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Meta-analysis combining new and existing data sets confirms that the TERT-CLPTM1L locus influences melanoma risk

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Letter

A number of GWAS have observed association between SNPs located in 5p15.33 with increased risk for a range of cancers, including some non-melanoma skin cancers (Baird, 2010). Contrary to the increased risk observed for other cancers the peak variant, rs401681 C allele, has been associated with a decreased risk for melanoma (OR=0.86, 95% CI 0.81–0.91 $p=5.0\times10^{-8}$) (Stacey *et al.*, 2009). There have been two attempts at independent replication. Nan et al., (2011) observed a similar direction of effect in a small sample (OR=0.73, 95% CI 0.59–0.91). However an additional replication study observed no evidence for association between rs401681 C allele and melanoma (OR=1.01, 95% CI 0.87–1.19) (Pooley *et al.*, 2010). As replication has been inconsistent, we present here unpublished Australian data and rationalize the findings.

The 5p15.33 SNPs are located within or adjacent to two genes in strong LD, encoding Telomerase Reverse Transcriptase (*TERT*, MIM: 187270) and *CLPTM1-like protein* (*CRR9p*; *CLPTM1L*, MIM: 612585). *CLPTM1L* was identified as up-regulated in cisplatin resistant cancer cells (Yamamoto *et al.*, 2001) and while a role for *CLPTM1L* should not be excluded little is known about its function. *TERT* is a striking candidate as it encodes the catalytic subunit of telomerase. Incomplete replacement of telomere repeat sequences by telomerase following their loss during S phase is a likely cause of cell senescence (Shawi *et al.*, 2008). While *TERT* expression is generally absent in adult tissues it is enhanced in most, but not all, cancerous cells (Engelhardt *et al.*, 1997; Kolquist *et al.*, 1998). Nevi (moles) result from melanocyte proliferation and nevus count is positively associated with melanoma

[3]Full author list available in supplementary material

Conflicts of Interest

All authors have no conflict of interest or financial interest in this work.

risk. Longer telomeres have been associated with increased nevus count and size as well as a non-significant increase in melanoma risk (OR 1.85, 95% CI 0.99–3.44) (Han *et al.*, 2009). Nan et al, (2011) reported a marginal association between the rs401681 C allele and shorter telomere length, an intriguing result given their earlier observation of decreased nevus count in those with shorter telomere length (Han *et al.*, 2009). Specifically, rs401681 C may associate with reduced melanoma incidence via shortened telomere mediated inhibition of nevi growth. However a far larger study observed no association between rs401681 and telomere length (Pooley *et al.*, 2010).

We recently performed a large melanoma GWAS in a Caucasian population by combining 2,168 cases from the Q-MEGA (Baxter *et al.*, 2008) and AMFS studies (Cust *et al.*, 2009) and 4,387 controls combined from 3 studies (Baxter *et al.*, 2008; Cust *et al.*, 2009; Painter *et al.*, 2011). This population gave sufficient power to detect effect sizes in line with other cancer GWAS (1.2<OR<1.5). Samples were genotyped on Illumina SNP arrays (Cases: Omni1-Quad or HumanHap610; Controls: Omni1-Quad or HumanHap610 or HumanHap670). Cases and controls were combined into a single data set for quality control, outlier removal and imputation. Imputation via MACH2 (Li *et al.*, 2010) based on the 1000 Genomes Project data, June 2010 release (Durbin *et al.*, 2010), allowed association testing for 5,480,804 well imputed SNPs (r^2 >0.5). Locuszoom (Pruim *et al.*, 2010) was used to plot SNP significance values across the region spanning *TERT* and *CLPTM1L*, which confirms there is indeed an association peak between *TERT* and *CLPTM1*, albeit below genome wide significance (Figure 1). Although imputation is able to fill in the missing data in cases where SNPs were not present on all arrays used, there remain regions where SNPs could not be well imputed, which in our case is a 30kb block within *TERT*. However those SNPs directly genotyped in this region were not meaningfully associated with melanoma (boxed squares, Figure 1) indicating the association signal between *TERT* and *CLPTM1L* does not extend into this region. Key SNP rs401681 is not on Omni1-Quad arrays. It was hence genotyped separately using the Sequenom platform (Brown *et al.*, 2008).

In the combined Australian dataset the rs401681 allele C was clearly inversely associated with melanoma as previously observed, but did not reach genome-wide significance (p=0.00107, Table 1). Meta-analysis of rs401681 C allele across all four studies supports the association with reduced melanoma rates (Stacey et al., 2009; Pooley et al., 2010; Nan et al., 2011). As the I^2 was high at 48.98 the random effect model is most appropriate (random effect $p=3.00\times10^{-4}$, OR=0.873 95% CI 0.812–0.939; fixed effect $p=9\times10^{-10}$, OR=0.871 95% CI 0.833–0.910). A forest plot is available in the supplementary data (Sup. Figure 1).rs401681 was not our highest association signal in this region. The strongest association for the *TERT-CLPTM1L* was observed at rs4975616 (Table 1), which has previously been associated with lung cancer (Broderick et al., 2009), and higher again at the fully imputed rs13356727 (Table 1). rs13356727 lies less than 10 kb from rs401681 and is also between *TERT* and *CLPTM1L* (Figure 1). All 3 SNPs exhibit strong LD (r^2>0.8) with one another (Supplementary Figure 3), and all fall within the same LD block that spans the *TERT* promotor and the 3' end of the *CLPTM1L* gene (Supplementary Figure 2). The signal at rs13356727 remained significant following covariation by rs401681 (Table 1). Similarly, covarying for rs13356727 abolished all signal at rs401681 (C allele p=0.512, OR=1.071,

95% CI 0.873–1.312). When each SNP was covaried by the other two only rs13356727 remains significant (p=0.046, OR=0.804, 95% CI 0.649–0.996). This suggests that rs13356727 represents a better proxy for the potential causal variant in this region leading to a reduced risk for melanoma. As nevi count is also associated with melanoma we hypothesized that the inverse association of rs13356727 with melanoma may result from an interaction with mole count. Self reported mole count ("None", "Few", "Some" and "Many") was available for 1398 controls and 1592 cases with melanoma. Co varying for mole count did not meaningfully change the association between rs13356727 and melanoma (subset melanoma association $p=4.88\times10^{-5}$ OR 0.800 95% CI 0.718–0.891; subset co-varied by mole count $p=3.01\times10^{-4}$, OR 0.816 95% CI 0.731–0.911). The protective rs13356727 A allele was also associated with a reduction in mole count (regression of self reported mole count on rs13356727 100,000 permutations p=0.00042). rs401681 C and rs4975616 A alleles were also associated with reduced mole count to a lesser extent (p_{perm}=0.00407 and p_{perm} 0.00069 respectively).

In conclusion we examined the role of *TERT-CLPTM1L* variants in determining melanoma risk by presenting new data on a large Australian case-control sample. Combining these data with inconclusive existing data clarifies that *TERT-CLPTM1L* variants do influence risk, albeit with a relatively small effect size. In our data there was an association with mole count and it is intriguing to speculate that the inverse association (relative to other cancers) may be due to an interaction with nevus propensity. However, the observed melanoma association was unchanged by correction with mole count and further work is required to dissect the specific role variation at *TERT-CLPTM1L* plays in mole count and melanoma. When considered in the light of studies by Nan et al (2011) and Han et al (2009), it may be that the apparently independent association we observed between this loci and melanoma or mole count was due to a functional variant influencing telomere length, which in turn altered melanoma and nevus development in a complex manner.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

TERT	Telomerase Reverse Transcriptase
CLPTM1L	CLPTM1-like proteinl
Q-MEGA	Queensland study of Melanoma: Environment and Genetic Associations
AMSF	Australian Melanoma Family Study

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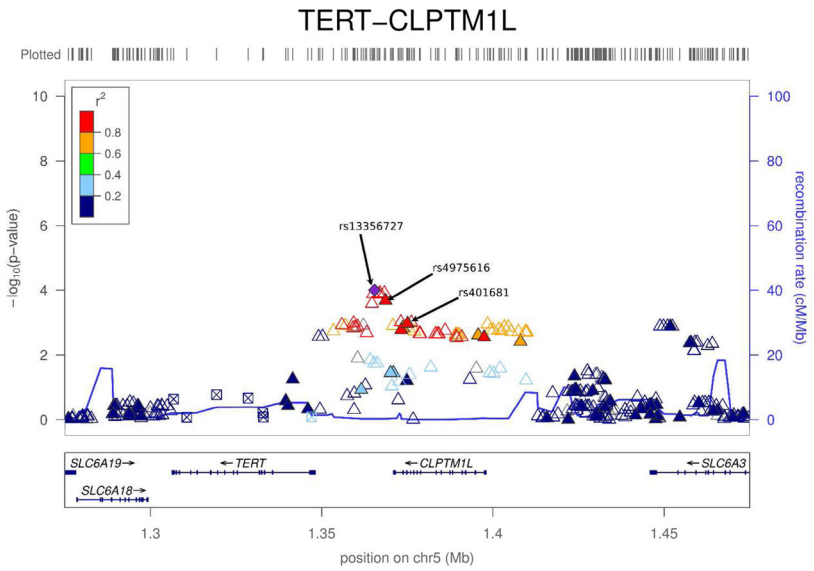

Figure 1.

Genome wide association results for the *TERT-CLPTM1L* locus. Solid triangles represent genotyped SNPs, and hollow triangles fully imputed SNPs. The top imputed SNP rs13356727 is displayed as a purple diamond and the degree of LD (r^2) with all other plotted SNPs indicated by their colour. The other discussed SNPs, the fully genotyped rs4975616 and rs401681 have been singled out. While all other plotted p values are derived from the imputed dosage scores there was insufficient information to well impute SNPs on the whole data set for a 30k region spanning the central part of TERT. Genotyped p values for this 30k region have been included as boxed squares, indicating the association signal does not extend into the central part of TERT.

Table 1

Association results at the *TERT- CLPTM1L* locus

SNP: Tested allele	N genotyped Case/Control	Tested allele freq Case/ Control	Association of genotyping results with melanoma		Association of imputed dosage scores with melanoma[a]			Results when co-varied by rs401681	
			p value	OR (95% CI)	r^2 [b]	p value	OR (95% CI)	p value	OR (95% CI)
rs401681: C	2035/4345	0.5388/0.5697	0.00107[c]	0.883 (0.819–0.951)	NA	NA	NA	NA	NA
rs4975616: A [c]	2168/4361	0.5542/0.5899	0.000101	0.864 (0.803–0.930)	0.988	0.00021	0.869 (0.807–0.937)	0.126	0.848 (0.686–1.048)
rs13356727: A [d]	NA	NA	NA	NA	0.907	9.96×10^{-5}	0.858 (0.795–0.926)	0.0455	0.803 (0.649–0.996)

[a] Total population following imputation was 2168 cases and 4387 controls; rs4975616's imputation p value is generated using the combination of genotyped and imputed data, while rs13356727 is fully imputed.

[b] r^2 is a measure of imputation quality; it is equivalent to the ratio between the variance of the imputed genotypes and the expected binomial variance 2p(1-p) at HWE where p is the estimated allele frequency (Li *et al.*, 2010).

[c] genotyped SNP most associated with melanoma.

[d] imputed SNP most associated with melanoma

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