[a]	1	.838		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.042	1	.838		
Fisher's Exact Test				1.000	.505
Linear-by-Linear Association	.041	1	.839		
McNemar Test				.880[c]	
N of Valid Cases	87				

CT * hasta_kontrol_grubu

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
CT	NO	Count	25	21	46
		% within CT	54.3%	45.7%	100.0%
		% within hasta_kontrol_grubu	53.2%	52.5%	52.9%
		% of Total	28.7%	24.1%	52.9%
	YES	Count	22	19	41
		% within CT	53.7%	46.3%	100.0%
		% within hasta_kontrol_grubu	46.8%	47.5%	47.1%
		% of Total	25.3%	21.8%	47.1%
Total		Count	47	40	87
		% within CT	54.0%	46.0%	100.0%
		% within hasta_kontrol_grubu	100.0%	100.0%	100.0%
		% of Total	54.0%	46.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.004[a]	1	.949		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.004	1	.949		
Fisher's Exact Test				1.000	.560
Linear-by-Linear Association	.004	1	.949		
McNemar Test				1.000[c]	
N of Valid Cases	87				

TT * hasta_kontrol_grubu

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
TT	NO	Count	43	36	79
		% within TT	54.4%	45.6%	100.0%
		% within hasta_kontrol_grubu	91.5%	90.0%	90.8%
		% of Total	49.4%	41.4%	90.8%
	YES	Count	4	4	8
		% within TT	50.0%	50.0%	100.0%
		% within hasta_kontrol_grubu	8.5%	10.0%	9.2%
		% of Total	4.6%	4.6%	9.2%
Total		Count	47	40	87
		% within TT	54.0%	46.0%	100.0%
		% within hasta_kontrol_grubu	100.0%	100.0%	100.0%
		% of Total	54.0%	46.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.057[a]	1	.811		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.057	1	.811		
Fisher's Exact Test				1.000	.549
Linear-by-Linear Association	.057	1	.812		
McNemar Test				.000[c]	
N of Valid Cases	87				

C * hasta_kontrol_grubu

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
C	NO	Count	5	4	9
		% within C	55.6%	44.4%	100.0%
		% within hasta_kontrol_grubu	10.6%	10.0%	10.3%
		% of Total	5.7%	4.6%	10.3%
	YES	Count	42	36	78
		% within C	53.8%	46.2%	100.0%
		% within hasta_kontrol_grubu	89.4%	90.0%	89.7%
		% of Total	48.3%	41.4%	89.7%
Total		Count	47	40	87
		% within C	54.0%	46.0%	100.0%
		% within hasta_kontrol_grubu	100.0%	100.0%	100.0%
		% of Total	54.0%	46.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.009[a]	1	.922		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.010	1	.922		
Fisher's Exact Test				1.000	.603
Linear-by-Linear Association	.009	1	.923		
McNemar Test				.000[c]	
N of Valid Cases	87				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 4.14.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	.010	.107	.096
Ordinal by Ordinal	Spearman Correlation	.010	.107	.096
Measure of Agreement	Kappa	.006	.061	.097
N of Valid Cases		87		

Symmetric Measures

		Approximate Significance
Interval by Interval	Pearson's R	.924[c]
Ordinal by Ordinal	Spearman Correlation	.924[c]
Measure of Agreement	Kappa	.922
N of Valid Cases		

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for C (NO / YES)	1.071	.267	4.293
For cohort hasta_kontrol_grubu = kontrol	1.032	.555	1.917
For cohort hasta_kontrol_grubu = over ca	.963	.446	2.077
N of Valid Cases	87		

T * hasta_kontrol_grubu

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
T	NO	Count	21	17	38
		% within T	55.3%	44.7%	100.0%
		% within hasta_kontrol_grubu	48.8%	47.2%	48.1%
		% of Total	26.6%	21.5%	48.1%
	YES	Count	22	19	41
		% within T	53.7%	46.3%	100.0%
		% within hasta_kontrol_grubu	51.2%	52.8%	51.9%
		% of Total	27.8%	24.1%	51.9%
Total		Count	43	36	79
		% within T	54.4%	45.6%	100.0%
		% within hasta_kontrol_grubu	100.0%	100.0%	100.0%
		% of Total	54.4%	45.6%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.020[a]	1	.886		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.020	1	.886		
Fisher's Exact Test				1.000	.533
Linear-by-Linear Association	.020	1	.887		
McNemar Test				.522[c]	
N of Valid Cases	79				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 17.32.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	.016	.112	.141
Ordinal by Ordinal	Spearman Correlation	.016	.112	.141
Measure of Agreement	Kappa	.016	.112	.143
N of Valid Cases		79		

Symmetric Measures

		Approximate Significance
Interval by Interval	Pearson's R	.888[c]
Ordinal by Ordinal	Spearman Correlation	.888[c]
Measure of Agreement	Kappa	.886
N of Valid Cases		

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for T (NO / YES)	1.067	.440	2.589
For cohort hasta_kontrol_grubu = kontrol	1.030	.688	1.542
For cohort hasta_kontrol_grubu = over ca	.965	.596	1.565
N of Valid Cases	79		

Crosstabs

Notes

Output Created		01-JUN-2022 17:01:52
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\OVER CANCER YAĞMUR SPSS (1)_1.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	40
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.

Syntax		CROSSTABS /TABLES=CXCL12_rs BY değişenmenapoz menopoz stage hücre_tipi hücretip_geniş metastaz nüks adjuvant_keMoterapi neoadjuvant_kemoterapi surgery gebeliksayısı doğumsayısı gebelikdurumu doğumyapmadurumu bmıgruplar yaşaralığı evreleme /FORMAT=AVALUE TABLES /STATISTICS=CHISQ CORR KAPPA RISK MCNEMAR /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.03
	Dimensions Requested	2
	Cells Available	524245

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CXCL12_rs * değişenmenapoz	36	90.0%	4	10.0%	40	100.0%
CXCL12rs * menopoz	35	87.5%	5	12.5%	40	100.0%
CXCL12_rs * evre	33	82.5%	7	17.5%	40	100.0%
CXCL12rs * hücre_tipi	29	72.5%	11	27.5%	40	100.0%
CXCL12rs * hücretip_geniş	29	72.5%	11	27.5%	40	100.0%
_ CXCL12rs * metastaz	34	85.0%	6	15.0%	40	100.0%

_ CXCL12rs * nüks	34	85.0%	6	15.0%	40	100.0%
CXCL12rs * adjuvant_keMoterapi	34	85.0%	6	15.0%	40	100.0%
CXCL12_rs * neoadjuvant_kemoterapi	34	85.0%	6	15.0%	40	100.0%
CXCL12_rs * surgery	31	77.5%	9	22.5%	40	100.0%
CXCL12rs * gebeliksayısı	35	87.5%	5	12.5%	40	100.0%
CXCL12_rs * doğumsayısı	35	87.5%	5	12.5%	40	100.0%
CXCL12_rs * gebelik durumu	35	87.5%	5	12.5%	40	100.0%
CXCL12_rs * doğum durumu	35	87.5%	5	12.5%	40	100.0%
CXCL12_rs * bmı grupları	30	75.0%	10	25.0%	40	100.0%
CXCL12_rs * yaşaralığı	35	87.5%	5	12.5%	40	100.0%
CXCL12_rs * evreleme	33	82.5%	7	17.5%	40	100.0%