Libertas Academica
FREEDOM TO RESEARCH

Open Access: Full open access to this and thousands of other papers at http://www.la-press.com.

Biomarkers in Cancer

Susceptibility to Cutaneous Squamous Cell Carcinoma in Renal Transplant Recipients Associates with Genes Regulating Melanogenesis Independent of their Role in Pigmentation

Per A. Andresen[1], Dag A. Nymoen[1], Kristina Kjærheim[2], Torbjørn Leivestad[3] and Per Helsing[4]

[1]Department of Pathology, Oslo University Hospital, Oslo, Norway. [2]Cancer Registry of Norway, Oslo, Norway. [3]Norwegian Renal Registry, Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway. [4]Department of Dermatology, Oslo University Hospital, Oslo, Norway.

ABSTRACT: The highly polymorphic melanocortin 1 receptor (*MC1R*) gene plays a crucial role in pigmentation. Variants of the gene have been implicated in risk of cutaneous squamous cell carcinoma (SCC) in the general population. In renal transplant (RT) recipients these cancers are more aggressive and very common. To evaluate the risk of SCC relative to *MC1R* and the pigmentation-associated genes *ASIP*, *TYR*, and *TYRP1*, a group of 217 RT recipients with and without SCC was genotyped. Associations with SCC risk were indicated in carriers of the red hair color associated *MC1R* variant p.Arg151Cys (OR = 1.99; 1.05–3.75), and in carriers of two of any of the *MC1R* variants disclosed (OR = 2.36; 1.08–5.15). These associations appeared independent of traditionally protective phenotypes, also supported by the stratifications from skin phototype and hair color. A tendency towards an increased SCC risk was observed for a specific *ASIP* haplotype (OR = 1.87; 0.91–3.83), while no such associations were observed for the *TYR* and *TYRP1* variants. Thus, the risk of developing SCC in RT patients is modulated by *MC1R* variation irrespective of phenotypes considered to be protective. Heterozygous combinations of *MC1R* variants appear to be more relevant in assessing SCC risk than the effects of variants individually.

KEYWORDS: squamous cell carcinoma, melanocortin 1 receptor, MC1R, genetic polymorphisms

CITATION: Andresen et al. Susceptibility to Cutaneous Squamous Cell Carcinoma in Renal Transplant Recipients Associates with Genes Regulating Melanogenesis Independent of their Role in Pigmentation. *Biomarkers in Cancer* 2013:5 41–47 doi:10.4137/BIC.S12754.

TYPE: Original Research

FUNDING: PAA and PH have received grants from The Norwegian Radium Hospital Research Foundation, Comprehensive Cancer Center (CCC).

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: per.arne.andresen@ous-hf.no

Introduction

Improved long term graft survival and better quality of life in organ transplant recipients are explained by increasingly better graft versus host compatibility and harmonization between allograft functionality and immunosuppression.[1] However, this progression comes with an overall increased risk of developing cancer, in particular non-melanoma skin cancer.[2,3] Squamous cell carcinoma (SCC) represents the major challenge conferring both increased morbidity and mortality.[4] Standardized incidence ratios for developing SCC in graft recipients are 65–250 times higher than in the general population.[2,5] The main reason is immunosuppressive therapy causing reduced host immunosurveillance and direct oncogenic effects.[6]

The melanocortin 1 receptor (*MC1R*) gene encodes a seven pass transmembrane G protein-coupled receptor in melanocytes with a key role in regulation of melanogenesis.[7,8] The gene is highly polymorphic in populations with lighter skin and some variants have been associated with both melanoma and non-melanoma skin cancer.[9–11] The ability of MC1R to bind α-melanocortin stimulating hormone (αMSH) and activate adenylate cyclase to catalyze the production cAMP

is crucial to pigmentation. UV induced αMSH stimulation regulates the ratio between the black-brown eumelanin and the yellow-red pheomelanin and increases melanocyte proliferation, dendricity, and melanosome transfer to keratinocytes.[12] Other cytoprotective roles of *MC1R* involve antioxidant defense, DNA repair, and regulation of inflammatory responses through NF-κB signaling pathways.[7,13,14] More than 100 *MC1R* variants resulting in alternative amino acids at specific codons, normally termed as non-synonymous variants, have been described with highly variable frequencies.[15] Some are referred to as the RHC (red hair color) variants (p.Asp84Glu, p.Arg151Cys, p.Arg160Trp, and p.Asp294His) because of their associations with red hair, fair skin, and poor tanning. In contrast, those less associated with these phenotypic traits (p.Val60Leu, p.Val92Met, and p.Arg163Gln) are categorized as non-RHC (NRHC) variants.[8,16] The only study committed to the impact of *MC1R* variation on risk of SCC in RT patients is a thesis stating that an increased SCC risk was observed in carriers of the RHC associated variants p.Asp84Glu and p.Arg151Cys, and that the increased risk was independent of pigmentation.[17]

The agouti signaling protein encoded by the *ASIP* gene antagonizes ligand-induced basal cAMP activity resulting in default pheomelanin pigmentation.[18] A specific *ASIP* haplotype (AH) based on two single nucleotide polymorphisms has been associated with red and blond hair, tanning sensitivity, and increased risk of skin cancer.[19,20] Downstream of *MC1R*, a tyrosinase (*TYR*) is involved in both eumelanin and pheomelanin synthesis, while a tyrosinase related protein (*TYRP1*) is involved in eumelanin synthesis exclusively.[21] Polymorphic variants of *TYR* and *TYRP1* associate with blue eye color, increased skin sensitivity to sun, and melanoma risk.[20]

With reference to skin type, hair and eye color, the aim of this study was to assess the impact of *MC1R* variation on SCC risk in RT patients. Also the relationship between the naturally occurring variants of *ASIP*, *TYR*, and *TYP1* and susceptibility to SCC was explored. This is the first comprehensive report dedicated to the correlation between genetic variation associated with phenotypic traits and susceptibility to SCC risk in RT patients.

Materials & Methods

Study subjects. All participants invited (n = 555) were recruited through the Norwegian Renal Registry. They were all above the age of 18 with functional renal grafts at time of invitation. At least one invasive SCC was diagnosed in 185 (33.3%) of the invitees. Two SCC negative controls were matched by gender, year of birth (+/– 3 years), and duration of grafts (+/– 3 years) for each case according to the incidence density sampling method.[22] Among those who eventually volunteered to participate (n = 217; 39.1%), SCC was diagnosed in 80 patients (36.9%). All study participants provided informed consent, delivered EDTA-blood for DNA analyses, and responded to a questionnaire reporting skin phototype, hair and eye color, and the presence of skin lesions considered as warts or "warty-like" lesions (Table 1). In short, the typing of skin refers to skin phototype 1 (SPT1) as white, always burns and never tans; SPT2 as having some tanning response, otherwise as SPT1; SPT3 have white skin with a gradual and moderate tanning potential with minimal burns; whereas SPT4 has light brown skin with good tanning response and minimal burns.[23] The representation of transplant recipients with and without SCC were evenly distributed throughout the different therapeutic eras administrating azathiopurine (Aza), prednisone (Pred), cyclosporine A (CsA), mycophenolate mofetil, and tacrolimus (1968–1983; Aza+Pred; 1983–1985: CsA+Pred; 1985–1987: CsA+Pred, or Csa+Aza+Pred, randomized; 1987–2000: Csa+Aza+Pred; 2000-onwards: gradually conversion from Aza to mycophenolat mofetil, and CsA to tacrolimus). Initial immunosuppression is normally used during the grafts entire life. The study was approved by the Regional Ethical Committee and performed according to the Helsinki declaration.

***MC1R* DNA sequencing.** The complete coding region of *MC1R* (NM_002386.3) was amplified and sequenced to anticipate *MC1R* variation in the Norwegian population (details available upon request to communicating author). Seven non-synonymous variants were identified, all previously recognized as common single nucleotide polymorphisms (SNPs) (Supplemental data; Table S1). These were the red hair (RHC) associated variants p.Asp84Glu (rs1805006), p.Arg151Cys (rs1805007), p.Arg160Trp (rs1805008), and p.Asp294His (rs1805009), and the non-red hair color (NRHC) variants p.Val60Leu (rs1805005), p.Val92Met (rs2228479), and p.Arg163Gln (rs885479).

The common SNPs of *ASIP*, *TYR*, and *TYRP1* were analyzed on a MALDI-TOF mass spectrometry platform (Sequenom, San Diego, CA) platform, at Centre of Integrative Genetics (CIGENE) at the Norwegian University of Life Sciences, Ås, Norway (www.cigene.no). To assess the reproducibility of MALDI-TOF, all the initially sequenced RT patient samples were included and retested. Two intergenic SNPs near the *ASIP* locus, rs1015362 (G>A) and rs4911414 (G>T), define a specific *ASIP* haplotype (AH; alleles G and T, respectively). The *TYR* SNP results in an alternative amino acid at codon 402 (p.Arg402Gln; rs1126809), and the *TYRP1* variant is an inter-gene nucleotide transition with unknown consequence (rs1408799; C>T). PCR and extension primers were designed using the Sequenom Spectro DESIGNER software (version 3.0).

Statistics

The statistical models were adjusted for gender, age at transplantation, and age at inclusion. Only the results from the unconditional analyses are presented as they resulted in essentially the same risk estimates as the conditional analyses. All analyses were conducted using Stata statistical software, release 11 (Stata Corporation; 2009. College Station, TX).

Cross-tabulations were used to assess clinical (SCC *versus* non-SCC) relative to phenotypic categorization and carrier status. Two-sided P values less than 0.05 indicated statistical significance. Hardy Weinberg equilibrium (HWE) was assessed for *MC1R* genotype distribution among the participants (Supplemental data, Table S1). *MC1R* genotype categorization; *Wild type*: absence of any of the identified variants; *Any variant*: combinations of any or number of variants; *1 NRHC/RHC*: NRHC/WT or RHC/WT; *2 NRHC/RHC*: NRHC/NRHC, NRHC/RHC, or RHC/RHC; *1–2 NRHC*: NRHC/WT or NRHC/NRHC; *1–2 RHC*: RHC/WT or RHC/RHC. Odds ratio (OR) with a 95 % confidence interval (CI) according to multivariable unconditional logistic regression models were used to assess interactions between genotypes, phenotypic traits, and relative risk of SCC. Estimation of *ASIP* haplotype distribution (based on genotyping the rs1015362 and rs4911414 SNPs) was done by an expectation-maximization algorithm using the Haploview program.[24]

Results

Based on Hardy Weinberg equilibrium the entire group of RT patients appeared representative of the general (Norwegian) population (Supplemental data; Table S1). As presented in Table 1 the matching between RT cases with and without SCC appeared unaffected by the overall low response rate (39.1%). The representation of males dominated equally (~63%) in both

Table 1. Clinical variables and phenotypic characteristics of renal transplant patients with (SCC positive) and without (SCC negative) squamous cell carcinoma of the skin.

CLINICAL VARIABLES	SCC POSITIVE (n = 87) % (*n*)		SCC NEGATIVE (n = 130) % (*n*)		
Males (n)	63.2 (55)		62.3 (81)		
Females (n)	36.8 (32)		37.7 (49)		
Mean age at transplantation	**Years**	**Range/St.dev.**	**Years**	**Range/St.dev.**	**p-value**
All	45.0	13–74/16.2	45.1	6–80/14.9	0.94
Males	47.3	19–74/15.9	46.9	6–72/13.7	0.89
Females	41.1	13–70/16.3	42.3	14–80/16.4	0.75
Mean age at inclusion	**Years**	**Range/St.dev.**	**Years**	**Range/St.dev.**	**p-value**
All	56.4	26–75/11.4	56.8	32–89/10.7	0.77
Males	58.0	27–75/10.7	58.2	37–87/8.7	0.89
Females	53.6	26–74/12.3	54.6	32–89/13.1	0.75
Mean duration of graft	**Years**	**Range/St.dev.**	**Years**	**Range/St.dev.**	**p-value**
All	11.7	1–32/7.7	11.2	0–35/7.6	0.65
Males	11.1	1–30/7.6	10.6	1–35/7.3	0.70
Females	12.8	1–32/7.9	12.3	0–30/8.0	0.79
Phenotypes	***n***	**%**	***n***	**%**	**p-value**
Skin phototypes					
1	6	7.0	13	10.1	
2	18	20.9	17	13.2	
3	51	59.3	70	54.3	0.15
4	11	12.8	29	22.5	
Hair color					
Dark Blond	40	46.0	66	51.2	
Blond	30	34.5	43	33.3	0.56
Light blond	8	9.2	13	10.1	
Red	9	10.3	7	5.4	
Eye color					
Blue	68	78.2	102	80.3	0.70
Non blue	19	21.8	25	19.7	
Warts					
Negative	12	14.8	47	38.2	0.00
Positive	69	85.2	76	61.8	

groups. Mean ages at first transplantation (~45 years) and age of allograft (11–12 years) did not differ significantly between the groups. No significant associations between skin phototypes, hair and eye color, on one side, and SCC on the other, were observed.

Seven non-synonymous variants of *MC1R* were characterized by minor allele frequencies ranging from 1.6% (p.Asp84Glu) to 12.2% (p.Arg151Cys). The only variant apparently associated with risk of SCC specifically was the RHC allele p.Arg151Cys (OR = 1.99; CI: 1.05–3.75) (Supplemental data; Table S1). When adjusted for the concurrent presence of other *MC1R* variants or red hair, the basis for estimating the significance of p.Arg151Cys on an individual basis, diminished (not shown). Carriers of any variant or combination of variants implied a non-significant risk (OR = 1.85; CI: 0.92–3.69), while carriers of two variants reached a significant association (OR = 2.36; CI: 1.08–5.15) (Table 2). These estimates were unaffected when adjusted for eye, skin, or hair phenotypes (Supplemental data; Table S2). However, when stratified by phenotypic traits (Table 3), a significant elevation in SCC risk was observed in carriers of any *MC1R* variant combination with the darker skin phototype (SPT3) (OR = 3.94; CI: 1.37–11.30). Against this background, it appeared sufficient to carry one of any variant combined with the wild type allele (OR = 3.48; CI: 1.14–10.60); not differing significantly from those being carriers of two *MC1R* variants of any type (OR = 4.62; CI: 1.47–14.60) (Table 3 and Supplemental data; Table S3). When stratified by hair color, two of any of the *MC1R* variants indicated a higher risk of SCC in blond haired individuals (OR = 10.50; CI: 1.86–59.27). Assessing the NRHC and RHC genotype groups individually revealed that only carriers of NRHC alleles reached significance (OR = 7.29; CI: 1.39–38.20) (Table 3). All red-haired individuals (n = 16) were consistently positive for at least one RHC variant and negative for any NRHC variant; a representation observed evenly distributed between those with and without SCC (Supplemental data; Table S4). When stratified by eye color, carriers of two of any *MC1R* variant and carriers of 1–2 NRHC variants indicated an increased risk within the blue-eyed group of patients (OR = 2.80; CI: 1.15–6.83, and OR = 2.50; CI: 1.02–6.16, respectively) (Table 3 and Supplemental data; Table S5). The presence of self-reported warts correlated with a relatively high SCC risk apparently independent of *MC1R* (Tables 1 and 3, and Supplemental data; Table S6). This was consistent with the observed independence between *MC1R* and warts (Supplemental data; Table S7).

In the SCC-positive and -negative groups, 80 to 86, and 118 to 128, respectively, were informative for the assessment of *ASIP*, *TYR*, and *TYRP1* variants relative to SCC (Supplemental data; Table S8). None of the individual *ASIP* polymorphisms were significantly associated with SCC, although an overrepresentation was observed for the *ASIP* G>T transversion (rs4911414) in the SCC group. The non-synonymous *TYR* (p.Arg402Gln) and *TYRP1* variants appeared insignificant in imposing risk (Supplemental data; Table 8). Seven of nine possible *ASIP* genotypes were observed of which the estimated *ASIP* haplotype (AH) representation tended towards increased risk of SCC (OR = 1.87; CI: 0.91–3.83) (Table 4 and Supplemental data; Table S8).

Table 2. Odds ratio (OR) with 95% confidence intervals (CI) for risk of SCC in renal transplant patients related to *MC1R* variation (RHC variants: p.Asp84Glu, p.Arg151Cys, p.Arg160Trp, and p.Asp294His. NRHC-variants: p.Val60Leu, p.Val92 Met, and p.Arg163Gln). Adjustments for phenotypic traits are provided in supplemental data (Table S2).

VARIABLE	SCC POSITIVE *n* (%)	SCC NEGATIVE *n* (%)	OR[a]	95% CI
Wild type	14 (16.1)	34 (26.2)	1.00	
Any variant	73 (83.9)	96 (73.8)	1.85	0.92–3.69
Number of variants				
1 NRHC/RHC	39 (44.8)	61 (46.9)	1.55	0.74–3.26
2 NRHC/RHC	34 (39.1)	35 (26.9)	**2.36**	**1.08–5.15**
Type of variant				
1–2 NRHC	31 (36.6)	39 (30.0)	1.93	0.88–4.21
1–2 RHC	42 (48.3)	57 (43.8)	1.79	0.85–3.75

[a]Significant odds ratios are indicated by bold numbers.

Discussion

Among the more than 100 genes implicated in pigmentation, the key signaling regulator *MC1R*, its antagonist *ASIP*, and the downstream melanization regulatory genes *TYR* and *TYRP1*, remain the most extensively studied in relation to skin cancer.[25–27] Here we demonstrate that *MC1R* variation has a significant impact on risk of developing SCC in RT patients independent of conventional risk phenotypes. Carriers of the RHC variant p.Arg151Cys, or carriers of two *MC1R* variants independent of being represented by NRHC or RHC alleles, indicated a significant risk of SCC. After stratifications by phenotypic traits the significance of *MC1R* was supported even further as traits traditionally associated with skin cancer protection were shown inferior to the impact of *MC1R*. None of the other pigmentation-associated genes were found to have a significant influence on SCC risk in RT patients.

No more than two of any of the *MC1R* variants were carried simultaneously among the participants in this study. This is in line with previous population- and haplotyping-based studies indicating lack of linkage disequilibrium between Caucasian-prevalent *MC1R* variants.[26,28] In accordance with an anticipated south to north gradient in Western Europe, Norway appears to have the highest frequencies of common European *MC1R* alleles.[28,29] Because of the high *MC1R* variability and the low allele frequencies for some of the variants, the categorization into genotypes related to strong or weak associations with risk phenotypes (RHC and NRHC,

Table 3. Associations between *MC1R* genotypes and SCC risk reflected by odds ratios after stratifications by phenotypic traits.

PHENOTYPIC TRAITS	SKIN PHOTOTYPE				HAIR COLOR				EYE COLOR		WARTS (UNSPECIFIED)	
	1	2	3	4	DARK BLOND	BLOND	LIGHT BLOND	RED	BLUE	NON-BLUE	NEGATIVE	POSITIVE
SCC/non-SCC[a]	6/13	18/17	51/70	11/29	40/46	30/43	8/13	9/7	68/102	19/25	12/47	69/76
						OR 95% CI[b]						
Wild type	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	1.00	1.00	1.00	1.00
Any variant	0.36 0.04–3.52	1.08 0.22–5.22	**3.94** **1.37–11.30**	0.85 0.18-4.10	1.50 0.60–3.72	5.42 0.11–26.40	0.30 0.04–2.42	–	2.19 0.99–4.88	0.94 0.21–4.10	1.35 0.25–7.19	**3.08** **1.31–7.21**
1 NRHC/RHC	0.43 0.04–4.64	0.67 0.12–3.75	**3.48** **1.14–10.60**	0.93 0.18-4.86	1.41 0.54–3.69	3.65 0.71–18.79	0.40 0.04–3.96	–	1.83 0.78–4.30	0.80 0.17–3.82	0.80 0.13–5.08	**2.84** **1.14–7.05**
2 NRHC/RHC	0.25 0.01–4.73	2.00 0.32–12.10	**4.62** **1.47–14.60**	0.67 0.08-5.30	1.71 0.55–5.34	**10.50** **1.86–59.27**	0.22 0.02–2.45	–	**2.80** **1.15–6.83**	1.25 0.22–7.08	2.50 0.41–15.23	**3.43** **1.32–8.91**
1–2 NRHC	0.25 0.01–4.73	2.00 0.22–17.90	**3.83** **1.23–12.00**	1.04 0.17-6.23	1.44 0.50–4.14	**7.29** **1.39–38.20**	0.17 0.01–2.82	–	**2.50** **1.02–6.16**	0.83 0.16–4.44	0.71 0.09–5.96	**3.47** **1.32–9.08**
1–2 RHC	0.43 0.04–4.64	0.91 0.18–4.64	**4.04** **1.32–12.40**	0.72 0.12-4.16	1.54 0.57–4.14	3.88 0.73–20.80	0.38 0.04–3.34	–	2.01 0.86–4.70	1.02 0.21–4.98	0.22 0.04–1.27	**3.65** **1.39–9.61**

[a]SCC/non-SCC: Number of patients within the SCC positive (SCC) and negative (non-SCC) groups with informative genotypes.
[b]Significant odds ratio estimates indicated in bold numbers.

respectively), seemed appropriate. The risk of developing SCC in the general population has been evaluated based on similar genotype categorizations.[26,30]

The impact on SSC risk appears to depend on combinations of alleles rather than on the individual allele or whether the allelic variants are associated with a particular phenotype. This may explain the discordance between SCC risk and traditional risk phenotypes. When stratified by skin phenotypes, patients with the darker skin phototype SPT3 had significant and similar odds ratios for all allelic combinations when compared to wild type *MC1R*. Being a carrier of any of these *MC1R* genotypes appeared to negate the protection normally afforded by darker skin. In the general population, individuals with darker or olive colored skin being carriers of loss-of-function *MC1R* have been shown to be at an increased risk of developing skin cancer.[10,11,31] The influence of NRHC variants was prominent when stratified by hair color. Initially, when assessing the distribution of the individual NRHC variants independent of phenotypes, only p.Arg163Gln was overrepresented in the SCC group, but without indicating significant risk. The other two (p.Val60Leu and p.Val92Met) were more or less equally distributed between those with and without SCC. This suggests that the effects on risk associate with variable dominant negative action where the impact of the individual NRHC alleles are masked and affected by the combination of alleles.

Unless stratified by phenotypic traits only the strongly red hair associated p.Arg151Cys variant and a dual representation of any *MC1R* variants were found associated with SCC. This is consistent with the only previous report that to our knowledge addresses the correlation between *MC1R* and SCC in RT patients.[17] However, the significance of p.Arg151Cys is obscured when adjusting for the concurrent presence of other *MC1R* variants or red hair; again pointing towards the necessity of assessing the overall *MC1R* genotype. In the general population a three-fold increase in risk has been indicated in carriers of NRHC/RHC compound heterozygotes followed by a two-fold increase in heterozygous WT/NRHC and WT/RHC carriers, and with the least impact on risk associated with homozygous representation of variants, all independent of traditional risk phenotypes.[26] Signaling conveyed by dimeric and oligomeric proteins like MC1R is vulnerable to structural changes caused by non-synonymous polymorphisms in the parental alleles. Such polymorphisms apparently have an effect on signaling because of variation in cell surface expression and density, organelle retention affecting trafficking, and G-protein coupling.[12,16,32–34] The considerable variation in residual signaling generated from the numerous allelic combinations complicates the establishment of meaningful correlations between *MC1R* variation and phenotypes.[15] Similar challenges in assessing risk of SCC are conceivable. Also because RT recipients are treated with combinations of immunosuppressive drugs at different strengths and that these regimens have changed over time, the impact of changes in therapeutic conditions on cancer risk is difficult to assess.

Table 4. The significance of the *ASIP* haplotype (AH) relative to SCC risk as indicated by odds ratio (OR) with 95% confidence intervals in RT patients with (SCC) and without (Non-SCC) squamous cell carcinoma.

ASIP HAPLOTYPE (AH)[a]	SCC-POSITIVE *n* = 160	%	SCC-NEGATIVE *n* = 236	%	OR	95% CI
Non-AH	142	88.8	221	93.6	1.0	
AH	18	11.2	15	6.4	1.87	0.91–3.83

[a]Background data are given under Supplemental data (Table S8).

The *ASIP* haplotype (AH) based on two specific polymorphisms tended towards an increased SCC risk. However, it appeared that only one of the polymorphisms (*ASIP* G>T; rs4911414) contributed to this tendency. An overrepresentation was observed for this variant among the *ASIP* genotypes and estimated haplotypes in the SSC group of patients (Supplemental data; Table S8). This particular polymorphism has been associated with increased SCC risk in the general population.[30] A strong association between AH, red hair, and reduced tanning ability supports gain-of-function for this haplotype with the potential of inhibiting MC1R signaling.[20,35] A broader systematic study is necessary to conclude on the significance of this haplotype relative to SCC risk in RT patients. The Gln-allele of the *TYR* p.Arg402Gln variant was overrepresented in the SCC positive patients but without reaching a significant association. A haplotype based on p.Arg402Gln and another *TYR* variant (p.Ser192Tyr) has been associated with SCC risk in the general population and should be assessed together in future studies.[30] The lack of a correlation between SCC and *TYRP1* was consistent with observations in the general population.[20,21]

Warts reported by the patients themselves appeared significantly associated with SCC. No dosage effect was observed when estimated in concert with *MC1R* variants indicating that these lesions and *MC1R* are mutually independent. This was also supported by the observation that *MC1R* apparently does not modulate risk of contracting warts (Supplemental data; Table S7). As self-reported, no reference to histological classification exists and the correlations should therefore be evaluated accordingly. A recent study of organ transplant patients demonstrated a significant association between rare subtypes of warts like verrucokeratotic lesions and verrucous papilloma and SCC, but not with the more common verrucae vulgares and flat warts.[36] Solar keratosis is an indicator of excessive sun exposure and SCC risk in the immunocompetent population where the majority of keratotic lesions regress spontaneously.[37] Whether the potential of regression in immunocompromised patients is affected by marginal or substantial differences in keratinocyte differentiation imposed by *MC1R* remains to be seen. To address this issue further a histological differentiation between SCC and other possible non-pigmented epithelial tumors of the skin is necessary.[38]

The increasing incidence of skin cancers and the general lack of public awareness encourage the institution of guidance protocols for supervising patients and health personnel about SCC risk factors and precautionary measures. The criteria for stratifying risk in relation to cumulative sun-exposure, pigmentation, individual and family history of solar-associated skin cancer, are uncontroversial.[39] Before implementing genetic testing of candidate genes like *MC1R* as an integral part of risk assessment, broader and more systematic studies searching meaningful correlations between *MC1R* variation, phenotypes, and risk of histological verified SCC, are essential. At this point a sober message to be carried forward is that traditionally low and high risk phenotypes do not necessarily imply a reduction or an increase in SCC risk, respectively. The modest statistical power in this study as indicated by wide confidence intervals warrants cautious consideration of the presented data. However, we conclude that a correlation exists between *MC1R* genotype variation and the risk of developing SCC in RT patients independent of traditional risk phenotypes. The current data do not allow an explicit differentiation between the SCC risk in immunocompromised patients and the risk in the general population.

Acknowledgements

We thank Drs. Helge Scott and Øivind Nilssen for reading and commenting on the manuscript during preparation. We also appreciate the support concerning Haploview analysis given by Dr. Stefan Johansson.

Author Contributions

Conceived and designed the experiments: PAA, PH. Analyzed the data: PAA, KK. Wrote the first draft of the manuscript: PAA. Contributed to the writing of the manuscript: PAA, PH. Agree with manuscript results and conclusions: PAA, DAN, KK, TL, PH. Jointly developed the structure and arguments for the paper: PH. Made critical revisions and approved final version: PAA, PH. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

Supplemental Data

Supplement table 1. The distribution and frequencies of *MC1R* alleles and genotypes in all renal transplant (RT) patients (All), with (SCC positive), and without (SCC negative) squamous cell carcinoma, and estimated odds ratios (OR) with 95% confidence intervals (95% CI) as indicators of SCC risk.

Supplement table 2. Adjustments for phenotypic traits and odds ratio (OR) with 95% confidence intervals (CI) indicative of SCC in RT patients related to *MC1R*.

Supplement table 3. Stratifications by skin phototypes (SPT); Associations between *MC1R* genotypes and SCC risk indicated by odds ratio (OR) with 95% confidence intervals (95% CI).

Supplement table 4. Stratifications by hair color; Associations between *MC1R* genotypes and SCC risk indicated by odds ratio (OR) with 95% confidence intervals (95% CI).

Supplement table 5. Stratifications by eye color; Associations between *MC1R* genotypes and SCC risk indicated by odds ratio (OR) with 95% confidence intervals (95% CI).

Supplement table 6. Relation between the presence of wart-like lesions (WLL) and risk of SCC as reflected by odds ratios (OR) and 95% confidence intervals (95% CI) independent (**A**) and dependent on *MC1R* genotypes (**B**).

Supplement table 7. *MC1R* genotypes related to the incidence of self-reported warty-like lesions (WLL).

Supplement table 8. Odds ratios with confidence intervals after distribution of specific *ASIP*, *TYR*, and *TYRP1* alleles (**A**), observed *ASIP* genotypes (**B**), and estimated *ASIP* haplotypes (**C**) in patents informative for assessment (All), SCC positive (SCC), and SCC negative (Non-SCC) patients.

REFERENCES

1. Penn I. Post-transplant malignancy: the role of immunosuppression. *Drug Saf.* 2000;23(2):101–3.
2. Jensen P, Hansen S, Møller B, et al. Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. *J Am Acad Dermatol.* 1999;40(2):177–86.
3. Webster AC, Craig JC, Simpson JM, Jones MP, Chapman JR. Identifying high risk groups and quantifying absolute risk of cancer after kidney transplantation: a cohort study of 15,183 recipients. *Am J Transplant.* 2007;7(9):2140–51.
4. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. *N Engl J Med.* 2003;348(17):1681–91.
5. Hartevelt MM, Bavinck JN, Kootte AMM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in the Netherlands. *Transplantation.* 1990;49(3):506–9.
6. Madan V, Lear JT, Szeimies RM. Non-melanoma skin cancer. *Lancet.* 2010;375(9715):673–85.
7. Böhm M, Luger TA, Tobin DJ, García-Borrón JC. Melanocortin receptor ligands: new horizons for skin biology and clinical dermatology. *J Invest Dermatol.* 2006;126(9):1966–75.
8. Valverde P, Healy E, Jackson I, Rees JL, Thody AJ. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat Genet.* 1995;11(3):328–30.
9. Rana BK, Hewett-Emmett D, Jin L, et al. High polymorphism at the human melanocortin 1 receptor locus. *Genetics.* 1999;151(4):1547–57.
10. Bastiaens MT, ter Huurne JA, Kielich C, et al. Melanocortin-1 receptor gene variants determine the risk of nonmelanoma skin cancer independently of fair skin and red hair. *Am J Hum Genet.* 2001;68(4):884–94.
11. Kennedy C, ter Huurne J, Berkhout M, et al. Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skintype and hair color. *J Invest Dermatol.* 2001;117(2):294–300.
12. Beaumont KA, Shekar SN, Newton RA, et al. Receptor function, dominant negative activity and phenotype correlations for MC1R variant alleles. *Hum Mol Genet.* 2007;16(18):2249–60.
13. Yoon SW, Goh SH, Chun JS, et al. alpha-Melanocyte-stimulating hormone inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in leukocytes by modulating protein kinase A, p38 kinase, and nuclear factor kappa B signaling pathways. *J Biol Chem.* 2003;278(35):32914–20.
14. Doyle JR, Fortin JP, Beinborn M, Kopin AS. Selected melanocortin 1 receptor single-nucleotide polymorphisms differentially alter multiple signaling pathways. *J Pharmacol Exp Ther.* 2012;342(2):318–26.
15. Pérez Oliva AB, Fernéndez LP, Detorre C, et al. Identification and functional analysis of novel variants of the human melanocortin 1 receptor found in melanoma patients. *Hum Mutat.* 2009;30(5):811–22.
16. Sturm RA, Duffy DL, Box NF, et al. The role of melanocortin-1 receptor polymorphism in skin cancer risk phenotypes. *Pigment Cell Res.* 2003;16(3):266–72.
17. Moloney FJ. Epidermological, genetic and cellular studies of skin cancer in renal transplant recipients. MD thesis. NUI, Galway. October 2005.
18. Furumura M, Sakai C, Abdel-Malek Z, Barsh GS, Hearing VJ. The interaction of agouti signal protein and melanocyte stimulating hormone to regulate melanin formation in mammals. *Pigment Cell Res.* 1996;9(4):191–203.
19. Sulem P, Gudbjartsson DF, Stacey SN, et al. Genetic determinants of hair, eye and skin pigmentation in Europeans. *Nat Genet.* 2007;39(12):1443–52.
20. Gudbjartsson DF, Sulem P, Stacey SN et al. ASIP and TYR pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. *Nat Genet.* 2008;40(7):886–91.
21. Sturm RA, Teasdale RD, Box NF. Human pigmentation genes: identification, structure and consequences of polymorphic variation. *Gene.* 2001;277(1–2): 49–62.
22. Richardson DB. An incidence density-sampling program for nested case-control analyses. *Occup Environ Med.* 2004;61(12):e59.
23. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol.* 1988;124(6):869–71.
24. Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005;21(2):263–5.
25. Wong TH, Rees JL. The relation between melanocortin 1 receptor (MC1R) variation and the generation of phenotypic diversity in the cutaneous response to ultraviolet radiation. *Peptides.* 2005;26(10):1965–71.
26. Han J, Kraft P, Colditz GA, Wong J, Hunter DJ. Melanocortin 1 receptor variants and skin cancer risk. *Int J Cancer.* 2006;119(8):1976–84.
27. Scherer D, Kumar R. Genetics of pigmentation in skin cancer—a review. *Mutat Res.* 2010;705(2):141–53.
28. Helsing P, Nymoen DA, Rootwelt H, et al. MC1R, ASIP, TYR, and TYRP1 gene variants in a population-based series of multiple primary melanomas. *Genes Chromosomes Cancer.* 2012;51(7):654–61.
29. Gerstenblith MR, Goldstein AM, Fargnoli MC, Peris K, Landi MT. Comprehensive evaluation of allele frequency differences of MC1R variants across populations. *Hum Mutat.* 2007;28(5):495–505.
30. Nan H, Kraft P, Hunter DJ, Han J. Genetic variants in pigmentation genes, pigmentary phenotypes, and risk of skin cancer in Caucasians. *Int J Cancer.* 2009;125(4):909–17.
31. Palmer JS, Duffy DL, Box NF, et al. Melanocortin-1 receptor polymorphisms and risk of melanoma: is the association explained solely by pigmentation phenotype? *Am J Hum Genet.* 2000;66(1):176–86.
32. Beaumont KA, Liu YY, Sturm RA. The melanocortin-1 receptor gene polymorphism and association with human skin cancer. *Prog Mol Biol Transl Sci.* 2009;88:85–153.
33. Sánchez-Laorden BL, Herraiz C, Valencia JC, Hearing VJ, Jiménez-Cervantes C, García-Borrón JC. Aberrant trafficking of human melanocortin 1 receptor variants associated with red hair and skin cancer: Steady-state retention of mutant forms in the proximal golgi. *J Cell Physiol.* 2009;220(3):640–54.
34. Zanna PT, Sánchez-Laorden BL, Pérez-Oliva AB, et al. Mechanism of dimerization of the human melanocortin 1 receptor. *Biochem Biophys Res Commun.* 2008;368(2):211–6.
35. Suzuki I, Tada A, Ollmann MM, et al. Agouti signaling protein inhibits melanogenesis and the response of human melanocytes to alpha-melanotropin. *J Invest Dermatol.* 1997;108(6):838–42.
36. Joly P, Bastuji-Garin S, Frances C, et al. Squamous cell carcinomas are associated with verrucokeratotic cutaneous lesions but not with common warts in organ-transplant patients. A case-control study. *Transplantation.* 2010;89(10):1224–30.
37. Hofbauer GF, Bouwes Bavinck JN, Euvrard S. Organ transplantation and skin cancer: basic problems and new perspectives. *Exp Dermatol.* 2010;19(6):473–82.
38. Rosendahl C, Cameron A, Argenziano G, Zalaudek I, Tschandl P, Kittler H. Dermoscopy of squamous cell carcinoma and keratoacanthoma. *Arch Dermatol.* 2012;148(12):1386–92.
39. Skiveren J, Mortensen EL, Haedersdal M. Sun protective behaviour in renal transplant recipients. A qualitative study based on individual interviews and the Health Belief Model. *J Dermatolog Treat.* 2010;21(6):331–6.