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# Cyclin D1 gene polymorphism in Egyptian breast cancer women

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**ABSTRACT:**

**Background:**

Cyclin D1, a key regulator of G1 to S phase progression of the cell cycle, is strongly established as an oncogene with an important pathogenetic role in many human tumors; therefore any genetic variations that disturb the normal function of this gene product is ultimately a target for association with cancer risk and survival. Cyclin D1 silent mutation (G870A) in the splicing region of exon-4 enhances alternative splicing, resulting two CCND1 mRNA transcripts variant [a] and [b], in which transcript b has a longer half-life. It has been deduced that G870A polymorphism of the CCND1 gene may play a role in tumorigenesis. The aim of our study was to investigate the influence of CCND1 genotypes on the genetic susceptibility to breast cancer in Egyptian population.

**Patients and Methods:**

80 newly diagnosed females representing Egyptian population confirmed breast cancer patients and 40 healthy controls were included in the study. Single nucleotide polymorphism (SNP) in CCND1 (G870A) was determined in these samples by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP).

**Results:**

The frequencies of AG, AA genotypes between patients group and the healthy control group have shown a significant difference at (p=0,009). Subjects less than 45 years of age with AA genotype were at decreased risk (odds ratio 0.438, 95% confidence interval 0.251-0.763) and postmenopausal subjects with AA genotype were at increased risk of developing breast cancer (odds ratio 5.056, 95% confidence interval 1.239-20.626). We found that breast cancer females carrying A allele had longer DFS than did patients with GG genotype (p=0,001).

**Conclusion:**

This study provides the first indication that CCND1 870A alleles (AA/AG genotypes) are risk factors for breast cancer susceptibility in Egyptian women. Thus analysis of CCND1 G870A polymorphism may be useful for identifying females with higher risk to develop breast cancer.

**Keywords:**
Breast Cancer, Cyclin D1, Polymorphism, Egypt

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**INTRODUCTION**:

Breast cancer has become the leading cause of cancer death for females in Egypt. It represents 31% of all cancers diagnosed and 15% of all cancer death and the incidence is increasing worldwide (Coral and Amy, 2010). Molecular biological studies have clearly indicated that genetic alteration play significant role in the development of breast carcinoma in some cases and they addressed by better understanding of what genetic/ epigenetic events are likely to be associated with the earliest phases of the disease (Sadikovic *et al.,* 2008). Cyclin D1 protein (35-KDa) is established as an oncogene, gene considered as one of the human D-type cyclin genes which encoded by the 5 exons and mapped to chromosome bands 11q13 (Haber and Harlow, 1997). Cyclin D1 proto oncogene acts as a growth sensor target of proliferative signals in G1, by regulating the cell cycle progression from G1-to- S phase transition in different cell type from various tissues (Donnellan and Chetty, 1998; Baldin *et al.,*1993). Cyclin D1 active complexes that phosphorylate and inactivate the retinoblastoma tumor suppressor protein (RB), are formed by the binding of cyclin D1 to its dependent kinases 4 and 6 (CDK4/6). Hyperphosphorylation of RB in early G1 phase allows to bind active RB to E2F transcription factors and stimulates the cell cycle entry into S phase (Sherr, 1993; Alao *et al.,*2006). Several studies have demonstrated that cyclin D1 can also act as a transcriptional co-factor for steroid hormone receptors e.g., estrogen receptor (Neuman *et al.,*1997; Tashiro *et al.,*2007). CCND1 overexpression occurs in a number of cancers including breast cancer, conversely repression of CCND1 gene expression is a hallmark of cell differentiation (Gillett *et al.,*1996; James *et al.,* 2006). Moreover, Robert and Elizabeth (Sutherland and Musgrove, 2002) reported that the cyclin d1 gene is amplified in up to 20% of breast cancer patients and overexpression occurs in more than 50% of mammary tumors, and this appears to be an early event in the breast cancer (Buckley *et al.,*1993). On the other hand it is also demonstrated by a correlation between CCND1 overexpression and cellular metastasis (Drobnjak *et al.,* 2000). Silent polymorphism (G870A, pro241pro) occurs in cyclin D1 coding gene, this commonly available SNP, affects the exon 4/intron 4 splice donor site and leads to two different variants of the cyclin D1 mRNA (Betticher *et al.,*1995). Diverse studies demonstrated that variant transcript (a) has carried all exons whereas variant (b) lack exon 5 including a PEST domain, which was hypothesized to acts as a degradation motif. It has been shown that variant transcript b lead to a longer half- life of cyclin D1 (Betticher *et al.,*1995; Sawa *et al.,*1998). Furthermore, cyclin D1 transcript (b) was appear to be weakly catalyst of RB phosphorylation / inactivation and significantly enhanced cell transformation activity compared to cyclin D1 transcript (a) (Solomon *et al.,* 2003). It has been proved that the cyclin D1 isoform (cyclin D1b) is an unclear oncogene which is generated via CCND1 mRNA alternative splicing and involved in tumorigenesis through promoting the transition between G1 and S phases (Sawa *et al.,*1998; Solomon *et al.,* 2003; Lu *et al.,* 2003). Numerous studies have been examined on the correlation between cyclin D1 polymorphism and risk of breast cancer, but those studies yielded conflicting results (Grieu *et al.,* 2003; Ceschi *et al.,* 2005; Yu *et al.,*2008; Forsti *et al.,* 2004; Krippl *et al.,* 2003; Wang *et al.,* 2002). The aim of our study was to investigate the influence of CCND1 genotypes on the genetic susceptibility to breast cancer in the Egyptian population.

**MATERIALS AND METHODS:**

All patients (n=80) who had experienced primary invasive breast carcinoma, with median age 52.0 (range 32.0-77.0) years, at the Experimental and Clinical Surgery and Cancer Management and Research Departments, Medical Research Institute, Alexandria University From 2008 to 2012, were enrolled in this

study. The samples were collected before surgery or any chemotherapeutic treatment. Blood samples were taken from patients who had pathological diagnosis and had not undergone blood transfusion or receiving immunomodulatory agent. The non tumor control group (n=40), with median age 49.50 (range 36.0-71.0) years, was composed of healthy women volunteers clinically free from any chronic disease. Questionnaires, medical records, and pathological reports were used to confirm the diagnosis and cancer status. This study protocol was approved by the Local Ethical Committee at Alexandria University.

**CCND1 genotyping**

5-mL blood samples were obtained from cases and controls. The samples were collected in tubes containing EDTA and genomic DNA was purified from peripheral whole blood using a ready- for use DNA extraction kit (QIA amp DNA Blood mini kit, Qiagen, Hilden, Germany). Genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) (Enayat, 2002; Onay *et al.,* 2008), using semi quantitatively conventional polymerase chain reaction (PCR) kits (Qiagen, Germany) according to producer's instructions.

For amplifying CCND1 gene we used the following primers, Forward primer:5´- GTTTTCCCAGTCACGAC -3´;Reverse primer: 5´ GGGACATCACCCTCACTTAC -3´_; The CCND1 G870A polymorphism specific primers were ordered from QIAGEN system (QIAGEN, Germany) to amplify a 167-bp fragment of CCND1 gene at exon 4/intron 4. The PCR reactions were performed on a thermal cycler (Biometra- TProfessional Thermocycler -Germany) and the cycling program was programmed according to the manufacturer's protocol. Specifically, these reactions were carried out in a total volume 50 µl of QIAGEN Multiplex PCR Master Mix 25 µl, primer mix (2 µl taken from each 20µM primer working solution) 4 µl and Template DNA 21 µl.

Each PCR started within the initial heat-activation program to activate Hot Star Tag DNA polymerase (95°C for 15 min), followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 90 sec, and extension at 72°C for 90 sec, with a final extension step at 72°C for 10 minutes. For RFLP analyses, each PCR product was subjected to ScrF1 restriction enzyme (New England, BioLabs Inc, UK). According to the manufacture's protocol, 1 unit of restriction enzyme digests 1 µg of substrate DNA in a 50 µl reaction in 60 minutes. Agarose gel electrophoresis was used as the appropriate detection system. This gave a satisfactory signal with our PCR product. The DNA fragments were separated using 2% agarose gel containing ethidium bromide and the bands on the gel were visualized by using UV Transilluminator.

The allele types were determined, GG genotype showed two fragments (145 and 22bp), AG genotype showed three fragments (167, 145, and 22 bp) and AA genotype showed single fragment (167-bp).

**Statistical Analysis**

Predictive Analytics Software (PASW Statistics 18) for Windows (SPSS Inc, Chicago, USA) was used for statistical analysis. Chi-square test and Firsher's Exact test (When more than 20% of the cells have expected count less than five) were used for testing Association between categorical variables. Quantitative data were described using median, minimum and maximum as well as mean and standard deviation. Parametric and non-parametric tests were applied for analyzed normal data and abnormally distributed data, respectively. Odd ratio (OR) and 95% Confidence Interval (CI) were used. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

**RESULTS**

The clinical profile of breast cancer patients included in the current study presented in table (1). The

**Table 1: Characteristics of normal healthy controls and breast cancer patients**

| Clinical characteristics | Normal subjects (n = 40) | | Breast cancer patients n = 80) | | Test of significance (*P*- value) |
|---|---|---|---|---|---|
| | No | % | No | % | |
| **Age (years)** | | | | | |
| < 45 | 15 | 37.5 | 11 | 13.8 | $X^2$ test ($P$ = 0.454) |
| ≥ 45 | 25 | 62.5 | 69 | 86.3 | |
| Range | 36.00 –71.00 | | 32.00 – 77.00 | | |
| Mean ± SD | 50.15 ± 9.43 | | 52.62 ± 10.07 | | *Student T test* ($P$ = 0.198) |
| Median | 49.50 | | 52.0 | | |
| **Menopausal status** | | | | | |
| Premenopausal | 20 | 50.0 | 37 | 46.3 | $X^2$test $X^2P$ = 0.698 |
| Postmenopausal | 20 | 50.0 | 43 | 53.8 | |

$x^2p$: p value for Chi square test *: Statistically significant at $p < 0.05$

frequencies of GG, AG and AA genotypes were 37.5%, 20% and 42.5% respectively, in healthy controls and16.3%, 28.8% 55.0% respectively, in patients group. The statistical analyses of these results revealed that, in comparison with that in control group CCND1 (G870A) AG and AA genotypes frequencies in breast cancer patients were insignificantly higher, whereas CCND1 (G870A) GG genotype frequency was significantly lower (p= 0.009).Our results revealed that, frequencies of the three genotypes GG, AG and AA between patients and controls were significantly different (p =0.034, table 2).

Table 3 shows the results of the CCND1 genotype effects on breast cancer risk. AA, AG were at increased risk for developing breast cancer compared with the GG genotype [OR= 2.986, 95%CI (1.178-7.569); p= 0.019 and OR= 3.317, 95% CI (1.110-9.915); p= 0.029, respectively]. In addition AA also had a higher risk in postmenopausal women [OR=5.056, 95% CI (1.239-20.626); p= 0.019] than premenopausal ones [OR= 1.870, 95% CI (0.530-6.603); p= 0.328], table (3a), and had reduced risk in younger women [<45 y/o, OR=0.438, 95% CI (0.251-0.763); p= 0.046] than elder ones[≥ 45 y/o, OR= 2.423, 95% CI (0.804-7.300); p= 0.111], table (3b). Association of different CCND1 G870A polymorphic variants among breast cancer patients with pathological features were shown in table (4). There was no significant differences with (p=0.688)

**Table 2: Frequencies of CCND1 G870A genotype in breast cancer patients and controls**

| | Normal healthy controls (n=40) | | Breast cancer patients (n = 80 ) | | *p* |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| **Polymorphic variants** | | | | | |
| GG | 15 | 37.5 | 13 | 16.33 | 0.009* |
| AG | 8 | 20.0 | 23 | 28.80 | 0.302 |
| AA | 17 | 42.5 | 44 | 55.00 | 0.197 |
| *p* | 0.034* | | | | |

p: p value for Chi-square test *: Statistically significant at $p \leq 0.05$

**Table (3): Association of *CCND1* G870A polymorphism with breast cancer risk**

| | Healthy control group (n=40) | | Breast cancer patients (n=80) | | Test of sig | OR ( 95% CI) (lower– upper) |
|---|---|---|---|---|---|---|
| | No | % | No | % | | |
| **All participants** | | | | | | |
| **GG®** | 15 | 37.5 | 13 | 16.33 | | 1.000 (reference) |
| **AG** | 8 | 20.0 | 23 | 28.80 | P = 0.029* | 3.317 (1.110-9.915) |
| **AA** | 17 | 42.5 | 44 | 55.00 | P = 0.019* | 2.986 (1.178-7.569) |
| **AA+ AG** | 25 | 62.5 | 67 | 83.80 | P = 0.009* | 3.092 (1.291-7.405) |

*p*: p value for Chi-square tes FEp : p value for Fisher Exact test
*: Statistically significant at $p \leq 0.05$

in the CCND1 genotypes distribution between stage T3 and T4 tumors. Breast cancer patients carrying the CCND1 A allele had a 1.04-fold increased risk for lymph node metastasis but this was not statistically significant (p=1.000). The CCND1 genotypes were furthermore not associated with vascular invasion in carrier A allele patients was higher when compared with G allele carriers and this difference was statistically insignificant (p=0.717). In addition breast cancer patients carrying A allele (AA/AG genotypes) were at reduced risk of metastasis [OR= 0.247, 95%CI (0.072-0.848); p= 0.020] when compared with those carrying GG genotype. Kaplen Meir disease free survival (DFS) curve was constructed to study the prognostic value of CCND1 G870A genotypes. The median fallow up period 25 months (range 18-48 months) in which 22(27.5%) out of 80 patients had metastasis. The incidence of metastasis was observed in 53.9% of patients with GG genotype and 46.2% of patients carrying A allele (AA / AG genotypes) (table 5). Survival curve of the different

**Table (3a): Association of *CCND1* G870A polymorphism with breast cancer risk**

| | Healthy control group (n=15) | | Breast cancer patients (n=11) | | Test of sig | OR ( 95% CI) (lower– upper) |
|---|---|---|---|---|---|---|
| | No | % | No | % | | |
| **Women ages <45 years** | | | | | | |
| **GG®** | 6 | 40.0 | 0 | 00.0 | | 1.000 (reference) |
| **AG** | 2 | 13.3 | 2 | 18.2 | FEp = 0.133 | 0.500 (0.188-1.332) |
| **AA** | 7 | 46.7 | 9 | 81.8 | FEp = 0.046* | 0.438 (0.251-0.763) |
| **AA+ AG** | 9 | 60.0 | 11 | 100.0 | FEp= 0.024* | 0.450 (0.277-0.731) |
| | **Healthy control group (n=25)** | | **Breast cancer patients(n=69)** | | **Test of sig** | **OR ( 95% CI) (lower– upper)** |
| | **No** | **%** | **No** | **%** | | |
| **Women ages ≥ 45 years** | | | | | | |
| **GG®** | 9 | 36.0 | 13 | 18.8 | | 1.000 (reference) |
| **AG** | 6 | 24.0 | 21 | 30.4 | p = 0.158 | 2.423 (0.699-8.400) |
| **AA** | 10 | 40.0 | 25 | 50.7 | p = 0.111 | 2.423 (0.804-7.300) |
| **AA+ AG** | 16 | 64.0 | 56 | 81.2 | P = 0.083 | 2.423 (0.878-6.689) |

*p*: p value for Chi-square tes FEp : p value for Fisher Exact test
*: Statistically significant at $p \leq 0.05$

**Table (3b): Association of *CCND1* G870A polymorphism with breast cancer risk**

| | Healthy control group (n=21) | | Breast cancer patients(n=34) | | Test of sig | OR ( 95% CI) (lower– upper) |
|---|---|---|---|---|---|---|
| | No | % | No | % | | |
| **Premenopausal status** | | | | | | |
| **GG®** | 8 | 83.1 | 7 | 20.6 | | 1.000 (reference) |
| **AG** | 2 | 9.5 | 9 | 26.5 | FEp = 0.109 | 5.143 (0.819-32.302) |
| **AA** | 11 | 52.4 | 18 | 52.9 | p = 0.328 | 1.870 (0.530-6.603) |
| **AA+ AG** | 13 | 61.9 | 27 | 79.4 | P = 0.157 | 2.374 (0.707-7.969) |
| | **Healthy control group (n=19)** | | **Breast cancer patients (n=46)** | | **Test of sig** | **OR ( 95% CI) (lower– upper)** |
| | **No** | **%** | **No** | **%** | | |
| **Postmenopausal status** | | | | | | |
| **GG®** | 7 | 36.8 | 6 | 13.0 | | 1.000 (reference) |
| **AG** | 6 | 31.6 | 14 | 30.4 | p = 0.171 | 2.722 (0.638-11.610) |
| **AA** | 6 | 31.6 | 26 | 56.5 | p = 0.019* | 5.056 (1.239-20.626) |
| **AA+ AG** | 12 | 63.2 | 40 | 87.0 | P = 0.029* | 3.889 (1.095-13.806) |

*p*: p value for Chi-square tes FEp : p value for Fisher Exact test
*: Statistically significant at $p \leq 0.05$

genotypes are shown in Fig. 1. A significant association between the genotypes and survival was found in the patients ($p < 0.001$). Furthermore, patients with GG genotype had a worse prognosis and short survival (24.0±1.13 months) than patients carrying A allele (AA / AG genotypes) (41.92±1.20 months).

**DISCUSSION:**

Cyclin D1 (CCND1) is considered as one of the proteins that acts within a regulatory circuit that dominate cell cycle G1 to S-phase transition (Diehl, 2002). Moreover, it is proved that cyclin D1 acts as a dual function in promoting cell proliferation and inhibiting drug- induced apoptosis; these finding are attributed to the presence of a chemoresistance during overexpression (Biliran *et al.,* 2005). In a normal breast, cyclin D1 protein plays uncompensated roles in mammary gland development during different growth cycles, whereas, enhanced oncogenic transformation and tumorigenesis, of the CCND1 gene may be a primary and early step in breast cancer formation (Fu *et al.,* 2004). It is found that 45-50% of human breast carcinoma types are over expressed by the oncogenic CCND1 mRNA (Sutherland and Musgrove, 2002).

Possible correlations between CCND1 gene polymorphism and breast cancer susceptibility were studied in different population and produced inconsistent results. In the present study, we noticed that CCND1 AA, AG and AA/AG genotype frequencies were more frequently observed in cases, whereas GG genotype frequency was significantly higher in controls. Furthermore, genotype distribution between patient group and controls are markedly different, suggesting that CCND1 G870A polymorphism is associated to breast cancer susceptibility. These observations were in concordance with previous findings suggesting that CCND1 genotype is associated with the breast cancer risk (Yu *et al.,* 2008; Forsti *et al.,* 2004). Multiple and specialized studies were conducted to evaluate the CCND1 polymorphic variants and breast cancer patients from different ethnic groups. Yu *et al.,* (2008) conducted a study in China and found that cyclin D1 G870A polymorphism lead a potential contribution to breast cancer with superiority occurrence of breast cancer in young women.

In the present series, Lu *et al.,* (2009) conducted a Meta analysis on the association between CCND1 G870A polymorphism and breast cancer susceptibility,

**Table (4): Association of *CCND1* G870A polymorphism with clinicopathological features of breast cancer**

| | AA+AG | | GG® | | Test of sig | OR ( 95% CI) (lower– upper) |
|---|---|---|---|---|---|---|
| | No | % | No | % | | |
| **Tumor pathological grade** | | | | | | |
| II ® | 56 | 83.6 | 12 | 92.3 | FEp =0.679 | 2.357 (0.277-20.033) |
| III | 11 | 16.4 | 1 | 7.7 | | |
| **Clinical stage** | | | | | | |
| II ® | 35 | 52.2 | 6 | 46.2 | p = 0.688 | 0.784 (0.238-2.579) |
| III | 32 | 47.8 | 7 | 53.8 | | |
| **Tumor size (cm)** | | | | | | |
| < 5® | 35 | 52.2 | 5 | 38.5 | p = 0.363 | 0.571 (0.169-1.928) |
| ≥ 5 | 32 | 47.8 | 8 | 61.5 | | |
| **Lymph node involvements** | | | | | | |
| -ve® | 15 | 22.4 | 3 | 23.1 | FEp= 1.000 | 1.040 (0.253-4.270) |
| +ve | 52 | 77.6 | 10 | 76.9 | | |
| **Estrogen receptor status** | | | | | | |
| -ve® | 3 | 4.5 | 1 | 7.7 | FEp= 0.515 | 1.778 (0.170-18.560) |
| +ve | 64 | 95.5 | 12 | 92.3 | | |
| **Progesterone receptor status** | | | | | | |
| -ve® | 6 | 9.0 | 2 | 15.4 | FEp=0.610 | 1.848 (0.330-10.367) |
| +ve | 61 | 91.0 | 11 | 84.6 | | |
| **Her2/neu expression** | | | | | | |
| -ve® | 59 | 88.1 | 10 | 76.9 | FEp= 0.374 | 0.452 (0.102-1.999) |
| +ve | 8 | 11.9 | 3 | 23.1 | | |
| **Vascular invasion** | | | | | | |
| -ve® | 13 | 19.4 | 3 | 23.1 | FEp= 0.717 | 1.246 (0.300-5.182) |
| +ve | 54 | 80.6 | 10 | 76.9 | | |
| **Metastasis** | | | | | | |
| -ve® | 52 | 77.6 | 6 | 46.2 | p = 0.020* | 0.247 (0.072-0.848) |
| +ve | 15 | 22.4 | 7 | 53.8 | | |

*p*: p value for Chi-square test FEp : p value for Fisher Exact test *: Statistically significant at p ≤ 0.05

he observed that the Caucasian population which increased breast cancer susceptibility were carrying a variant 870 A allele, however, it is not observed in the Asians. The study reviewed that genetic and environmental factors might also contribute to the ethnic difference. In contrast, some studies reported that there was no association between CCND1 polymorphic variants and susceptibility to breast cancer (Grieu *et al.,* 2003; Krippl *et al.,* 2003; Shu *et al.,* 2005).

In the present study, We found that individuals carrying A allele of CCND1 G870A polymorphism (AA, AG, AA/AG) had a 2.9, 3.3 and 3.1 fold increased risk for the development of breast cancer compared with those carrying GG genotype (P=0.019, P=0.029, P=0.009) respectively. These finding could be interpreted in view of Betticher *et al.,* (1995) who indicated that the alternative splicing and production of altered transcript b occurs in individuals those carrying

**Table (5): Association of CCND1 G870A genotypes with breast cancer disease free survival (DFS)**

| | Metastasis N =22 | Non Metastasis N = 58 | Median (Mean ± SE) DFS (months) | Log rank | *p* |
|---|---|---|---|---|---|
| GG (N= 13) | 7 (53.9%) | 6 (46.2%) | 24.0 (23.14 ± 1.30) | 26.617* | <0.001 |
| AG/AA (N=67) | 15 (22.4%) | 52 (77.6%) | 44.0 (41.92 ± 1.20) | | |

*: Statistically significant at p<0.05

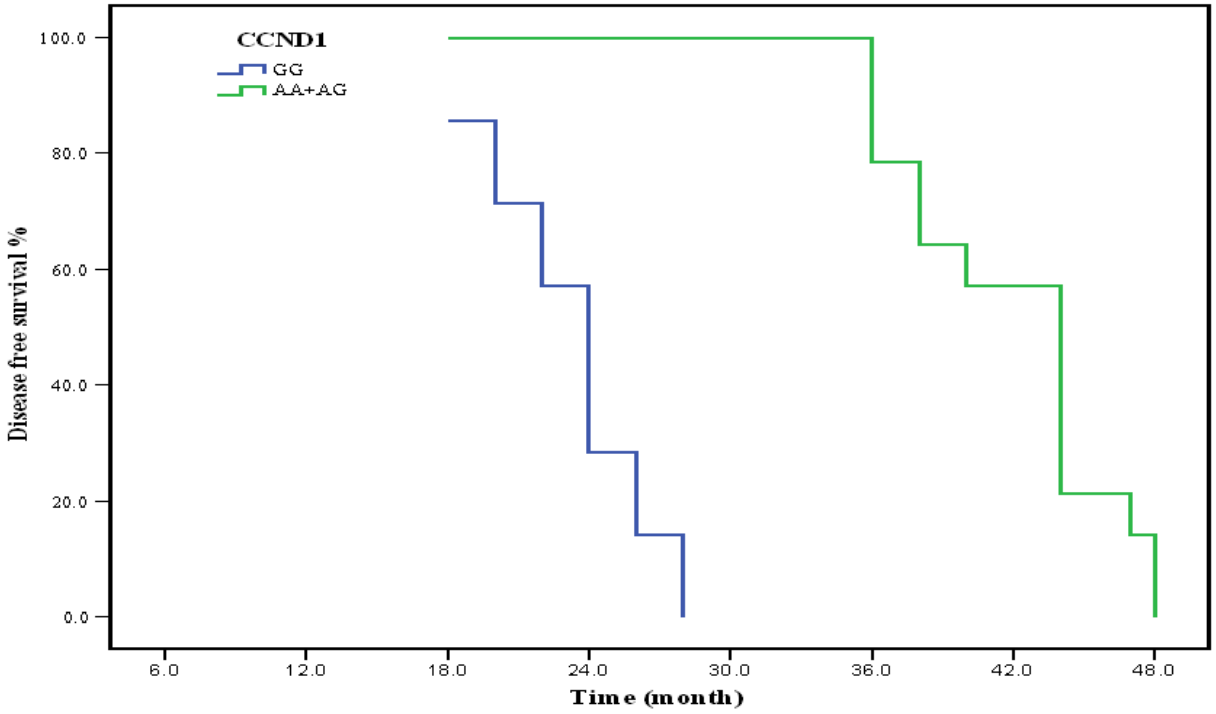


**Figure 1: Kaplan-Meier disease free survival for *CCND1* G870A genotypes**

the homozygosity for CCND1 A allele that may have longer half-life. Therefore cells will damaged DNA carrying A allele of CCND1 G870A polymorphism may bypass G1/S check point easily compared to GG genotype. Also the study of Sawa *et al.,* (1998) shown that inhibition to the entry of the S phase in the cell cycle is occurred within high level of normal transcript a occurrence. All these observations lead to proved that different polymorphic CCND1 variants affect the biological behavior of the cells, thus altering the risk of developing breast cancer.

Moreover, our results revealed that breast cancer female patients < 45 years of age carrying AA or combined variant AA/AG genotypes were at decreased risk of breast cancer than those with GG genotype. These finding are confirmed with the report of Shu *et al.,* (2005) who stated that the A allele of the CCND1 G870A polymorphism was only weakly associated with the risk of breast cancer among women ages < 45 years. These results lead us to predict that variant 870A allele may play a role in increasing estrogen metabolism and inhibiting cell proliferation (Sutherland and Musgrove, 2002). On the other hand postmenopausal females carrying AA or combined variant (AA/AG genotypes) were at increased risk for breast cancer when compared with those carrying GG genotype. These findings agreed with the report of Grieu *et al.,* (2003) who stated that A allele of CCND1 G870A polymorphism might play a more important role in the development of breast cancer among postmenopausal females.

Furthermore, we evaluated the association of CCND1 G870A polymorphism with clinicopathological

features of breast cancer patients. We did not find any significant association of carrying the A allele with tumor pathological grade III, clinical stage III, tumor size ≥ 5, axillary lymph node involvement, +ve hormone receptors status, +ve Her2/neu expression or vascular invasion. These results may be attributed to the small sample size which limited our ability to detect a significant difference.

The correlation between CCND1 (A870G) polymorphism and cancer progression produced different results. It is found that, carrying of 870A allele in patients with advanced preinvasive neoplasia of the larynx and/or oral cavity was positively correlated with CCND1 expression and poor disease prognosis (Izzo *et al.,* 2003).

Also in non-small cell lung cancer the A allele of CCND1 (G870A) polymorphism had a more favorable disease free-survival and showed positive association with increasing risk of local relapse (Betticher *et al.,*1995). In contrast to results, a study on ovarian cancer revealed that CCND1 polymorphic variants were not associated with the overall survival. On the other hand there was a positive correlation between 870A allele and early disease occurrence (Dhar *et al.,* 1999). Also the results of the study including 339 patients in CCND1 G870A polymorphism with breast cancer survival appear to be a null association with breast cancer prognosis (Grieu *et al.,* 2003). These different results on CCND1 genotype and cancer prognosis may be attributable to the cancer features in the study, preparation design and treatment systems. Notably after a median 25 months of fallow up, only 27.5% of our patients had metastasis of their breast cancer, suggesting that 72.5% of those patients are doing well in the short term with improvement in therapy. In the present study we found that carrying the A allele of CCND1 G870A polymorphism was related to a longer disease free survival for breast cancer than patients with GG genotype ($p < 0.001$). The favorable DFS for breast cancer patients carrying the A allele of CCND1 G870A despite its positive association with increased risk of breast cancer could be attributed to the induction of cyclin D1 degradation by chemotherapy, causing cell death and apoptosis (Zhou *et al.,* 2001).

In conclusion, this study provides the first indication that CCND1 870A allele (AA/AG genotypes) is risk factors for breast cancer susceptibility in Egyptian women. Thus analysis of CCND1 G870A polymorphism may be useful for identifying females with higher risk to develop cancer. As compared with CCND1 870A allele and, CCND1 GG genotypes were significantly associated with shorter disease free survival in breast cancer patients. Therefore analysis of these genes may also be useful in identifying the breast cancer patients that have a high risk of relapse and most likely to be benefit from the adjuvant chemotherapy.

## REFERENCES

**Alao JP, Gamble SC, Stavropoulou AV, Pomeranz KM, Lam EW, Coombes RC, Vigushin DM. 2006.** The cyclin D1 proto-oncogene is sequestered in the cytoplasm of mammalian cancer cell lines. Mol Cancer 5: 7.

**Baldin V, Lukas J, Marcote MJ, Pagano M, Draetta G. 1993.** Cyclin D1 is a nuclear protein required for cell cycle progression in G1. Genes Dev. 7(5): 812-21.

**Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, Heighway J.1995.** Alternate splicing produces a novel cyclin D1 transcript. Oncogene 11: 1005-11.

**Biliran H Jr, Wang Y, Banerjee S, Xu H, Heng H, Thakur A, Bollig A, Sarkar FH, Liao JD. 2005.** Overexpression of cyclin D1 promotes tumor cell growth and confers resistance to cisplatin-mediated apoptosis in an elastase-myc transgene-expressing pancreatic tumor cell line. Clin Cancer Res., 11(16):6075-86.

**Buckley MF, Sweeney KJE, Hamilton JA, Sini RL, Manning DL, Nicholson RI, deFazio A, Watts CKW, Musgrove EA, Sutherland RL .1993**. Expression and amplification of cyclin genes in human breast cancer. Oncogene 8:2127-2133.

**Ceschi M, Sun CL, Van Den Berg D, Koh WP, Yu MC, Probst-Hensch N. 2005.** The effect of cyclin D1 (CCND1) G870A-polymorphism on breast cancer risk is modified by oxidative stress among Chinese women in Singapore. Carcinogenesis 26: 1457-64.

**Coral O and Amy T. 2010.** The Differences between Male and Female Breast Cancer: In Principles of gender-specific medicine (2th ed.). Marianne J L (eds). Elsevier Inc (pub) 42(7): 459-69.

**Dhar KK, Branigan K, Howells REJ Musgrove C, Jones PW, Strange RC, Fryer AA, Redman CWE, Hoban PR. 1999.** Prognostic significance of cyclin D1 gene (CCND1) polymorphism in epithelial ovarian cancer. Int J Gynecol Cancer. 9(4):342 –347.

**Diehl JA. 2002.** Cycling to cancer with cyclin D1. Cancer Biol Ther., 1(3):226-31.

**Donnellan R and Chetty R. 1998.** Cyclin D1 and human neoplasia. Mol Pathol., 51: 1-7.

**Drobnjak M, Osman I, Scher HI, Fazzari M, Cordon-Cardo C. 2000.** Overexpression of cyclin D1 is associated with metastatic prostate cancer to bone. Clin Cancer Res., 6:1891-5.

**Enayat MS. 2002.** Restriction fragment length polymorphism. In: Methods of in molecular biology, PCR mutation detection protocols. Theophilus B D M, Rapley R (eds). Human press Inc (pub), 5: 39-35.

**Försti A, Angelini S, Festa F, Sanyal S, Zhang Z, Grzybowska E, Pamula J, Pekala W, Zientek H, Hemminki K, Kumar R.2004.** Single nucleotide polymorphisms in breast cancer. Oncol Rep., 1:917-22.

**Fu M, Wang C, Li Z, Sakamaki T, Pestell RG. 2004.** Minireview: Cyclin D1: normal and abnormal functions. Endocrinology 145(12):5439-47.

**Gillett C, Smith P, Gregory W, Richards M, Millis R, Peters G, Barnes D. 1996**. Cyclin D1 and prognosis in human breast cancer. Int J Cancer. 69(2):92-9.

**Grieu F, Malaney S, Ward R, Joseph D, Iacopetta B. 2003**. Lack of association between CCND1 G870A polymorphism and the risk of breast and colorectal cancers. Anticancer Res 23: 4257-9.

**Haber D, Harlow E. 1997.** Tumour-suppressor genes: Evolving definitions in the genomic age. Nature Genetics 16: 320-322.

**Izzo JG, Papadimitrakopoulou VA, Liu DD, den Hollander PL, Babenko IM, Keck J, El-Naggar AK, Dong M. Shin, Jack Lee J, Waun K. Hong and Walter N. Hittelman. 2003.** Cyclin D1 genotype, response to biochemoprevention, and progression rate to upper aerodigestive tract cancer. J Natl Cancer Inst 95 (3):198 – 205.

**James CG, Woods A, Underhill TM, Beier F. 2006.** The transcription factor ATF3 is upregulated during chondrocyte differentiation and represses cyclin D1 and A gene transcription. 7:30.

**Krippl P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, Paulweber B, Weitzer W, Leithner A, Samonigg H. 2003.** The 870G>A polymorphism of the cyclin D1 gene is not associated with breast cancer. Breast Cancer Res Treat 82: 165-8.

**Lu C, Dong J, Ma H, Jin G, Hu Z, Peng Y, Guo X, Wang X, Shen H. 2009.** CCND1 G870A polymorphism contributes to breast cancer susceptibility: a meta-analysis. Breast Cancer Res Treat 116: 571-5.

**Lu F, Gladden AB, Diehl JA . 2003.** An alternatively spliced cyclin D1 isoform, cyclin D1b, is a nuclear oncogene. Cancer Res., 63: 7056-61.

**Neuman E, Ladha MH, Lin N, Upton TM, Miller SJ, DiRenzo J, Pestell RG, Hinds PW, Dowdy SF, Brown M, Ewen ME. 1997.** Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4. Mol Cell Biol., 9: 5338-47.

**Onay UV, Aaltonen K, Briollais L, Knight JA, Pabalan N, Kilpivaara O, Andrulis IL, Blomqvist C, Nevanlinna H, Ozcelik H. 2008.** Combined effect of CCND1 and COMT polymorphisms and increased breast cancer risk. BMC Cancer 8: 6.

**Sadikovic B, Al-Romaih K, Squire JA and Zielenska M. 2008**. Cause and Consequences of Genetic and Epigenetic Alterations in Human Cancer. Current Genomics 9(6): 394 - 408.

**Sawa H, Ohshima TA, Ukita H, Murakami H, Chiba Y, Kamada H, Hara M, Saito I. 1998.** Alternatively spliced forms of Cyclin D1 modulate entry into the cell cycle in an inverse manner. Oncogene 16: 1701-12.

**Sherr CJ. 1993.** Mammalian G1 cyclins. Cell.73: 1059-1065.

**Shu XO, Moore DB, Cai Q, Cheng J, Wen W, Pierce L, Cai H, Gao YT, Zheng Wl. 2005.** Association of cyclin D1 genotype with breast cancer risk and survival. Cancer Epidemiol Biomarkers Prev., 1(14): 91-7.

**Solomon DA, Wang Y, Fox SR, Lambeck TC, Giesting S, Lan Z, Senderowicz AM, Conti CJ, Knudsen ES. 2003**. Cyclin D1 splice variants: Differential effects on localization, RB phosphorylation, and cellular transformation. J Biol Chem., 278: 30339-30347.

**Sutherland RL, Musgrove EA. 2002.** Cyclin D1 and mammary carcinoma: new insights from transgenic mouse models. Breast Cancer Res 4(1):14-7. Epub 2001 Nov 30.

**Tashiro E, Tsuchiya A, Imoto. 2007.** Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression. Cancer Sci., 98:629-35.

**Wang L, Habuchi T, Takahashi T, Mitsu K, Kamoto T, Kakehi Y, Kakinuma H, Sato K, Nakamura A, Ogawa O and Kato T. 2002.** Cyclin D1 gene polymorphism is associated with an increased risk of urinary bladder cancer. Carcinogenesis 23(2): 257-264.

**Yu CP, Yu JC, Sun CA, Tzao C, Ho JY, Yen AM. 2008**. Tumor susceptibility and prognosis of breast cancer associated with the G870A polymorphism of CCND1. Breast Cancer Res Treat. 107: 95-102.

**Zhou Q, Hopp T, Fuqua SA, Steeg PS. 2001.** Cyclin D1 in breast premalignancy and early breast cancer: implications for prevention and treatment. Cancer Lett., 162(1):3 – 17.