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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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TO THE EDITOR

A number of genome-wide association studies have observed an association between single-nucleotide polymorphisms (SNPs) located in 5p15.33 and an 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 (odds ratio (OR) = 0.86, 95% confidence interval (CI) 0.81–0.91, $P = 5.0 \times 10^{-8}$; 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 As replication has been inconsistent, we present here unpublished Australian data and rationalize the findings.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 linkage disequilibrium (LD), encoding telomerase reverse transcriptase (*TERT*, MIM: 187270) and CLPTM1-like protein (*CRR9p*; *CLPTM1L*, MIM: 612585). CLPTM1L was identified as upregulated in cisplatin-resistant cancer cells (Yamamoto *et al.*, 2001) and, although 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 and Autexier, 2008). Although *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 risk. Longer telomeres have been associated with increased nevus count and size, as well as with 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 nevus growth. However a far larger study observed no association between rs401681 and telomere length (Pooley *et al.*, 2010).

We recently conducted a large melanoma genome-wide association study in a Caucasian population by combining 2,168 cases from the Q-MEGA (Queensland study of Melanoma: Environment and Genetic Associations; Baxter *et al.*, 2008) and AMFS (Australian Melanoma Family Study; Cust *et al.*, 2009) studies

Abbreviations: AMFS, Australian Melanoma Family Study; CLPTM1L, CLPTM1-like protein; Q-MEGA, Queensland study of Melanoma: Environment and Genetic Associations; TERT, telomerase reverse transcriptase

and 4,387 controls combined from three 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 genome-wide association studies ($1.2 < OR < 1.5$). 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 that 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 in which SNPs were not present on all arrays used, there remain regions in which SNPs could not be well imputed, which in our case is a 30 kb block within *TERT*. However, those SNPs directly genotyped in this region were not meaningfully associated with melanoma (boxed squares, Figure 1), indicating that the association signal between *TERT* and *CLPTM1L* does not extend into this region. The 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 data set, 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 the 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 value was high at 48.98, the random-effect model was most appropriate (random effect $P = 3.00 \times 10^{-4}$, OR = 0.873, 95% CI 0.812–0.939; fixed effect $P = 9 \times 10^{-10}$, OR = 0.871, 95% CI 0.833–0.910). A forest plot is available in the Supplementary Figure S1 online. rs401681 was not our highest association signal in this region. The strongest association for TERT–CLPTM1L was observed at rs4975616 (Table 1), which has previously been associated with

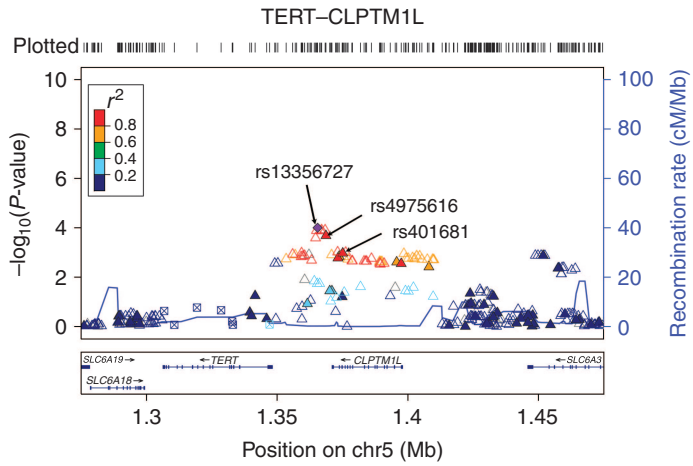

Figure 1. Genome-wide association results for the *TERT–CLPTM1L* locus. Solid triangles represent genotyped single-nucleotide polymorphisms (SNPs), and hollow triangles represent fully imputed SNPs. The top imputed SNP rs13356727 is displayed as a purple diamond, and the degree of linkage disequilibrium (r^2) with all other plotted SNPs is indicated by their color. The other discussed SNPs, the fully genotyped rs4975616 and rs401681, have been singled out. Although all other plotted *P*-values are derived from the imputed dosage scores, there was insufficient information to impute SNPs well on the whole data set for a 30 k region spanning the central part of *TERT*. Genotyped *P*-values for this 30 k region have been included as boxed squares, indicating that the association signal does not extend into the central part of TERT. CLPTM1L, CLPTM1-like protein; TERT, telomerase reverse transcriptase.

Table 1. Association results at the *TERT–CLPTM1L* locus

			Association of genotyping results with melanoma		Association of imputed dosage scores with melanoma[1]			Results when covaried by rs401681	
SNP: tested allele	*N* genotyped, case/control	Tested allele freq, case/control	*P*-value	OR (95% CI)	r^2[2]	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)
rs401681: C	2,035/4,345	0.5388/0.5697	0.00107[3]	0.883 (0.819–0.951)	NA	NA	NA	NA	NA
rs4975616: A[3]	2,168/4,361	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[4]	NA	NA	NA	NA	0.907	9.96×10^{-5}	0.858 (0.795–0.926)	0.0455	0.803 (0.649–0.996)

Abbreviations: CI, confidence interval; CLPTM1L, CLPTM1-like protein; NA, not applicable; OR, odds ratio; SNP, single-nucleotide polymorphism; TERT, telomerase reverse transcriptase.
[1]Total population following imputation was 2,168 cases and 4,387 controls; rs4975616's imputation *P*-value is generated using the combination of genotyped and imputed data, while rs13356727 is fully imputed.
[2]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 Hardy–Weinberg equilibrium, where *P* is the estimated allele frequency (Li *et al.*, 2010).
[3]Genotyped SNP most associated with melanoma.
[4]Imputed SNP most associated with melanoma.

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 three SNPs exhibit strong LD ($r^2 > 0.8$) with one another (Supplementary Figure S3 online), and all fall within the same LD block that spans the *TERT* promoter and the 3′ end of the *CLPTM1L* gene (Supplementary Figure S2 online). The signal at rs13356727 remained significant following covariation by rs401681 (Table 1). Similarly, covariation by rs13356727 abolished all signals 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 remained 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 nevus count is also associated with melanoma, we hypothesized that the inverse association of rs13356727 with melanoma may have resulted from an interaction with mole count. Self-reported mole count (''None'', ''Few'', ''Some,'' and ''Many'') was available for 1,398 controls and for 1,592 cases with melanoma. Covarying for mole count did not meaningfully change the association between rs13356727 and melanoma (subset melanoma association $P = 4.88 \times 10^{-5}$, OR = 0.800, 95% CI 0.718–0.891; subset covaried by mole count $P = 3.01 \times 10^{-4}$, 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, $P = 0.00042$). The 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 because of 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 that *TERT–CLPTM1L* has 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.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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