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GG genotype of *cyclin D1 G870A* polymorphism is associated with increased risk and advanced colorectal cancer in patients in Singapore

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Abstract

Recent studies have implicated *cyclin D1 G870A* single-nucleotide polymorphism (SNP) in susceptibility to and early onset of colorectal cancers (CRC). We investigated the role of *cyclin D1 G870A* SNP in Singapore CRC patients without dominant family history by genotyping 254 patients and 101 controls. The risk of cancer for AA individuals was less than half that of GG individuals (odds ratio (OR) 0.41; 95% confidence interval (CI) 0.18–0.96). Furthermore, AA and AG patients whose tumours were Dukes C and D (OR 0.38; 95% CI 0.17–0.83), poorly differentiated (OR 0.28; 95% CI 0.09–0.84) and left-sided (OR 0.45; 95% CI 0.21–0.98) were associated with significantly lower risk than GG patients. Young (aged 50 years or less) GG patients had a 5-year lower mean age at onset than AA/AG patients ($P = 0.02$). Young male GG patients had worse disease-specific survival than AA/AG patients ($P = 0.002$). Thus, contrary to Caucasians, the GG (rather than AA) genotype is associated with increased susceptibility and advanced CRC in Singapore patients, suggesting a more complex relationship between the SNP and CRC risk, possibly modulated by population differences.

Keywords: Colorectal cancer risk; Cyclin D1; Single-nucleotide polymorphism; Age-at-onset; Disease-specific survival

1. Introduction

Colorectal cancer (CRC) is the cancer with highest incidence and accounts for the second highest cancer mortality in Singapore [1]. Mutations in the dominant *adenomatous polyposis coli* (*APC*) and *mismatch repair* genes account for only a small proportion (up to 5%) of familial CRC, namely familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC), respectively. There are at least another 10% of early onset CRCs attributable to other unknown, less penetrant genes [2].

Cyclin D1 is a key cell cycle regulator that is up-regulated by the β-catenin/Tcf pathway in colorectal tumourigenesis [3,4]. Studies on Caucasian populations have implicated *cyclin D1 G870A* SNP in a range of cancers, including CRC. The *G* to *A* variant at nucleotide 870 at the splice donor site of exon 4 may modulate alternative splicing [5]. It is postulated that in the "*G*" allele, splicing from exon 4 to exon 5 is favoured, giving rise to the full-length transcript a. Conversely, in the "*A*" allele, the pre-mRNA may splice less frequently at this junction, thus allowing the coding sequence reading into and terminating at intron 4, resulting in a shorter transcript b. Both transcripts encode the cyclin D1 functional domain [6]. The protein encoded by transcript b, cyclin D1b, however, lacks the degradation signal encoded

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by exon 5 and hence may have a longer half-life, resulting in deregulated cell proliferation.

One study from TX, USA, reports that HNPCC patients with AG/AA genotypes developed CRC 11 years earlier than patients with the GG genotype [7]. In a subsequent study, non-syndromic CRC patients aged 60 years or less with AA were found to have a 2.5 times increased risk compared with patients with AG/GG genotypes [8]. Another study from England shows that the "*A*" allele confers increased risk only in non-HNPCC familial cases [9]. However, a report on Finnish HNPCC patients finds that the presence of transcript b but not cyclin D1 genotype was inversely correlated with the age of onset of cancer [10]. These conflicting results could be due to population differences.The role of *cyclin D1* SNP in CRC risk thus remains controversial.

In the present study, we investigated the role of *cyclin D1 G870A* SNP in risk of cancer, disease-specific survival and age at onset in 254 Singapore CRC patients. The patients were without dominant family history and were predominantly Chinese. Interestingly, we found that the GG genotype (rather than AA) is associated with higher risk and advanced cancer. The GG genotype is also associated with earlier age of onset and worse disease-specific survival in young patients.

2. Materials and methods

2.1. Patient and control selection

Archival normal mucosa specimens from 254 consecutive CRC patients who had undergone surgery in Singapore General Hospital (SGH) between late 1989 and 1993 were included in the study. Specimens from FAP or HNPCC patients were excluded. There were 141 males and 113 female patients, mean age 60 years (range 24–93 years). At the time of surgery, 79 (31%) patients were aged 50 years or less. Tumour staging was according to Dukes' parameters [11]. Dukes A/B are early tumours confined to the colon and with no metastatic secondaries; Dukes C tumours have lymph node metastasis and Dukes D tumours have distant metastasis.

Archival blood specimens were available from 101 individuals undergoing health screening in SGH and whose full blood counts were normal, as well as from spouses and unaffected individuals of FAP families. Full blood counts comprise the counts for haemoglobin (g/dl), white blood cells (10^9/l), blood platelets (10^9/l) and differential counts of various white blood components (10^9/l). There were 51 males and 50 females; mean age 43 years (range 15–82 years).

The referral procedures for the controls were similar to the patients, that is, through doctors in general practice or polyclinics; SGH being the major primary care provider in Singapore. The majority of the patients (94%) and controls (90%) were Chinese of Han origin who had migrated to Singapore from Southern China in the first half of the 20th century. The patients and controls were thus from a genetically homogenous population.This study was approved by the Ethics Committee of SGH.

2.2. Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analyses

DNA was obtained from normal mucosa or lymphocytes by a simple salting out procedure [12]. A 234 base-pair (bp) sequence encompassing the exon 4 splice donor site of cyclin D1 (*CCND1*, GenBank X59798) was amplified in a 25 μl reaction mixture with forward primer 3-1 located in exon 4 and reverse primer 2 in intron 4 [7]. Restriction digest with the *ScrF1* enzyme gave a different band pattern for each genotype (AA, one 168-bp band; GG, one 145-bp band; AG, one of each band; Fig. 1(a)).

2.3. Denaturing high-performance liquid chromatography (dHPLC) analyses

The *cyclin D1* SNP was also determined by the Transgenomic WAVE nucleic acid fragment analysis system at 63.5 °C, the optimum denaturing temperature based on the software prediction of melting temperature of the fragment. An 8-μl volume of the same 234-bp PCR product was injected directly into the column after 4 min denaturation at 95 °C. Samples were eluted in a linear gradient over a period of 8.8 min at a constant flow rate of 0.9 ml/min. Samples that were heterozygous (AG) showed two peaks, whereas homozygous samples (AA or GG) had only one peak (Fig. 1(b)). The homozygous samples were then spiked with AA control and ran through the dHPLC columns again. Samples with one peak were scored as AA and those with two peaks were scored as GG.

2.4. Sequencing analyses

The restriction fragment length polymorphism analyses (RFLP) and dHPLC patterns were confirmed by PCR-based sequencing using primers 3-1 or Cy1F [10] with Applied Biosystems automated sequencer.

2.5. Reverse-transcriptase-polymerase chain reaction analyses

Total RNA was extracted from normal mucosa tissues using the Promega (Madison, WI, USA) Total RNA extraction kit. The expressions of transcripts a and b were investigated by Reverse-transcriptase (RT)-PCR and nested PCR, as previously described [10]. Briefly, RT-PCR reactions were performed with Promega

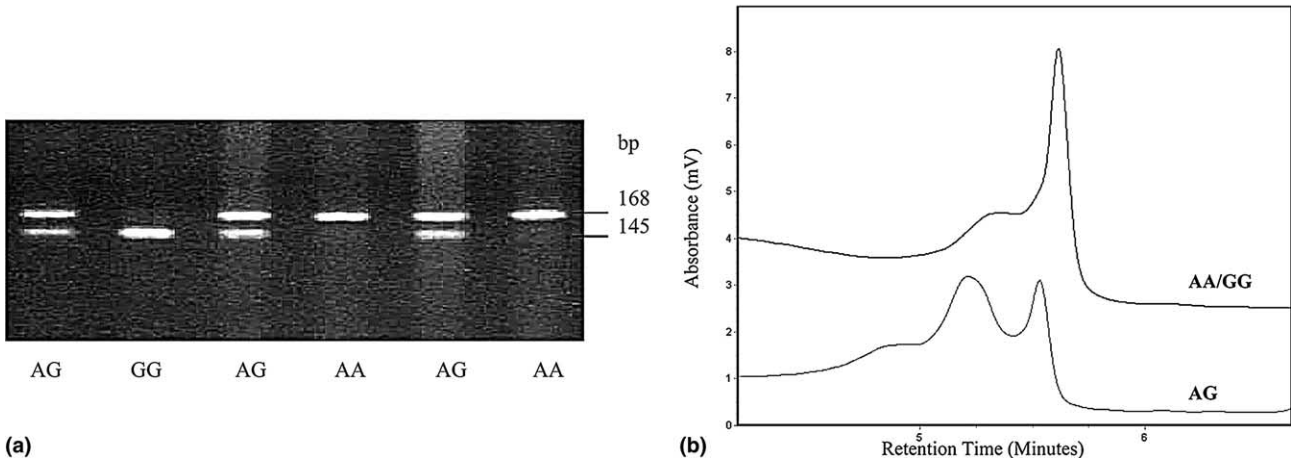

Fig. 1. (a) *Scr F1* restriction digest pattern of the three different *cyclin D1 G870A* genotypes. (b) Representative homozygous and heterozygous denaturing high-performance liquid chromatography (dHPLC) profiles for *cyclin D1 G870A* polymorphism.

RT-PCR Access System and primer sets Cy10/Cy 6 (transcript a) or primer sets Cy10/Cy 32 (transcript b) at 55 °C annealing temperature for 35 cycles. This is followed by nested PCR using 1:50 dilution and primer sets Cy1F/Cy4R (transcript a, 389 bp) or Cy1F/Cy27 (transcript b, 324 bp) at 60 °C annealing temperature for another 32 cycles.

ScrF1 restriction digest of transcripts yielded different major bands if they were spliced from allele "*A*" or "*G*". When spliced from allele "*A*", *ScrF1* digestion of transcript a results in a 279-bp band and transcript b a 290-bp band, respectively. If spliced from allele "*G*", both transcripts give a 270-bp major band.

2.6. Statistical analyses

The χ^2 test for Hardy–Weinberg equilibrium was used for each group (patient and control; d$f = 1$) to determine whether there was any population stratification. Pearson's χ^2 test was used to determine whether there was any significant difference in allele and genotype frequencies between patients and controls. Multivariate logistic regression analysis was used to assess the association between CRC and cyclin D1 genotypes, with sex and age as covariates. Kaplan–Meier survival analysis with log-rank test was used to evaluate the relationship between cyclin D1 genotype and disease-specific survival. Survival was calculated from the date of surgery to date of death or, if alive, to December 31, 2000, which was the latest death entry in the Singapore Cancer Registry when the patients were recruited in 2002. Patients who were still alive or who had died of causes other than cancer were censored. Patients whose survival status was unknown were omitted from the survival analysis. Disease-specific survival data were available for 205 patients. The Cox regression was used to estimate the risk ratio of death for cyclin D1 genotype with age group (above and below 50 years), sex and tumour stage (low and high malignancy) as covariates and interaction between the covariates, if any. The non-parametric Mann–Whitney *U* test was used to estimate the association between age of onset and the cyclin D1 genotypes.

All statistical analyses were performed using the SPSS package version 10.0 (SPSS, Chicago, IL, USA). All tests of statistical significance were two-sided and differences were taken as significant when *P*-value is less than 0.05. For sub-group analyses stratified by age and by age and gender, significance was defined at $P < 0.025$ (0.05/2) and $P < 0.017$ (0.05/3), respectively.

3. Results

3.1. Genotypic frequencies are in Hardy–Weinberg equilibrium

The genotypic frequencies of the controls ($n = 101$; $\chi^2 = 0.444$; d$f = 1$; $P = 0.505$) were in Hardy–Weinberg equilibrium, suggesting that there was no population stratification and no sampling bias. The patients' frequencies were also in Hardy–Weinberg equilibrium ($n = 254$; $\chi^2 = 0.036$; d$f = 1$; $P = 0.850$). There were thus no excess homozygotes, indicating that there was no risk of allelic loss due to contamination by tumour tissues.

3.2. GG genotype is associated with higher risk and advanced cancer

The allelic frequencies of the patients ($A = 0.53$; $G = 0.47$) were significantly different from the controls ($A = 0.63$; $G = 0.37$; $\chi^2 = 6.319$, d$f = 2$, $P = 0.04$). The GG genotype was significantly higher in the patients (see also Table 1).

The risk of CRC for individuals with the AA genotype is less than half of that for those with GG genotype

Table 1
Cyclin D1 G870A genotypic frequency and logistic regression analysis

Genotype	Patient no. (%)	Control no. (%)	Multivariate analysis		*P*-value
	254	101	Adjusted OR[a]	95% CI	
GG	55 (21.7)	12 (11.9)	1		
AA	71 (28)	39 (38.6)	0.414 (AA/GG)	0.179–0.955	0.039
AG	128 (50.4)	50 (49.5)	0.539 (AG/GG)	0.243–1.194	0.128
AA+AG	199 (78.3)	89 (88.1)	0.485 (AA+AG/GG)	0.227–1.038	0.062

[a] Odds ratio (OR) adjusted for sex and age.

(Table 1; OR = 0.414; $P = 0.039$), as determined by multivariate logistic regression analysis. Conversely, the risk of cancer for patients with GG genotype is 2.4 times that of those with AA genotype. A similar trend is observed when comparing the risk of both AA and AG with that of the GG genotype (Table 1; OR = 0.485; $P = 0.062$).

Furthermore, the AA/AG patients with Dukes C and D (Table 2; OR = 0.376; $P = 0.015$), poorly differentiated (OR = 0.276; $P = 0.023$) and left-sided (OR = 0.445; $P = 0.043$) tumours are associated with significantly lower risk than the corresponding tumours of GG patients.

3.3. Young GG patients have lower age at onset than young AA/AG patients

There was no significant difference in the age of onset of patients with the GG genotype ($n = 55$; mean age ± SD = 61 ± 17 years) compared with those with AA/AG genotypes ($n = 199$; 59 ± 15 years) for the whole series ($P = 0.264$). However, young patients with GG genotype ($n = 14$) have approximately a 5-year lower mean age of onset of cancer (37 ± 7 years) than young patients with AA/AG genotypes ($n = 65$; 42 ± 6 years; $P = 0.021$).

3.4. GG is associated with worse disease-specific survival in young male patients

Interestingly, the Cox regression analysis shows a significant increase in death risk for the interaction between age group, genotype and sex (OR 4.85; 95% CI 1.6–14.9; $P = 0.006$). Further Kaplan–Meier survival analysis stratified by sex and age group reveals that young male patients with GG genotype had worse disease-specific survival than those with the other two genotypes ($n = 29$, log-rank test, $P = 0.002$; Fig. 2(a)). The relationship did not hold for young female patients ($n = 17$, Fig. 2(b)).

3.5. Relationship between genotypes and transcripts

RNA was available from a subset of young (⩽50 years old) healthy controls ($n = 20$) and CRC patients ($n = 49$). RT-PCR analysis (Fig. 3) indicates that both transcripts a and b were present in all 20 healthy controls regardless of genotype.Transcript a was also found in all 49 young patients. However, transcript b was detected in 18/49 (37%) of the young patients only. Patients with GG genotype have a higher frequency of transcript b (6/10) compared with those with AG/AA

Table 2
Cyclin D1 genotype and risk of colorectal cancer stratified by tumour characteristics[b]

Tumour characteristics	Genotype				*P*-value
	GG		AG + AA		
	Cases/controls	OR[a]	Cases/controls	OR[a] (95% CI)	
Tumour stage					
A/B	16/12	1.0	79/89	0.880 (0.309–2.502)	0.810
C/D	39/12	1.0	118/89	0.376 (0.171–0.826)	0.015
Tumour differentiation					
Well/moderate	45/12	1.0	163/89	0.559 (0.253–1.235)	0.150
Poor	10/12	1.0	33/89	0.276 (0.091–0.863)	0.023
Tumour site[c]					
Left	49/12	1.0	159/89	0.445 (0.203–0.975)	0.043
Right	6/12	1.0	38/89	0.578 (0.175–1.914)	0.370

CI, confidence interval.
[a] Odds ratio (OR) adjusted for sex and age.
[b] Not all tumour characteristics were available for every patient.
[c] Left or right tumours are demarcated by the splenic flexture.

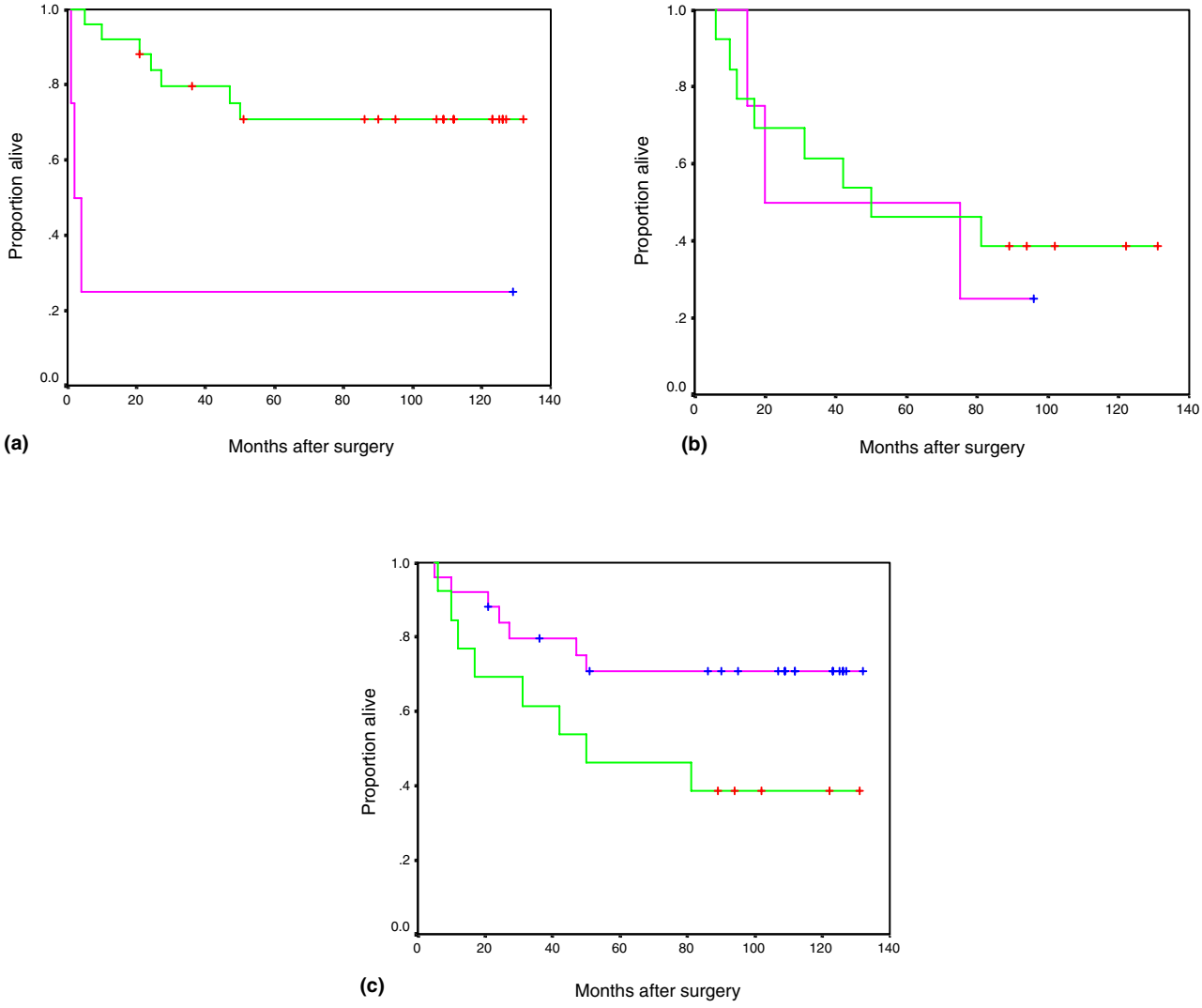

Fig. 2. (Colour on-line) Kaplan–Meier survival analysis for cyclin D1 genotype ((a) and (b)) and gender (c) for young patients. Cyclin D1 genotype is AA/AG (grey line) or GG (black line). (a) Male patients, *n* (AA/AG) = 25; *n* (GG) = 4; log-rank test $P = 0.002$. (b) Female patients, *n* (AA/AG) = 13; *n* (GG) = 4; log-rank test $P = 0.727$. (c) Young AA/AG female (gray line, *n* = 13) and young AA/AG male (black line, *n* = 25) patients, log-rank test $P = 0.061$.

genotypes (12/39) although this has not reached statistical significance ($P = 0.087$).

In addition, *ScrF1* digest on transcripts a and b from young heterozygous (AG) patients reveals that transcript a was spliced from both alleles in 7/7 cases. Interestingly, transcript b was also spliced from both alleles in 5/7 cases and mainly from the "*G*" allele in 2 cases.

There was no significant difference in mean age of onset between young patients expressing transcript a alone (*n* = 31; 43 ± 6 years) or those expressing both transcripts (*n* = 18; 42 ± 5 years; Mann–Whitney *U* test, $P = 0.448$).

4. Discussion

We investigated the role of *cyclin D1 G870A* SNP on CRC risk, disease status, age at onset and disease-specific survival in a series of 254 patients and 101 controls. Based on a 10% difference in genotypic frequencies (Table 1), an allocation ratio of 2.5:1 (case: control = 254:101) and a 5% type I error ($\alpha = 0.05$), the power for detecting significant differences between the cases and control is 65%.

In contrast to Caucasian studies, which reported that the "*A*" allele is associated with increased risk in some disease sub-groups of CRC [8,9,13], the GG genotype is associated with higher risk in Singapore CRC patients (Table 1). Two earlier studies on Southern Chinese [14] and Taiwanese Chinese [15] respectively, have reported very similar *cyclin D1 G870A* allelic frequencies to that found in this study, and these are quite different from the Caucasian's (Table 3). The mean allelic frequency for the "*A*" allele from the three studies on the Chinese population is 0.63, compared with 0.42 from the four

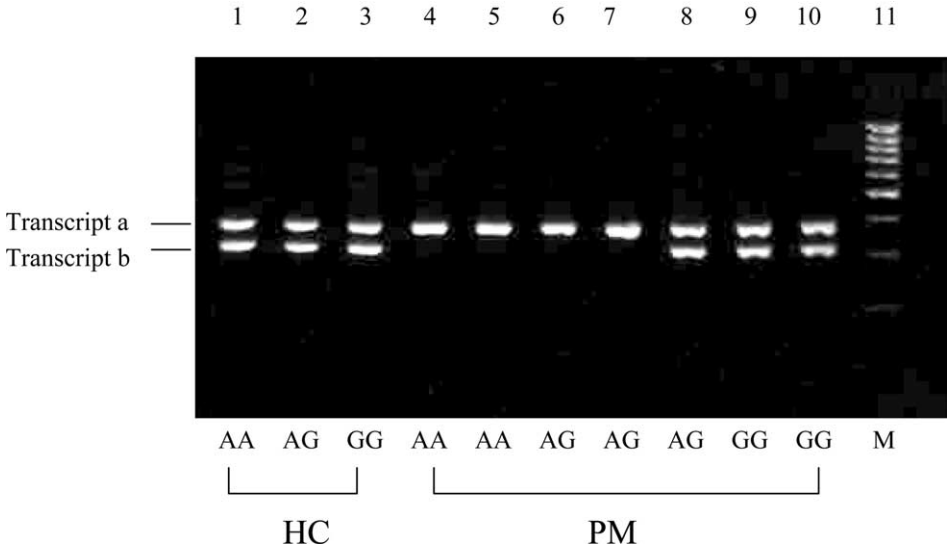

Fig. 3. Representative gel image showing the alternatively spliced transcripts a (389 bp) and b (324 bp) in healthy controls (HC) in lanes 1–3 and patients' mucosa (PM) in lanes 4–10, respectively. M (lane 11) shows the 100-bp DNA marker. Cyclin D1 genotype of each individual is shown at the bottom of lanes 1–10.

studies on the Caucasian population. The allelic frequencies of the patients from this study ($A = 0.53$; $G = 0.47$), however, are similar to that of the Caucasian CRC patients [8,13]. Furthermore, in a study with ethnic data, only the whites (but not the Japanese and Hawaiians) with AA genotype have significantly increased risk of CRC compared with those with GG genotype (Table 2 of reference 13). The latter two ethnic groups, incidentally, have allelic frequencies ($A = 0.49 - 0.57$) closer to the Chinese patients. The data thus suggest that the effect of cyclin D1 polymorphism on cancer risk could be population-specific.

Two earlier studies [13,16] have indicated that cyclin D1 polymorphism was associated with advanced CRC and adenomas, respectively. In this study, we found that the increased risk with the GG genotype is also more pronounced in individuals with advanced (Dukes C and D and poorly differentiated) tumours, suggesting that cyclin D1 may also play a role in tumour progression in the local series (Table 2).

We also found that young patients with GG genotype have approximately a 5-year lower mean age at onset of cancer than patients with AA/AG genotype. This is in contrast to the results of an earlier report that indicates that AA/AG genotypes decrease age at onset of HNPCC patients by 11 years [7]. The diverse results could be due to genetic differences attributable to different population and/or disease sub-groups, since the young patients in our series were non-FAP and non-HNPCC. On the other hand, several studies have failed to show that cyclin D1 genotype has any effect on the age at onset even for sub-group analysis [9,10]. We did not find any significant association of cyclin D1 genotype with disease-specific survival for the whole series. This is perhaps not surprising as more than half of the patients in the series (54%) are aged 60 years or more and are therefore sporadic cases. In sporadic CRC, the effect of environmental determinant on survival probably predominates over the effect of susceptibility genes. An earlier study on sporadic CRC [17] also did not detect a significant association between *cyclin D1 G870A* polymorphism and overall survival, due perhaps to the lack of early onset-cases.

Nevertheless, we found that the GG genotype was significantly associated with worse disease-specific survival for young male patients (Fig. 2). An earlier study in squamous cell carcinoma of head and neck (SCCHN) has reported gender-specific risk for the AA genotype [18]. To our knowledge, however, this is the first report that shows that an interaction between cyclin D1 genotype and gender has prognostic significance in CRC. It is intriguing that for young AA or AG patients, the

Table 3
Cyclin D1 G870A allelic frequencies of controls from Caucasian and Chinese populations

Populations	*n*	Allelic frequencies		References
		A	*G*	
Caucasian (USA)	152	0.43	0.57	[8]
Caucasian (UK)	171	0.41	0.59	[9]
Caucasian (UK)	101	0.42	0.58	[17]
Whites (USA)	161	0.43	0.57	[13]
Southern Chinese (China)	91	0.62	0.38	[14]
Chinese (Taiwan)	35	0.64	0.36	[15]
Chinese (Singapore)	101	0.63	0.37	This study

females, though not yet statistically significant, tend to survive less well than the males (Fig. 2(c), log-rank test, $P = 0.061$). This observation may be due to the contrasting interaction of cyclin D1 when it binds to the androgen and oestrogen receptors, respectively. It has been reported that cyclin D1 inhibits the transcriptional potential of the androgen receptor [19] but activates the oestrogen receptor [20]. Nevertheless, it cannot be ruled out at present that this finding could be due to chance in the sub-group analysis since the sample size is small and needs to be validated in larger studies. The GG genotype has previously been associated with worse survival in SCCHN [21] and von Hippel–Lindau haemangioblastoma [22].

Both transcripts a and b were expressed in the normal mucosa of all healthy controls irrespective of genotype, suggesting that both transcripts can be spliced from both alleles in the normal colon. However, only a subset (37%) of the patients' mucosa expressed transcript b, demonstrating allelic specific expression in young CRC patients. It is noteworthy that in our series, the GG genotype tends to be associated with more transcript b and that transcript b was spliced from both alleles and not predominantly from allele "*A*". This is contrary to some earlier studies in Caucasian populations. The study on non-small cell lung cancer has reported that the "*G*" allele favours splicing to transcript a although no statistical analysis and no splicing data from normal controls were reported [5]. Using limited specimens, the study on SCCHN indicated that transcript b is spliced mainly from the "*A*" allele [23]. However, a study on leukaemia patients has shown that the predominant transcript in GG patients was transcript b, which supports our observation [24]. The only CRC study with transcript data shows that the presence of transcript b is associated with earlier onset of HNPCC [10]. Although the presence of transcript b in our young patients (37%) is similar to this Finnish study (35%), our data indicates that the GG genotype, but not the presence of transcript b, is associated with earlier age at onset in young non-FAP and non-HNPCC patients. Again, it is possible that the diverse results could be due to population or disease sub-group specific deregulation of cyclin D1 mRNA splicing.

In conclusion, the findings of this study support the candidacy of *cyclin D1 G870A* polymorphism as a low penetrant gene associated with increased susceptibility and advanced CRC. The polymorphism also affects the age at onset and eventual survival of young Singapore male patients. The mechanism of its action, however, may be population-, tissue- and gender-specific and may be influenced by other cyclin D1 polymorphisms, such as the 1722 SNP (23) and thus merits further investigation in larger series and other populations.

Conflict of interest statement

None declared.

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References

1. Chia KS, Seow A, Lee HP, *et al.* Cancer incidence in Singapore 1993–1997. *Singapore Cancer Registry Report* 2000, **5**, 6–14.
2. Houlston RS, Tomlinson IPM. Polymorphisms and colorectal tumour risk. *Gastroenterology* 2001, **121**, 282–301.
3. Tetsu O, McCormick F. β-Catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999, **398**, 422–426.
4. Shtutman M, Zhurinsky J, Simcha I, *et al.* The cyclin D1 gene is a target of the β-catenin/LEF-1 pathway. *Proc Natl Acad Sci USA* 1999, **96**, 5522–5527.
5. Betticher DC, Thatcher N, Altermatt HJ, *et al.* Alternate splicing produces a novel cyclin D1 transcript. *Oncogene* 1995, **11**, 1005–1011.
6. Motokura T, Bloom T, Kim HG, *et al.* A novel cyclin encoded by a bcl1-linked candidate oncogene. *Nature* 1991, **350**, 512–515.
7. Kong S, Amos CI, Luthra R, *et al.* Effects of cyclin D1 polymorphism on age of onset of hereditary nonpolyposis colorectal cancer. *Cancer Res* 2000, **60**, 249–252.
8. Kong S, Wei Q, Amos CI, *et al.* Cyclin D1 polymorphism and increased risk of colorectal cancer at young age. *J Nat Cancer Inst* 2001, **93**, 1106–1108.
9. Porter TR, Richards FM, Houlston RS, *et al.* Contribution of cyclin D1 (CCND1) and E-cadherin (CDH1) polymorphisms to familial and sporadic colorectal cancer. *Oncogene* 2002, **21**, 1928–1933.
10. Bala S, Petlomaki P. Cyclin D1 as a genetic modifier in hereditary nonpolyposis colorectal cancer. *Cancer Res* 2001, **61**, 6042–6045.
11. Dukes CE. The classification of cancer of the rectum. *J Pathol Bacteriol* 1932, **35**, 323–332.
12. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1998, **16**, 1215.
13. Le Marchand L, Seifried A, Lum-Jones A, *et al.* Association of the cylcin D1 A870G polymorphism with advanced colorectal cancer. *JAMA* 2003, **290**, 2843–2848.
14. Deng L, Zhao X, Pan KF, *et al.* Cyclin D1 polymorphism and the susceptibility to NPC using DHPLC, *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao* 2002, **34**, 16–20.
15. Zhang Y, Chen S, Chen C, *et al.* Polymorphisms in cyclin D1 gene and hepatocellular carcinoma. *Mol Carcinog* 2002, **33**, 125–129.
16. Lewis RC, Bostick RM, Xie D, *et al.* Polymorphism of the cyclin D1 gene, CCND1, and risk for incident sporadic colorectal adenomas. *Cancer Res* 2003, **63**, 8549–8553.

17. McKay JA, Douglas JJ, Ross VG, *et al*. Cyclin D1 protein expression and gene polymorphism in colorectal cancer. Aberdeen colorectal initiative. *Int J Cancer* 2000, **88**, 77–81.
18. Zheng Y, Shen H, Sturgis EM, *et al*. Cyclin D1 polymorphism and risk for squamous cell carcinoma of the head and neck: a case-control study. *Carcinogenesis* 2001, **22**, 1195–1199.
19. Knudsen KE, Cavenee WK, Arden KC. D-Type cyclins complex with the androgen receptor and inhibit its transcriptional transactivation ability. *Cancer Res* 1999, **59**, 2297–2301.
20. Zwijsen RM, Wientjens E, Klompmaker R, *et al*. CDK-independent activation of estrogen receptor by cyclin D1. *Cell* 1997, **88**, 405–415.
21. Matthias C, Branigan K, Jahnke V, *et al*. Polymorphism within the cyclin D1 gene is associated with prognosis in patients with squamous cell carcinoma of head and neck. *Clin Cancer Res* 1998, **4**, 2411–2418.
22. Zatyka M, da Silva NF, Clifford SC, *et al*. Identification of cyclin D1 and other novel targets for the von Hippel Lindau tumour suppressor gene by expression array analysis and investigation of cyclin D1 genotype as a modifier in von Hippel Lindau disease. *Cancer Res* 2002, **62**, 3803–3811.
23. Holley SL, Parkes G, Matthias C, *et al*. Cyclin D1 polymorphism and expression in patients with squamous cell carcinoma of the head and neck. *Am J Pathol* 2001, **159**, 1917–1924.
24. Howe D, Lynas C. The cyclin D1 alternative transcripts [a] and [b] are expressed in normal and malignant lymphocytes and their relative levels are influenced by the polymorphism at codon 241. *Haematologica* 2001, **86**, 563–569.