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Review of: Association of cyclin D1 genotype with breast cancer risk and survival

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Abstract of the original article
Cyclin D1 (CCND1) is a key cell cycle regulatory protein that governs cell cycle progression from the G1 to S phase. A common polymorphism (A870G) in exon 4 of the CCND1 gene produces an alternate transcript (transcript-b) that preferentially encodes a protein with enhanced cell transformation activity and possible prolonged half-life. We evaluated the association of CCND1 A870G polymorphism with breast cancer risk and survival in 1130 breast cancer cases and 1196 controls who participated in the Shanghai Breast Cancer Study. Approximately 81% of cases and 79% of controls carried the A allele at A870G of the CCND1 gene [odds ratio, 1.1; 95% confidence interval (95% CI), 0.9–1.4]. A slightly stronger but non-significant association was found for the A allele among younger women (odds ratio, 1.3; 95% CI, 0.9–1.8). However, this polymorphism seems to modify the effect of hormonal exposures on postmenopausal breast cancer, as the positive associations of postmenopausal breast cancer with body mass index (*P* for interaction = 0.02) and waist-to-hip ratios (*P* for interaction < 0.03; all *P*s are two sided) were only observed among women who carry the A allele at A870G of the CCND1 gene. Follow up with this cohort of patients for an average of 4.84 years, we found that the CCND1 A870G polymorphism was inversely associated with overall and disease-free survival, particularly among women with late stage or estrogen/progesterone receptor-negative breast cancer. The adjusted hazard ratios for disease-free survival associated with GA and AA genotypes were 0.94 (95% CI, 0.49–1.82) and 0.41 (95% CI, 0.19–0.91) for tumor-node-metastasis stage III to IV breast cancer, and 0.35 (95% CI, 0.15–0.80) and 0.32 (95% CI, 0.13–0.79) for estrogen/progesterone receptor-negative breast cancer. This study suggests that CCND1 A870G polymorphism may modify the postmenopausal breast cancer risk associated with hormonal exposure and predict survival after breast cancer diagnosis.

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Review

Cyclin D1 (CCND1) belongs to a family of key regulatory proteins that govern the G1 to S phase progression of the cell cycle [1]. It is encoded by the *CCND1* gene located at band q13 on chromosome 11, a region commonly amplified in epithelial cancers,

including breast cancer [2]. Overexpression of CCND1 occurs in approximately 50% of primary breast cancer tissue, appearing to be an early event in disease progression [3]. Attempts to associate overexpression of CCND1 with clinicopathological features including prognosis/outcome and/or oestrogen/progesterone receptor status have proved controversial (reviewed in ref. [4]).

The *CCND1* gene, consisting of five exons, encodes a 295 amino acid protein (isoform a). A common single nucleotide polymorphism (SNP) (rs603965) located at the last nucleotide of exon 4 at codon 241 (Pro241 G>A), affects the intron 4 slicing mechanism at the exon 4 donor site resulting in an alternatively spliced transcript which in turn encodes for a truncated 273 amino acid protein with 33 alternate C-terminal amino acids (isoform b) [5]. Thus isoform b lacks exon 5 and the C-terminal PEST-rich region, implicated in destabilising CCND1, and therefore predicted to have a longer half-life. A number of studies have investigated the role of this functional polymorphic marker on cancer risk and/or outcome, including breast cancer [6–8], with inconsistent findings. In the current study, Shu *et al*. evaluated the association of the *CCND1* Pro241 G>A polymorphism (refereed to as the A870G polymorphism) with breast cancer risk and survival in a case–control study based in Shanghai, China.

Current literature on the association of this *CCND1* SNP and breast cancer risk/outcome has been limited to four studies as summarised in Table 1. The two Caucasian-based studies found no association with the presence of the A allele and breast cancer risk [6,7] or with tumour characteristics/patient survival [6]. Neither study included data on known modifiable risk factors, such as reproductive history or hormone replacement therapy. A more recent study (2005) of Chinese women from Singapore, presented an overall reduced risk for breast cancer in women carrying the heterozygous GA genotype, while the AA genotype did not influence breast cancer risk, thus implicating molecular heterosis (heterozygosity is associated with an effect where no association is associated with homozygosity of either allele) [8]. The protective effect of the GA genotype was accentuated when restricting for advanced stage disease and was further restricted by situations of elevated oxidative stress. Although this study did stratify for known breast cancer risk factors, including markers of oxidative stress, its major limitation was the relatively low number of cases studied.

Shu and co-authors, in the largest breast cancer population-based case–control association study reported to date, showed a weak association between the presence of the A allele and an overall risk of breast cancer, in particular among women aged <45 years. This association was driven predominantly by the presence of the GA genotype. Although there was also a strong indication that this polymorphism may modify the association of sex hormone exposure with postmenopausal breast cancer (in particular

Table 1. Summary of *CCND1* Pro241 G>A breast cancer case–control association studies in order of publication.

Study	Case–control	Characteristics	Genotyping	Overall association
Grieu *et al*. (2003) [6]	Caucasian (Australian) 339 cases, 327 controls	Hospital-based study, breast cancer risk factors were not considered, correlation with pathological features and patient survival was performed	SSCP and pyro-sequencing	Null
Krippl *et al*. (2003) [7]	Caucasian (Austrian) 497 cases, 498 controls	Cases were hospital-based with breast cancer risk factors not considered, no survival analysis performed; controls were population-based and age-matched	RFLP	Null
Shu *et al*. (2005) This study	Asian (Chinese) 1130 cases, 1196 controls	Shanghai Breast Cancer Population-Based Case–Control Study (Shanghai Cancer Registry and Resident Registry), breast cancer risk factors were considered	RFLP	Weak association between the presence of the A allele and increased breast cancer risk
Ceshi *et al*. (2005) [8]	Asian (Chinese) 258 cases, 670 controls	Singapore Population-Based Cancer Registry, breast cancer risk factors were considered	TaqMan	Association between the presence of the GA genotype and reduced breast cancer risk

SSCP: single-stranded conformation polymorphism; RFLP: restriction fragment length polymorphism.

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hormone-related conditions such as higher body mass index and larger waist-to-hip ratio, or endogenous hormone levels such as increased oestrone and testosterone levels or decreased sex hormone binding globulin (SHBG) levels), no significance for multiplicative interactions was observed. This polymorphism was associated with reduced risk of death and/or disease progression.

A possible shortcoming of this paper may be in the chosen method of genotyping, which has the potential of false A allele calling due to incomplete digestion. Quality control was performed on only 2% (56/2326) of the total study population with a genotype consistency rate of 98.2%. Keeping this in mind, consistency with Hardy–Weinberg equilibrium is reported.

This study is certainly the most comprehensive in assessing the role of this altered transcript in breast cancer risk, allowing for the evaluation of known non-genetic risk factors, and raises more questions in the understanding of the complex interactions involved in genetic susceptibility. The path forward for future studies has now been laid. Clearly we need to understand the true functional consequence of the alternate protein (isoform b) created by this polymorphism. Although one study suggested that isoform b has an increased half-life [5], another study did not confirm this and found isoform b to be a poor catalyst of RB phosphorylation/inactivation, but showed enhanced cell transformation activity [9]. Are the associations found in this study restricted to the Han Chinese ethnic group in which the study was performed? Subsequently an inverse association with breast cancer risk within a Singapore Chinese population was found, although this study was underpowered by the number of cases [8]. This association was further accentuated in patients with advanced stage disease, which coincides with the observations by Shu *et al*. in a similar subset of patients. Further studies are therefore required to determine the true nature of this polymorphic marker in breast cancer risk in Asian populations. Although two relatively small studies have excluded any association between this polymorphism and breast cancer risk in two Caucasian-based populations, this null effect requires further investigation, whereas the role of this polymorphic marker (frequency 16% in African Nigerians, National Center for Biotechnology Information (NCBI) SNP database) in breast cancer risk in African-based populations has not been addressed. This study also addresses the possible interaction between the *CCND1* polymorphism and oestrogen exposure, which although biologically plausible with the observation that oestrogens can induce *CCND1* gene transcription [10,11], requires validation at both the functional and genetic epidemiological level.

In conclusion, it is well established that *CCND1* is an essential regulator of cell-cycle progression, therefore any genetic variation that disrupts the normal functioning of this gene product is ultimately a target for association of cancer risk and/or survival. The Pro241 G>A polymorphism has been positively associated with risk of a number of cancers, although many of these studies are conflicting and may be as a direct result of low statistical power. The increasing availability of well-established case–control studies to address cancer risk, for example the population-based Shanghai Breast Cancer Study, and continuing advances in high-throughput technologies mean that the ability to address the issues raised by this paper are feasible in current genetic epidemiology climate.

References

1. Fu M, Wang C, Li Z, *et al*. Minireview: cyclin D1: normal and abnormal functions. *Endocrinology* 2004; **145**: 5439–5447.
2. Ormandy CJ, Musgrove EA, Hui R, *et al*. Cyclin D1, EMS1 and 11q13 amplification in human breast cancers. *Breast Cancer Res Treat* 2003; **78**: 323–335.
3. Alle KM, Henshall SM, Field AS, Sutherland RL. Cyclin D1 protein is overexpressed in hyperplasia and intraductal carcinoma of the breast. *Clin Cancer Res* 1998; **4**: 847–854.
4. Sutherland RL, Musgrove EA. Cyclins and breast cancer. *J Mamm Gland Biol Neoplasia* 2004; **9**: 95–104.
5. Betticher DC, Thatcher N, Altermatt HJ, *et al*. Alternate splicing produces a novel cyclin D1 transcript. *Oncogene* 1995; **11**: 1005–1011.
6. Grieu F, Malaney S, Ward R, *et al*. Lack of association between CCND1 G870A polymorphism and the risk of breast and colorectal cancer. *Anticancer Res* 2003; **23**: 4257–4259.
7. Krippl P, Langsenlehner U, Renner W, *et al*. The 870G>A polymorphism of the cyclin D1 gene is not associated with breast cancer. *Breast Cancer Res Treat* 2003; **82**: 165–168.
8. Ceschi M, Sun C-L, Van den Berg D, *et al*. The effect of cyclin D1 (CCND1) G870A-polymorphism on breast cancer risk is modified by oxidative stress among Chinese women in Singapore. *Carcinogenesis* 2005; advanced access. E-pub 21 April 2005.
9. Solomon DA, Wang Y, Fox SR, *et al*. Cyclin D1 splice variants. Differential effects on localisation, RB phosphorylation, and cellular transformation. *J Biol Chem* 2003; **278**: 30 339–30 347.
10. Altucci L, Addeo R, Cicatiello L, *et al*. 17-beta Estradiol induces cyclin D1 gene transcription, p36D1–p34cdk4 complex activation and p105Rb phosphorylation during mitogenic stimulation of G(1)-arrested human breast cancer cells. *Oncogene* 1996; **12**: 2315–2324.
11. Prall OW, Sarcevic B, Musgrove EA, Watts CK, Sutherland RL. Estrogen-induced activation of Cdk4 and Cdk2 during G_1–S phase progression is accompanied by increased cyclin D1 expression and decreased cyclin-dependent kinase inhibitor association with cyclin E-Cdk2. *J Biol Chem* 1997; **272**: 10 882–10 894.

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