rs34567942 a Novel Susceptibility Single Nucleotide Polymorphism for Cutaneous Squamous Cell Carcinoma in Organ Transplant Recipients

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Cutaneous squamous cell carcinoma (cSCC) is the second most common type of solid human tumour and a main cause of cancer-related death in the general population (1, 2). Typically, it emerges on ultraviolet (UV)-exposed sun-damaged skin from benign intraepithelial lesions called actinic keratosis (AK) (3, 4). High-risk patient groups, such as organ transplant recipients (OTR), display a 65–250-fold increase in cSCC, making cSCC the most frequent cancer in this group (5). However, not all OTR develop cSCC, while some develop a multiplicity of sSCC (1). This discrepancy is the major focus of the current study.

Specific genetic factors, such as single nucleotide polymorphisms (SNPs) determining cSCC susceptibility in OTRs, have been little-studied. Only a few papers have addressed this topic (6–8). Moreover, only one group has performed genome-wide association studies (GWAS), replicating 10 candidate SNPs previously associated with skin cancer in the general population; however, they did not show SNP-significant genome-wide association with cSCC in the OTRs (7). Our GWAS revealed novel OTR-specific SNP associated with cSCC susceptibility in OTR.

METHODS AND RESULTS

Patient data and material were collected prospectively from the Swiss Transplant Cohort Study (STCS), from adult solid-organ transplant recipients who received either kidney, liver, lung, heart, pancreas, small bowel or mixed organ transplant between 2008 and 2011. Skin cancer episodes after or during transplantation were reviewed by an independent clinician. All patients had provided written informed consent for participation in the STCS (including genetic analyses). The protocol was approved by the independent ethics committees of each Swiss participating centre (University Hospital of Lausanne; University Hospitals of Geneva; University Hospital Zürich; Cantonal Hospital St Gallen; Inselspital, Bern University Hospital; ClinicaLuganese, Lugano; and University Hospital of Basel and registered at ClinicalTrials.gov Identifier NCT01204944). To identify susceptibility SNPs for cSCC, GWAS were performed on 61 OTR patients with cSCC and 908 skin cancer negative-OTR patients. Genome-wide genotyping was performed on DNA samples extracted from EDTA blood using the Illumina® Human Omni Express chip Genome Studio software and default parameters were used to call genotypes. GWAS were conducted using frequentist association tests in SNPtest. Associations meeting the genome-wide threshold p-values of 5×10^{-8} were considered statistically significant.

The raw genotype data-set included 719,665 variants. After exclusion, 627,443 remained. The total number of imputed SNPs was 42,400,475. Exclusion criteria were: violation of Hardy-Weinberg-principle ($p<1^{E-6}$), call rate < 0.95, minor allele frequency (MAF) < 0.01, and minor allele count (MAC) < 3. Untyped variants were imputed using a combined reference panel of the 1,000 Genomes Project phase 3 (9) and Genome of the Netherlands v5 (10) totalling more than 90 million genetic variants across the genome. The software package SHAPEIT (11) was used for phasing and IMPUTE2 (12) for imputation. The info statistic was computed to establish imputation accuracy and markers with info < 0.7 were excluded from further analysis.

For the complex MHC region, imputation of SNPs, multi-allelic markers, amino acids, and classical HLA alleles using validated SNP2HLA pipelines were performed. Preliminary results in a subset of samples with HLA serology data showed that accuracy is very high for HLA-A, -B, -DRB1, and DQB1 (> 95%), and high for HLA-C (90%) in Europeans.

Manhattan plots were generated using the R package qqman (http://biorxiv.org/content/biorxiv/early/2014/05/14/005165.full.pdf). SNPs were queried against dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/) and the ensembl variation database (http://www.ensembl.org/info/genome/variation/index.html). The results were combined with annotation results obtained with snpEff (13).

GWAS identified 1 SNP, rs34567942, to be significantly associated with cSCC in OTRs at the p-value threshold of 5×10^{-8} (**Fig. 1**). **Table I** shows the characteristics of SNP rs345567942; namely the alternative nucleotide, imputation accuracy and im-

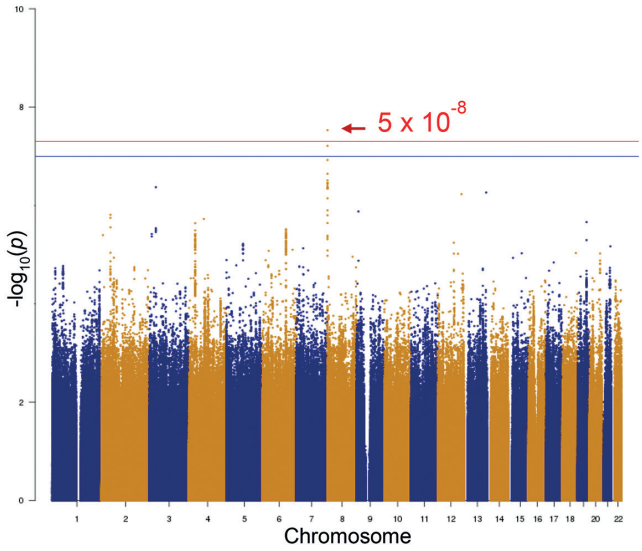

Fig. 1. Manhattan plot visualizing genome-wide association of 61 cutaneous squamous cell carcinoma (cSCC)-organ transplant recipients (OTRs) and 908 skin cancer negative-OTRs, showing –log10 p-value of SNPtest along chromosomes. *Horizontal red line* represents the significant threshold p-value of 5×10^{-8}. *Arrow* indicates single nucleotide polymorphism rs34567942 with the most prominent p-value.

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Table I. Characteristics of single nucleotide polymorphism (SNP) rs345567942, namely the position of the SNP on the chromosome, reference and alternative allele (R/A), minor allele, minor allele frequency (MAF), imputation accuracy (Info) and whether a SNP is located in repetitive region of the DNA (RM-repetitive masker)

Locus	Position	SNP	Gene	DNA region	R/A	Minor allele	MAF	*p*-value	Info	RM
Chr 8p23	284283	rs34567942	Upstream of *RP1163E5.6, FBXO25, OR4F21*	Intergenic	C/T	T	0.22	2.9×10^{-8}	0.95	NO

putation quality. rs34567942 is a non-protein-coding intergenic SNP, located in a non-repetitive DNA region (https://genome-euro.ucsc.edu) on chromosome 8.

DISCUSSION

Studies dealing with the specific genetic factors determining cSCC susceptibility in OTR are scarce. Burger et al. performed allele-specific sequencing and revealed gene-specific SNPs in OTRs with cSCC (6). However, this study could not find significant associations between the SNPs and the risk of cSCC development in OTRs. Sanders et al. were the first to perform GWAS on OTRs with cSCC, even though this study replicates 10 candidate SNPs previously associated with skin cancer in the general population, it did not show SNP-significant genome-wide association with cSCC in the OTRs (7). In our GWAS, we have identified one novel non-coding SNP meeting the genome-wide significance and previously unrelated to cSCC susceptibility in OTR. In addition, the Variant Effect Predictor (http://grch37.ensembl.org/Homo_sapiens/Tools/VEP) incorporated LoFtool (Loss-of-Function tool) revealed possibly damaging effects of rs34567942 on the nearby *FBXO25* gene (14). *FBXO25* was shown to be expressed and mutated in head and neck SCC (http://www.cbioportal.org/), suggesting possible SNP involvement.

Despite the small sample size, the current study describes a novel gene variation that could be functionally explored for development of safer individualized medication. Therefore, we will continue to expand the number of OTRs in this continuous project.

Bearing in mind that some GWAS hits are not replicated in subsequent populations, the results of this study should be considered preliminary until validated in a second set of cases and controls. In order to do this, we will continue expanding the number of OTRs in this continuous project. In conclusion, this study provides a novel gene variation that, once confirmed, could be functionally explored for development of safer individualized medication.

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The authors have no conflicts of interest to declare.

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