Polymorphisms in the syntaxin 17 gene are not associated with human cutaneous malignant melanoma

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The prevalence of cutaneous malignant melanoma (CMM) has increased significantly in most Caucasian populations in recent decades. Both genetic and environmental risk factors are involved in the development of CMM. A germline mutation in the syntaxin 17 (STX17) gene of horses was recently identified, which causes premature hair graying and is associated with susceptibility to melanoma. We hypothesized that common germline variants in the STX17 gene might be associated with a predisposition to human CMM or might interact with other melanoma risk genes. We genotyped 26 tagging single nucleotide polymorphisms (SNPs) across the STX17 gene region in an Australian sample of 1560 melanoma cases and 1650 controls and performed logistic regression analysis to identify potential SNP interactions in a combined dataset. Our results do not support an association between CMM and any of the STX17 SNPs and provide no evidence for interactions between the melanoma risk SNP rs910873 on chromosome 20 and any of the STX17 SNPs. We conclude that common variants in the STX17 gene region do not play a key role in the pathogenesis of human melanoma. Melanoma Res 19:80–86 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction
Cutaneous malignant melanoma (CMM) is a form of skin cancer that arises from melanocytes. The prevalence and incidence of melanoma have increased significantly over the past few decades; faster than for any other cancer worldwide, particularly in Caucasian populations [1,2]. The relative recurrence risk to siblings was estimated at 2.24 in a combined study of familial melanomas [3], suggesting that the familial cases carry an inherited susceptibility to CMM. Although the precise etiology of melanoma remains unknown, data from molecular and epidemiologic studies clearly indicate that both genetic and environmental risk factors are involved [4–8].

Several different genes have been identified that are associated with moderate-to-high risk of melanoma [5,9–12]. Multiple somatic and/or germline mutations in these genes increase the risk of developing melanoma through altering normal programs of cell proliferation, differentiation, and apoptosis [13,14]. Our genome-wide association study recently identified and replicated a new melanoma risk locus on chromosome 9q that causes premature hair graying and is also associated with susceptibility to melanoma [16]. Horses homozygous for the mutation showed more rapid graying and have a higher incidence of melanomas in glabrous skin. Strong associations between the TX17 germline mutation, ASIP genotype, and melanoma development were observed in gray horses [16]. One question that arises from the finding is whether variation in STX17 either on its own or through interaction with other melanoma risk variations such as rs910873 is associated with human CMM.

Study of a horse model identified a 4.6 kb duplication in intron 6 of the syntaxin 17 (STX17) gene on chromosome 9 that causes premature hair graying and is also associated with susceptibility to melanoma [16]. Horses homozygous for the mutation showed more rapid graying and have a higher incidence of melanomas in glabrous skin. Strong associations between the TX17 germline mutation, ASIP genotype, and melanoma development were observed in gray horses [16]. One question that arises from the finding is whether variation in STX17 either on its own or through interaction with other melanoma risk variations such as rs910873 is associated with human CMM.

In the STX17 genomic region, four genes are located in close proximity to each other including NR4A3 (nuclear receptor subfamily 4, group A, member 3; OMIM 600542), STX17 (OMIM 604204), TXNDC4 (thioredoxin domain containing 4; OMIM 609170), and INVS (inosin; OMIM 243305). They map to a region of horse chromosome 25, which is syntenic with human chromosome band 9q31.1 [17,18]. The long arm of chromosome 9 has been documented as a region to which a combined ocular–cutaneous melanoma risk gene has been located [19,20]. In addition, loss of heterozygosity on 9q21-33 has been documented in 49% of 76 melanoma cell lines and homozygous deletion of markers in this region were observed in five samples [21]. STX7 belongs to the...
syntaxin family and is expressed in the nuclei of some human melanoma cells [22,23]. These data, together with the cis-acting regulatory mutation that causes premature hair graying and susceptibility to melanoma in the horse, provide strong evidence for a locus on 9q in human CMM. We hypothesized that common germline variants in the region of the \textit{STX17} gene might be associated with predisposition to human melanoma and conducted a case–control study to test for association between SNPs in the \textit{STX17} genomic region and melanoma risk.

\textbf{Materials and methods}

\textbf{Participants}

An Australian case–control panel was made up of 1560 familial melanoma cases drawn from Queensland, unsolicited for age at the onset (The Queensland study of Melanoma: Environment and Genetic Associations, [24]), and 1650 controls drawn from parents of twins enrolled in the Brisbane Twin Nevus Study [25]. There were 5075 twin family members in 1100 pedigrees with some phenotypic data, and DNA was available for genotyping for 3839 individuals within 1037 of these pedigrees. All cases had incident primary melanomas. Tumor location and thickness were recorded. None of the controls had been diagnosed with melanoma. All samples were of European descent. The project was approved by the Human Research Ethics Committee of the Queensland Institute of Medical Research and the Australian Twin Registry. DNAs were extracted from peripheral blood lymphocytes by the salt precipitation method [26].

\textbf{Genotyping}

To cover the region of gray-causing mutation, we selected 26 functional and tagging SNPs ($r^2$ cut off 0.9) based on data from Pielberg's paper [16] and public databases including the International HapMap Project (http://www.hapmap.org/) and NCBI (http://www.ncbi.nlm.nih.gov/). There were eight SNPs selected from the \textit{NR4A3} gene, eight SNPs selected from the \textit{STX17} gene, six SNPs selected from the \textit{TXNDC4} gene, and four SNPs selected from the \textit{INVS} gene. A region of 473.4 kb on chromosome 9 was covered by these SNPs. SNP sequences were downloaded from the Chip Bioinformatics database (http://snpper.chip.org/) and the sequences were cross-checked with NCBI before assay design. Multiplex assays were designed for the 26 SNPs using the Sequenom MassARRAY Assay Design software (version 3.1; Sequenom Inc., San Diego, California, USA). SNPs were typed using iPLEX Gold chemistry (Sequenom Inc.) and analyzed using a Sequenom MassARRAY Compact Mass Spectrometer (Sequenom Inc.). Briefly, the 2.5 \mu l PCR reactions were performed in 384-well plates using 12.5 ng genomic DNA, 0.9 \textmu l of Taq polymerase (HotStarTaq, Qiagen, Valencia, California, USA), 500 \textmu M of each deoxynucleotide-triphosphate, 1.625 mM of MgCl$\textsubscript{2}$, and 100 \textmu M of each PCR primers (Bioneer, Korea). PCR thermal cycling was 15 min at 94\degree C, followed by 45 cycles of 20 s at 94\degree C, 30 s at 56\degree C, and 60 s at 72\degree C. The post-PCR reactions were performed in a final 5 \mu l of extension reaction containing 1x of termination mix, 1x of DNA polymerase, and 570–1240 nmol/l extension primers. A two-step 200 short cycles program was used for the iPLEX Gold reaction as described in our previous study [27]. The products were spotted on a SpectroChip (Sequenom Inc.), and data were processed and analyzed by MassARRAY TYPER 3.4 software (Sequenom Inc.).

\textbf{Statistical analysis}

SNP genotypes were tested for departures from the Hardy–Weinberg equilibrium for 1650 controls using Haploview version 4.1 [28]. Allelic associations between melanoma and the SNPs were tested using the PLINK program (http://pngu.mgh.harvard.edu/purcell/plink/). Associations between categorical groups were tested by use of $\chi^2$ statistics. The global significance level was derived from multiple tests and $P$ values less than 0.05 were considered to be statistically significant. Linkage disequilibrium (LD), haplotype frequencies and blocks were determined by Haploview using the default method of Gabriel et al. [29]. We conducted logistic regression for predicting the possible SNP interactions between the common variation in the \textit{STX17} gene region and the SNP rs910873 [30,31].

\textbf{Results}

We genotyped 26 SNPs across the \textit{STX17} gene in 1560 melanoma cases and 1650 controls after selecting tagging SNPs from the HapMap database ($r^2 < 0.9$) aiming to completely cover this genomic region. The overall genotype completion rate was 98.2\% and all control genotype frequencies were in the Hardy–Weinberg equilibrium. The mean difference in allele frequency between the HapMap database and our controls for the 26 SNPs is 0.007. Strong LD between SNPs was detected in \textit{STX17} (rs7024182 with rs10988912, $r^2 = 0.95$; with rs4742776, $r^2 = 0.82$; and rs10760704 with rs7038506, $r^2 = 0.999$), \textit{NR4A3} (rs7023690 with rs2416878, $r^2 = 0.84$), \textit{TXNDC4} (rs12552646 with rs1361668, $r^2 = 0.94$ and with rs1535667, $r^2 = 0.89$) and \textit{INVS} (rs7020636 with rs16918878, $r^2 = 0.95$). Allele frequencies did not differ significantly between cases and controls for any of the SNPs (Table 1) and therefore none was significantly associated with melanoma (Table 1, Fig. 1a).

As the horse melanomas occur primarily as firm, jet black nodules well circumscribed in the dermis of glabrous skin (nonhair bearing), we hypothesized that variation in the \textit{STX17} gene region may contribute to risk of CMM in a site-specific manner. The allele frequency differences between melanoma body site (glabrous versus non-glabrous) and controls were analyzed. A weak allele association with melanoma for the \textit{STX17} 5’ upstream SNP rs7024182 was detected in the 117 melanomas located on...
Table 1 Association analyses of the polymorphisms genotyped in 1560 familial melanoma cases and 1650 controls

<table>
<thead>
<tr>
<th>dbSNP ID</th>
<th>Position</th>
<th>Gene</th>
<th>Role</th>
<th>Alleles</th>
<th>Case</th>
<th>Control</th>
<th>OR (95% CI)</th>
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<td>0.107</td>
<td>3.365</td>
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</table>

Chr9, chromosome 9; CI, confidence interval; dbSNP, database single nucleotide polymorphism; OR, odds ratio.

Discussion

A recent study from our group identified a new melanoma risk locus close to the ASIP gene on chromosome 20 [15]. To identify possible SNP interactions between the STX17 common variation and the SNP rs910873, which was strongly associated with CMM on chromosome 20, we conducted logistic regression analysis in an attempt to identify the CMM risk conferred by SNP interactions. In the combined data, we did not detect SNP–SNP interactions between SNP rs910873 and any of the STX17 SNPs. Moreover, we investigated the effects of common variation at STX17 on melanocytic nevus count and on pigmentation classifications of eye and hair colors, but found no evidence for association between any of the STX17 SNPs and any of these phenotypes.

To further evaluate the association signal observed from SNP rs7024182, we performed analyses based on primary clinical phenotypic data available (Table 2). Stratification of cases according to site of melanoma produced the smallest P values of 0.008 and 0.023 for melanomas on the face for allelic association and genotypic association tests, respectively. Genotyping frequency differences between 96 facial tumors and 1650 controls gave a significant P value of 0.033 (\(\chi^2=4.54\)) for a dominant model and a significant P value of 0.022 (\(\chi^2=5.19\)) for a recessive model. The differences were, however, not significant after correcting for multiple testing.

A germline mutation in intron 6 of the STX17 gene has been reported to cause premature hair graying and susceptibility to melanoma in gray horses [16]. We screened STX17 for association with melanoma risk
because of the recent report of strong association between the \textit{STX17} germline mutation, \textit{ASIP} genotype, and melanoma development in horses, and because the location of the \textit{STX17} gene corresponds to a human melanoma susceptibility region on chromosome 9q.

\textit{STX17}, together with neighboring \textit{NR4A3} (encoding a member of the \textit{NR4A} orphan nuclear receptor family), which has been associated with cell-cycle regulation and has an established link with carcinogenesis [33], was highly expressed in horse melanomas. \textit{STX17} belongs to the syntaxin family, which encodes membrane proteins involved in synaptic vesicle fusion [22]. Expression of \textit{STX17} in the nuclei of some human melanoma cells indicated a distinct role for \textit{STX17} [23]. To determine whether an association exists between common variants of \textit{STX17} and human CMM, we genotyped 26 tag SNPs across the four genes in the region. The tagging SNPs we selected for this study could capture 100% of alleles with an $r^2$ cut-off at 0.9 based on HapMap Center d’Etude du Polymorphisme Humain population (CEU). If the genetic predisposition of melanoma is influenced by common variation in the region, we would expect to detect evidence of association in the SNPs and SNP haplotypes in our sample. Our results do not support an association between melanoma and common variation in the \textit{STX17} gene in human melanoma predisposition. Furthermore, we found no evidence for SNP–SNP interactions between rs910873 in the region of \textit{ASIP} and any of the \textit{STX17} SNPs. Haplotype analyses using sliding windows of 2–5 contiguous SNPs did not identify any evidence for association between the tested variants and melanoma. Our results, however, do not exclude the possibility that either unknown variants in weak LD with...
the genotyped SNPs or rare variants of large effect in this region influence melanoma risk.

Determination of the anatomic site distribution of CMM is not fully understood, although sun exposure is believed to be associated causally with the disease. A study of 844 patients with head and neck melanoma found that density of melanomas was increased by a factor of 2.6 on the face compared with 4858 patients with melanoma at other anatomical sites [34]. A similar association was observed in an Australian population [35,36], whereas others did not support site-specific theory [37–40].
It has been postulated that the \textit{STX17} duplication leads to proliferation of dermal melanocytes in glabrous (nonhairy) skin, thus predisposing to melanoma development [16]. We hypothesized that variation in the \textit{STX17} gene may contribute to risk of CMM in a site-specific manner. Data analysis using site-restricted cases provided weak evidence for association between the \textit{STX17} 5' upstream SNP rs7024182 and CMM on the face, but no association was observed for this SNP with CMM on the external ear. This result may be because of the size of site-restricted sample, which is too small to have sufficient power to detect a true association. As the overall results did not support an association between common variation in the \textit{STX17} gene region and CMM, we conclude that if the risk of melanoma on glabrous skin is influenced by the \textit{STX17} 5' upstream SNP rs7024182, the effect size would not be large. Replication studies are required to confirm or refute this result.

In summary, we examined the association between CMM and common SNPs or haplotypes in human CMM in the region syntenic to the horse gray-causing mutation containing the \textit{STX17}, \textit{NR4A3}, \textit{TXNDC4}, and \textit{INV3} genes. Our data do not support an association between common variation in these genes and melanoma risk. We found no evidence for SNP–SNP interactions between SNP rs910873 (\textit{ASIP}) and any of the \textit{STX17} SNPs. We conclude that common variants in the \textit{STX17} gene region do not play a key role in the pathogenesis of human CMM.

**Acknowledgements**

This study was supported by the National Cancer Institute (NCI) of the US National Institutes of Health (CA88363) and the National Health and Medical Research Council of Australia (NHMRC) (380385, 389892, 496675, and 402761). N.K.H. and G.W.M. are supported by the NHMRC fellowships scheme. The authors state no conflicts of interest.

**References**


