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LETTER TO THE EDITOR

WILEY Experimental Dermatology

GSTP1 does not modify *MC1R* effects on melanoma risk

1 | BACKGROUND

Glutathione S-transferases (GSTs) are a group of enzymes that act to detoxify reactive oxygen species resulting from oxidative stress processes and melanin production. *GSTP1* is a polymorphic gene encoding variant proteins involved in metabolism. The role of the rs1695*A/G *GSTP1* Ile105Val polymorphism in cutaneous malignant melanoma (CMM) susceptibility remains controversial. Two recent meta-analyses reported that the *GSTP1* 105Val allele was associated with an increased risk of CMM.[1,2] However, it was concluded by the authors of both publications that additional studies with larger cohorts were required.

The melanocortin 1 receptor (*MC1R*) gene encodes a G-protein coupled receptor involved in the regulation of melanin production and the response to solar ultraviolet.[3] *MC1R* RHC-variant alleles exercise major influence on hair colour, skin colour and sun sensitivity. The association between *MC1R* and susceptibility to CMM and skin cancer in general has been described previously.[4,5]

A 2011 Spanish study reported that the *GSTP1* polymorphism was associated with light coloured hair and increased CMM risk. Furthermore, the authors described a greater penetrance of CMM risk when the *GSTP1* 105Val allele was combined with *MC1R* RHC-variant alleles.[6,7]

2 | QUESTIONS ADDRESSED

This study has further examined the association between rs1695*A/G *GSTP1* Ile105Val polymorphism and CMM. In addition, we examined the previously described effect modification of the combination of *GSTP1* Ile105Val with *MC1R* RHC-variant alleles in a cohort of Queensland control and CMM cases and CMM control and patient samples from Germany. This study included the largest population to date, a total of 27 574 participants.

3 | EXPERIMENTAL DESIGN & RESULTS

The Brisbane Nevus Morphology Study (BNMS) included a total of 738 participants, 342 at high risk of developing CMM were recruited from the Melanoma Unit and Dermatology Department of the Princess Alexandra Hospital (Brisbane, Australia) or from community private dermatology clinics. High risk was defined by previous CMM or CMM affecting a first-degree relative. An additional 396 moderate- and low-risk control volunteers were recruited by public advertisement or by a direct letter sent to previous participants of the Brisbane Twin Nevus Study (BTNS). A total of 761 participants, including 558 CMM and 203 non-CMM patients, were recruited from the Department of Dermatology, University Hospital Tuebingen. To match our German CMM cases, we sourced an additional 1988 ethnically appropriate controls from Augburg Germany as part of the KORA study. QIMR-Berghofer provided access to a large panel of 23 724 individuals unaffected by CMM with direct and imputed genotypes from several rounds of typing in the Brisbane Longitudinal Twin Study (BLTS), combined with several other studies that were made available to be used as a control group (Duffy et al., in submission). A further 363 European samples were accessed from the 1K genome project. Please refer to the supplementary section for further explanation of the genotyping and statistical methods.

Six *GSTP1* polymorphisms were examined to assess for a potential effect on CMM risk (Table 1). *GSTP1* rs4891 was in strong linkage disequilibrium with rs1695 (r^2=0.92, Table S1). Although previous studies have suggested the involvement of rs1695 in CMM risk (also apparent when stratified by study, Table S2), when the number of controls was augmented using the BLTS (n=23 724), German KORA and European samples from the 1K genome project (n=363), the total combined analysis did not show any statistically significant association between rs1695 or rs4891 and CMM risk (*P*=0.45). Moreover, the recently published CMM meta-analysis[8] also does not support the role of *GSTP1* polymorphism in increasing risk (rs1695 *P*=0.62; rs1138272 *P*=0.95; rs4891 *P*=0.72, M. Law personal communication).

Consistent with the previously published literature,[4] this analysis demonstrated a highly statistically significant association between *MC1R* variant genotypes designated using WT, r and R allele classification and risk of CMM (Table 2). These effects were not modified by *GSTP1* rs1695 genotype (A/A, A/G, G/G) in the stratified analysis (overall *P*=0.79). This result was also confirmed when restricted to *MC1R* Arg151Cys genotype (WT/WT, WT/R, R/R), the *MC1R* allele of highest penetrance for CMM and red hair colour (Table S3). Moreover, examination of each *MC1R* allele individually did not show any interaction with *GSTP1* rs1695 genotype and CMM risk (Table S4).

The reported association of rs1695 and light hair colour [6] was not seen in our analysis. However, the rs1138272 *GSTP1* Ala114Val SNP showed weak association with darker hair colour in the BTNS and German case samples. This was not replicated in the analysis of the larger BLTS, Genes and Blood Clotting study (GABC) or UK 1958

TABLE 1 *GSTP1* polymorphisms in control and CMM participants

rs number	Base Change	Amino Acid Change	Polyphen/ SIFT	Frequency in ExAC (European, not Finnish)	Frequency in QIMR controls[a]	Frequency in BNMS unaffected controls[b] (number of alleles)	Frequency in German controls[c]	Frequency in the German KORA study controls[d,e]	Frequency in the 1K Genome project controls[f]	Frequency in BNMS CMM[g] (number of alleles)	Frequency in German CMM[h]	Total Combined Association with CMM[i] (*P* value)
rs192307201	[G/T]	A23S	Benign/ tolerated	0.003 932	N/A	0	0	0	N/A	0.0011 (3)[j]	0	
rs45543438	[G/A]	D58N	Benign/ tolerated	0.0 002 726	N/A	0	0	0	N/A	7×10^{-4} (2)[j]	0	
rs1695[j]	[A/G]	I105V	Benign/ tolerated	0.3191	0.3484	0.3043 (241)	0.2069	0.339[k]	0.2837	0.3392 (232)	0.3692	0.4
rs4986948	[C/G]	T110S	Benign/ tolerated	4.552e-05	N/A	4×10^{-4} (1)[j]	0	0	N/A	0	0	
rs1138272	[T/C]	A114V	Benign/ tolerated	0.07 827	0.084[l]	0.099	0.093	0.088	0.096	0.090	0.0995	0.2
rs4891[m]	[T/C]	S185S	Synonymous	0.3299	0.3577[n]	0.3194 (253)	0.2055	0.351	N/A	0.3491 (239)	0.3750	0.39

N/A, not assessed.
[a]Twenty three thousand seven hundred and twenty four individuals directly genotyped in the QIMR BLTS and other studies.
[b]Three hundred and ninety six individuals.
[c]Fifty eight genotyped of 203 individuals from University Hospital Tuebingen.
[d]One thousand eight hundred and sixty two genotyped of 1988 individuals.
[e]Michigan Imputation server pipeline (s4).
[f]Three hundred and sixty three 1K genome European individuals.
[g]Three hundred and forty two individuals.
[h]Five hundred and fifty eight individuals from University Hospital Tuebingen.
[i]Association shown comparing combined QIMR BLTS+BTNS+All German controls vs combined BTNS+German CMM groups.
[j]Combined for six rare alleles *P*=0.4 would need to have eight to approach significance.
[k]Direct genotypes.
[l]Only 12 808 individuals directly genotyped at rs1138272.
[m]r^2=0.92 between rs1695 and rs4891.
[n]Only 13 440 individuals directly genotyped at rs4891.

birth cohort studies. Neither of these SNPs was associated with skin colour.

4 | CONCLUSIONS

The role of *GSTP1* polymorphism in CMM has been examined in several candidate studies with some positive reports of association.[1,2] In the most recent meta-analysis, an association of rs1695 with CMM risk was detected (*P*=.033 in a dominant model).[1] However, this conclusion is not supported in larger GWAS analyses such as reported in Law et al.[8] who studied 16 000 CMM cases and 26 000 controls. In this study, we similarly saw an association in our German case subsample that did not replicate in the larger German KORA controls or our full sample. Our conclusion is that *GSTP1* polymorphisms do not have any large effect on risk of CMM. The effects of rare variants of *GSTP1* on CMM remain to be clarified in larger studies.

We also examined the hypothesis that *GSTP1* may interact with other CMM risk loci as recently reported.[6] This study is considerably larger than the group studied by Ibarrola-Villava et al. and should have good statistical power to replicate any true association. There was no modification effect found in the combination of *GSTP1* Ile105Val with *MC1R* RHC-variant alleles. Finally, we also excluded the effects of *GSTP1* on phenotypic risk factors for CMM, including hair and skin colour.

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GMD, AP, KJ, KJL and XLHY performed the research; GMD, DLD, KJ, HPS and RAS analysed the data; TKE, BW, BMS, NGM and CG contributed samples and genotypes to the study; and GMD, DLD, HPS and RAS designed the research study and wrote the manuscript. DLD, and RAS are Senior Research Fellows of the Australian NHMRC. This work was funded by project grant NHMRC APP1062935 and the Centre of Research Excellence for the Study of Naevi APP1099021.

CONFLICT OF INTERESTS

The authors have declared no conflicting interests.

Keywords
gene interaction, genetic epidemiology, glutathione S-transferases, polymorphism, susceptibility loci

TABLE 2 Interaction between *MC1R* RHC-variant and *GSTP1* rs1695 genotypes

MC1R[a]	*GSTP1* rs1695	Total	Control[b] N (proportion)	CMM[c] N (proportion)	Odds Ratio (95% CI)
WT/WT	A/A	7314	7185 (0.982)	129 (0.018)	1.00 (referent)
	A/G	7779	7655 (0.984)	124 (0.016)	0.90 (0.70–1.16)
	G/G	2145	2095 (0.977)	50 (0.023)	1.34 (0.96–1.86)
r/WT	A/A	1787	1721 (0.963)	66 (0.037)	2.14 (1.59–2.89)
	A/G	1922	1847 (0.961)	75 (0.039)	2.27 (1.70–3.03)
	G/G	483	467 (0.967)	16 (0.033)	1.96 (1.16–3.30)
R/WT	A/A	936	842 (0.900)	94 (0.100)	6.22 (4.73–8.19)
	A/G	982	869 (0.885)	113 (0.115)	7.24 (5.58–9.41)
	G/G	261	231 (0.885)	30 (0.115)	7.31 (4.82–11.08)
r/r	A/A	341	327 (0.959)	14 (0.041)	2.46 (1.41–4.27)
	A/G	285	274 (0.961)	11 (0.039)	2.32 (1.26–4.30)
	G/G	66	59 (0.894)	7 (0.106)	6.99 (3.21–15.24)
R/r	A/A	343	300 (0.875)	43 (0.125)	8.03 (5.59–11.54)
	A/G	344	299 (0.869)	45 (0.131)	8.43 (5.90–12.05)
	G/G	94	82 (0.872)	12 (0.128)	8.41 (4.52–15.62)
R/R	A/A	166	134 (0.807)	32 (0.193)	13.41 (8.80–20.42)
	A/G	155	125 (0.806)	30 (0.194)	13.48 (8.75–20.77)
	G/G	38	30 (0.789)	8 (0.211)	15.46 (7.09–33.73)
Total		25 441	24 542 [0.965]	899 [0.035]	

[a]WT, r and R alleles were as defined in Beaumont et al.[5]
[b]This includes all control samples from Table 1 excluding the German KORA data. As different genotyping platforms have been used, this has resulted in an underestimate of the true count of MC1R variant allele carriers. In the QIMR BLTS, a number have no MC1R variant alleles directly genotyped and are missing.
[c]This includes all CMM samples from Table 1.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1. Linkage disequilibrium between *GSTP1* rs1695 and rs4891
Table S2. CMM case-controlstatus and *GSTP1* rs1695 genotype by study
Table S3. Interaction between *MC1R* Arg151Cys and *GSTP1* rs1695 genotypes
Table S4. Test of interactions between CMM, *GSTP1* rs1695, and *MC1R* SNP variants
Data S1. Supplementary materials and methods
Data S2. Supplementary references