

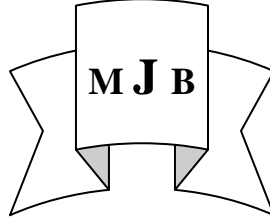
## Cancer Susceptibility and Caspase 8 Gene Variet in Local Iraqi Population

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### Abstract

**Background:** Breast cancer is the most common cancer in women .The incidence of breast cancer varies greatly around the world. Apoptosis occurs naturally at several stages of breast development. The gene caspase-8 (CASP8) plays a key role in the initiation of apoptosis. Suppression of apoptosis is one of the major mechanisms underlying the origin and progression of cancer. One variant in the CASP8 gene , D302H (rs1045485), has been confirmed to be associated with a reduced breast cancer risk.

**Aim:** to illustrate the role of the CASP8 gene variant with respect to breast cancer susceptibility. According to our knowledge, there is no previous association study concerning CASP8 gene variant (D302H) and breast cancer has been conducted in Iraq. So the frequency of this gene variant and its clinical impact on breast cancer was studied for the first time among Babylonian population.

**Materials and methods:** A genetic analysis was performed as single nucleotide polymorphism (SNP) to tag the D302H variant in four groups of Babylonian populations, including patients with breast cancer , healthy control people , first degree relatives, and patients with benign breast tumors.

**Results:** CASP8, D303H variant was shown to have a high degree of correlation with healthy control subjects in the present study in comparison with breast cancer patients. The presence of CASP8, D302H polymorphism not only diminishes the high risk of breast cancer, but also delays the onset of breast cancer until late age.

**Conclusions:** The CASP8 D302H polymorphism decreases the breast cancer risk and delays the onset of the disease.

### الخلاصة

يعتبر سرطان الثدي من أكثر الأمراض السرطانية شيوعاً. يلعب جين الكاسباز (CASP8) دوراً مهماً في الكثير من مراحل نمو الثدي، تثبيط عمل هذا الجين قد يؤدي إلى الإصابة بسرطان الثدي، بينما يعتبر المتغير الجيني D302H لجين الكاسباز عاملاً وقائياً ضد سرطان الثدي. الهدف من الدراسة هو الكشف عن الدور الذي يلعبه المتغير الجيني بالنسبة لقابلية الإصابة بسرطان الثدي.

تمت دراسة المتغير الجيني لجين الكاسباز عن طريق التحليل الوراثي لأربعة مجاميع شملت المرضى المصابين بسرطان الثدي، أقرباء من الدرجة الأولى، ناس أصحاء و مرضى مصابين بأورام حميدة في الثدي.

نتائج الدراسة أظهرت علاقة عكسية بين سرطان الثدي ووجود المتغير الجيني، كذلك أظهرت النتائج إن وجود هذا المتغير الجيني لدى النساء يؤخر ظهور السرطان.

### Introduction

Breast cancer is the most common malignancy affecting women worldwide [1]. The majority of breast and ovarian cancers are sporadic or not inherited [2]. Mutation in some genes such as the breast cancer susceptibility genes

*BRCA1* and *BRCA2* predispose to familial risk of breast cancer, much of the remaining variations in genetic risk is likely to be explained by the cumulative effect of multiple variations in genes that individually confer relatively small amount of risks [3].

Genetic instability is an essential feature in the development of all cancers, including those of the breast [4]. The effect of genetic alterations in a typical case of breast cancer are complex due to the high number of changes (both genetic and nongenetic) of normal host cells [5]. The growth of cancer is caused by an imbalance between the rates at which cancer cells are produced through cell division and the rate at which they die through a natural cell death process known as "apoptosis" [6].

Apoptosis is a genetically regulated form of cell death that plays an important role in eliminating infected, damaged, cancerous and other unwanted cells from the body [7]. The programmed cell death, apoptosis, is a physiological mechanism that eliminates damaged cells from an organism, thus controlling cell numbers and tissue size, and sustaining homeostasis [8]. Apoptosis results from a cascade of protease reactions carried out by caspases (aspartate-specific cysteine proteases). The caspases represent a group of structurally similar proteins that cleave other proteins, so called "proteases." These cell death proteases are the ultimate executioners responsible for apoptosis [6].

The final stage of apoptosis is caused by a type of molecule within cells, which are called "caspases". Mammalian caspases comprise a group of at least fourteen members. Each caspase may play a unique role [9]. Caspase 8 (*CASP8*) is a key regulator of apoptosis or programmed cell death, an essential defense mechanism against hyperproliferation and malignancy [10].

Variants are small changes that occur in a gene sequence. Mutations in genes causing common variations that contribute only small amounts to breast

cancer risk. Single nucleotide polymorphisms (SNPs) are the most common type of gene variants in which a single unit of DNA may vary from one person to the next. A SNP in *CASP8*, called D302H, is associated with a reduction in breast cancer risk. The biology of how variation in this gene may protect against the disease is that the protein produced by the *CASP8* gene participates in programmed cell death, or apoptosis, a defense mechanism that allows cells to commit suicide rather than develop into a tumor. DNA damage can trigger apoptosis, and one hypothesis is that *CASP8* SNP may enhance the body's ability to clear cancerous cells from the body and thereby lower the risk of breast cancer [3]. The variant D302H is the only confirmed common variant that has associated with a reduction in breast cancer risk thus far [5].

Up to our knowledge, genetic studies on *CASP8* gene variant, D302H, and its association with breast cancer risk have not been conducted in Iraqi population and the spectrum of this genetic polymorphism is not known. In this study, the frequency of *CASP8* gene variant, D302H, in a group of Iraqi population was determined, and the clinical phenotypes resulting from *CASP8* gene variant carriage were also clarified.

## **Patients and Methods**

### **Study population:**

The primary set of subjects were drawn from those attending the breast cancer center in Hilla General Teaching Hospital. Four groups were recruited ; 20 breast cancer patients, 10 healthy controls, 5 benign breast tumor patients and 5 first degree relatives to a known case of breast cancer.

Both of the breast cancer and the benign breast tumor patients were clinically, radiologically and

histopathologically confirmed as having breast cancer and benign breast tumor, respectively, while healthy control and first degree relatives to the breast cancer patients were clinically and radiologically confirmed as having no breast problems. All cases and controls were of Iraqi origin resident in Hilla city, and its peripheries. All subjects were screened for the presence of the polymorphism, *CASP8* D302H variant.

#### **DNA extraction:**

The screening of *CASP8* D302H variant and the study of polymorphisms were carried out on genomic DNA. DNA was extracted from blood samples using the conventional methodologies supplied by the Promega company ([www.promega.com](http://www.promega.com)), DNA was extracted from fresh whole blood collected in EDTA, heparin and citrate anticoagulant tubes. The extracted DNA was stored in 2-8°C.

#### **SNP selection:**

SNP was selected to tag the D302H variant in the *CASP8* gene, the primer sequence & PCR conditioning was supplied by Pittnam, *et.al.* 2008, [10]. The *CASP8* D302H polymorphism was detected by PCR amplification of a 138 bp region on exon 13.

#### **Genotyping:**

All cases & controls were successfully genotyped regarding the *CASP8* gene variant using a conventional PCR technique, the primer sequence and

PCR conditioning was supplied by Pittnam, *et.al.* (2008), amplicons were identified on agarose gel electrophoresis.

#### **Results**

In the present study a case-control study design was applied, composing of four groups; breast cancer patients, benign breast tumor patients, first degree relatives and healthy control people. All groups were successfully genotyped regarding the *CASP8* gene variant D302H. Figure (1) shows the PCR amplification results of the *CASP8* gene variant, D302H. The frequency of *CASP8* gene variant is represented in table (1).

*CASP8*, D303H variant was shown to have a high degree of correlation with healthy control subjects in the present study in comparison with breast cancer patients. The *CASP8* D302H polymorphism was highly associated with the absence of cancer (Table1). In fact, this polymorphism was harbored by 40% of healthy unaffected individual versus only 15% of the breast cancer patients, and 20% in both the benign breast tumor patients and the first degree relative groups. The high degree of association of *CASP8* D302H variant with the healthy control, and to lower extent with other non cancerous groups in the study may explain the "protective" nature of this allele.

**Table 1** Frequency of *CASP8* gene variant (D302H)

Phenotype (n.)	positive identification of <i>CASP8</i> gene variant (%)
BC patients (20)	3 (15%)
Control (10)	4 (40%)
First degree relatives (5)	1 (20%)
Benign tumor patients (5)	1 (20%)

The effect of the D303H variant carriage on age of onset of breast cancer is illustrated in table (2). The possible effect of the presence of *CASP8* D302H polymorphism in females with the age at diagnosis of breast cancer was analyzed. The

analysis showed that the *CASP8* D302H polymorphism was significantly associated with the late onset of breast cancer in the gene variant carriers in comparison with the patients negative for this polymorphism.

**Table 2** Age at onset of breast cancer in *CASP8* gene variant (D302H) carrier patients

Carrier status	Mean age at onset	Age range (year)
Non carrier (17)	52	28-76
Carrier (3)	63	54-72

The median age of onset of breast cancer in patients who do not have the polymorphism of *CASP8* gene variant was near 51 years, the lowest reported age was 28 years, which was different from the polymorphism carrier patients who have a median age of onset of about 62 years with a lowest reported age 54 years (Table-2).

**Discussion**

The growth and proliferation of normal cells are controlled by a complex interplay of several pathways, including programmed cell death. Two main types of programmed cell death occur, with both types ultimately activating caspases, of which the most important one is caspase-8 (*CASP8*) [11]. *CASP8* is highly polymorphic and has at least 500 variants. These polymorphisms may contribute to the inter-individual difference in susceptibility to malignancies [12].

This work emphasizes the critical importance of genetic correlational studies on breast cancer, especially in the view of its increasing frequency in Iraq. The genotyping data in the present study based on SNP analysis of the polymorphism of *CASP8* gene, D302H , provide a strong evidence that

this variant is associated with a reduced risk of breast cancer and support that the presence of *CASP8* D302H polymorphism decreases the breast cancer risk of *BRCA1/BRCA2* mutation carriers (OR = 0.40; 95% CI: 0.22–0.75, *P*=0.004) .

The results in the present work regarding the frequency of *CASP8* D302H variant agreed with other study which reported that these susceptibility alleles are common in population & high percentage of persons are carriers of this "protective" genotype [7].

Polymorphisms underlying polygenic susceptibility to breast cancer are considered low penetrance, a term often applied to sequence variants associated with a minimal to moderate risk. This is in contrast to "high-penetrance" variants or alleles that are typically associated with more severe phenotypes, for example those *BRCA1/BRCA2* mutations leading to an autosomal dominant inheritance patterns in a family. These types of sequence variants (also called low-penetrance genes, alleles, mutations, and polymorphisms such as *CASP8*, D302H variant) are relatively common in the population, their contribution to total cancer risk is estimated to be

much higher than the attributable risk in the population from mutations in *BRCA1* and *BRCA2* [13].

Observations about the frequency of D302H variant showed that it is not uncommon and is markedly different between the healthy control group and other groups in the study, this result came in parallel with that of Sigurdson and his colleagues, who found a similar positive correlation between the frequency of *CASP8* gene variant and the healthy people demonstrating conclusively that some of the variation in breast cancer risk is due to common alleles polymorphism[11].

The findings presented in this study regarding the effect of *CASP8* gene variant on the breast cancer susceptibility came in agreement with that of MacPherson and his colleagues who illustrated a hypothesis that apoptosis, is perturbed in many cancers, while the coding polymorphism of caspase-8 gene (*CASP8*), might act as low-penetrance breast cancer gene [14]. Single-nucleotide polymorphism (SNP) of this gene was genotyped in a series of breast cancer case patients and control subjects from U.K. in two independent studies. The reproducible, dose-dependent association of *CASP8* D302H with breast cancer indicates the potential importance of inherited variation in the apoptosis pathway in breast cancer susceptibility.

There is a remarkable differences in the distribution of caspase 8 gene polymorphisms between various ethnicities, however, it is noteworthy to conduct extensive investigations about the distribution of these genes in different ethnic groups. Genotype and allele distribution of *CASP8* D302H observed in this study was in contrast with that results obtained by George and Mittal,(2010) who measured the

*CASP8* gene D302H allele frequency in Indian population, the difference between the Iraqis' and Indian populations was explained by the same authors, as they highlight the fact that the ethnic background may influence the susceptibility to certain diseases [15].

The variation in our Babylonian population from other world population signifies the impact of ethnicity. Due to marked differences in the distribution of caspase gene polymorphisms between various ethnicities, the data from "normal healthy" for each population is of special interest for the adequate evaluation of the relevance of the investigated genetic markers in susceptibility of diseases.

Genetic polymorphisms in the caspase 8 gene may influence cancer risk by altering expression levels and functions of other important genes. In recent years, genetic variants in caspase 8 and their role in human breast cancer susceptibility have been getting more and more attention, especially the apoptosis initiator caspase 8 [15].

The "protective" role for *CASP8* gene variant in patients with breast cancer is reflected in the late age of onset of breast cancer in those patients. The presence of *CASP8* D302H polymorphism decreases the breast cancer risk of *BRCA1/BRCA2* mutation carriers and delaying the age of onset of breast cancer. The presence of the polymorphism could attenuate the high risk conferred by *BRCA1* and *BRCA2* mutations in predisposed individuals, making possible that some of them escape from suffering breast cancer [1].

The result regarding the effect of the D303H variant carriage on age of onset of breast cancer patients were supported by a similar study conducted

in Spain in which researchers proposed a fact that *CASP8* gene variant polymorphism delays the age of onset of breast cancer [1]. Their analysis showed that the *CASP8* D302H polymorphism was significantly associated with the late onset of breast cancer in comparison with the patients negative for this polymorphism. The patients with *CASP8* D302H polymorphism had a median age of onset of breast cancer in lower values than those subjects negatives for the polymorphism. Furthermore, the *CASP8* D302H positive subjects showed a high probability of being free of breast cancer by old age versus a low probability of the *CASP8* negative ones.

Obviously, the presence of *CASP8*, D302H polymorphism not only diminishes the high risk of breast cancer, but also delays the onset of breast cancer until late age, making possible that some of carriers could escape from being breast cancer patients along their lifespan.

### Conclusions

In conclusion, the *CASP8* D302H polymorphism decreases the breast cancer risk and delays the age of onset of the disease. The presence of the polymorphism could decrease the high risk conferred by *BRCA1* and *BRCA2* mutations in predisposed individuals, making possible that some of them escape from being patients with breast cancer throughout their life.

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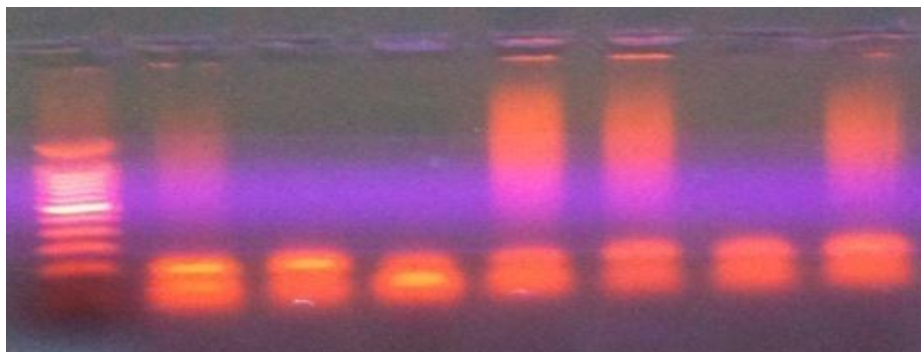
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Lane 1 2 3 4 5 6 7 8  
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**Figure1** Agarose gel electrophoresis of *CASP8* gene variant (D302H) amplicones. Lane 1 from the left is 100bp DNA ladder , lane 2,3,5,6,7,8 positive amplicones.