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Complete List of Authors:	Laing, mary; Beaumont Hospital, Dermatology Dicker, Patrick; Royal College of Surgeons in Ireland, Department of Molecular and Cellular Therapeutics Moloney, Fergal; Beaumont Hospital, Dermatology Murphy, Gillian; Beaumont Hospital, Dermatology Conlon, Peter; Beaumont Hospital, Nephrology Whitehead, Alexander; University of Pennsylvania, Pennsylvania, USA, Department of Pharmacology and Center for Pharmacogenetics Shields, Denis; Conway Institute, University College Dublin, Institute of Biomolecular and Biomedical Research
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Association of Methylenetetrahydrofolate reductase (MTHFR) polymorphism and the risk of Squamous Cell Carcinoma in renal transplant patients.

Mary E Laing[1], Patrick Dicker[2], Fergal J. Moloney[1], Gillian M. Murphy[1], Peter Conlon[3], Alexander S. Whitehead[4], Denis C. Shields[5]

[1]Department of Dermatology, Beaumont Hospital, Dublin 9.

[2]Department of Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland

[3]Department of Nephrology, Beaumont Hospital, Dublin 9.

[4]Department of Pharmacology and Center for Pharmacogenetics, University of Pennsylvania School of Medicine, Pennsylvania, USA

[5]UCD Conway Institute of Biomolecular and Biomedical Research, University

CollegeDublin, Dublin 4, Ireland.

Address for correspondence: Dr. Mary Elizabeth Laing. Department of

Dermatology, Beaumont Hospital, Dublin 9, Ireland 00353 87 7989603

mrylaing@yahoo.co.uk

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Abbreviations:

SCC: Squamous Cell Carcinoma

BCC: Basal Cell Carcinoma

MTHFR : Methylenetetrahydrofolate reductase

VDR: Vitamin D receptor

RTR: Renal Transplant Recipients

ABSTRACT

Background: The relative risk of developing cutaneous squamous cell carcinoma (SCC) is significantly increased following organ transplantation.

Objective: We investigated genetic association with SCC in two pathways associated with cancer risks, with potential for modification by vitamin supplementation.

Methods: 401 renal transplant recipients (117 with SCC and 250 without any skin cancer) were genotyped for key polymorphisms in the folate pathway (MTHFR:C677T; methylene tetrahydrofolate reductase), and the vitamin D pathway (VDR: Intron8G/T; vitamin D receptor).

Results: Individuals carrying the MTHFR 677T allele had a marked increase in risk of SCC (adjusted OR= 2.54, p=0.002, after adjustment for age, sex, skin type, sun exposure score and immunosuppression duration; lower 95% confidence boundary OR of 1.41). In contrast, VDR polymorphisms were not significantly associated.

Conclusion: Folate-sensitive pathways may play a critical role in the elevated rate of SCC in renal transplant recipients.

Risk factors for SCC in the general population include UV radiation, fair skintype and mutations in p53 tumour suppressor gene. [1] The most important of these risk factors is exposure to UV radiation. UV radiation induces local immunodeficiency as a result of a

decrease in the density of epidermal Langerhan cells. Immunosuppressive treatments, such as those used in organ transplant recipients also inhibit Langerhan cells. A recent population-based study demonstrated the risk for invasive SCC in renal transplant recipients (RTR) was increased 82-fold compared with the nontransplanted population.[2] Increased duration of patient survival and older average age at transplant has led to a steady increase in the incidence of post transplant squamous cell carcinoma (SCC). The current recommendations for the prevention of post-transplant skin cancer focus on reducing the cumulative ultraviolet radiation to which the RTR is exposed. The possibility that vitamin supplementation might reduce the subsequent risk of SCC has not been investigated.

The polymorphisms investigated in this study were the common polymorphisms in vitamin D receptor (*VDR*), and methylenetetrahydrofolate-reductase (*MTHFR*), Vitamin D has potent anti-tumour properties.[3] Calcitriol (1,25(OH)2D3), the hormonal derivative of vitamin D3, is an antiproliferative and prodifferentiation factor for squamous cells of the head and neck [3] and in vitro possesses multiple anti-cancer activities. [4] Folate deficiency is associated with genome-wide DNA hypomethylation [5]. Sequence specific alterations of DNA methylation in critical cancer-related genes may be involved in folate deficiency mediated colorectal carcinogenesis [6]. Long-term folic acid supplementation appears to reduce colorectal cancer risk.[7] The *MTHFR* C677 T polymorphism results in an Alanine to valine substitution at codon 222.[8] Individuals with this polymorphisms have reduced enzyme activity. [9] This polymorphism is associated with major pathologies ranging from neural tube defects [10] to cardiovascular diseases.[11] *MTHFR* C677T polymorphism is also implicated in colorectal cancer, pancreatic cancer endometrial cancer, gastric cancer and

leukaemia [9,12-16]. To date, there have been no investigations of *MTHFR* C677 T polymorphism association and the risk of SCC in transplant or non-transplant patients.

In the gene for VDR, the FokI (exon 2 C to T) transition creates a start codon three codons upstream of the usual translation start site. The ApaI (G1281T intron 8) variant is in close linkage disequilibrium with several other variants in Caucasians, including Bsm I and Taq I variants in introns 8 and 9. Thus, G1281T is representative of the major two haplotypes. The role of VDR polymorphism has been investigated in a number of cancers. In a meta-analysis, the TaqI/BsmI variants have been shown to confer little or no risk of prostate cancer; nor did the FokI variant make a significant contribution [17] ; there is, however, a suggestion that TaqI/BsmI variation may impact on BCC risk [18,] and malignant melanoma outcome.[19]

In this study, our objective was to determine whether the above *MTHFR* or *VDR* polymorphisms modify SCC risk in RTRs.

This study was carried out in Beaumont Hospital, the national renal transplant centre in Ireland. All RTR > 16 years old attending the renal and dermatology out patient departments were invited to participate in this study which was approved by the Beaumont ethics committee.

Each patient was interviewed and examined by one observer (FM). Data was collected on age, gender, patient skin type, duration since transplant, duration exposed to each immunosuppressive drug, and personal or family history of non-melanoma skin cancer, using a standardised questionnaire as previously described.[20]

Immunosuppressants used in these patients included Azathioprine, Cyclosporin and

Mycophenolate Mofetil. No patient was on the folate antagonist Methotrexate.

Genotyping was carried out using the Amplifluor™ method by K Biosciences (www.kbioscience.co.uk). Genomic DNA was isolated from a peripheral venous blood sample. Genotyping was performed in 384-well microplates using a fluorescence resonance energy transfer (FRET)-based genotyping method. Amplification relied on allele-specific primers and a common downstream primer.

The frequency of genotypes *MTHFR* C677T , *MTHFR* 677TT, a *VDR* FokI carrier, and a *VDR* ApaI carrier were each considered. The primary comparison was the genotype distributions in SCC patients, compared to those in patients with no skin cancer. Logistic regression was performed, correcting for the covariates of age, sex, time elapsed since transplant, skin type, and sun exposure score. All covariates were dichotomised into binary variables (similar results were obtained when the quantitative models were fitted) except skin type. The significance threshold adopted, allowing for testing of four genotypic hypotheses, was a p-value of 0.0125. Statistical analysis was performed using SAS ® Version 9.2.

The known risk factors for SCC in an RTR population are strongly overrepresented among the RTR presenting with SCC (Table 1). There was however, also a significant association of the *MTHFR* 677T variant with SCC (Table 2). The increase in risk estimated by the logistic regression analysis is substantial (OR= 2.54, Table 2). Based on the 95% confidence interval (Table 2), we can conclude that there is an Odds Ratio (OR) in excess of 1.41.

When *MTHFR* 677T allele carrier status in SCC was compared to the no cancer group, without adjustment for other risk factors, we found a weaker association (OR=1.52;

p=0.07). The lowered OR without correction for other risk factors largely reflected the importance of *MTHFR* genotype in the younger patients, fewer of whom have SCC (Table 1): 18% of carriers under 50 had SCC, compared to 7% of non-carriers. When those with SCC were compared to those with no SCC (including in the controls those patients with non-SCC skin cancers i.e. BCC, melanoma and Merkel cell carcinoma) there was still an association of SCC with *MTHFR* carrier status (odds ratio= 2.37, p-value=0.0027). Other cancers were less frequent than SCC: we noted no strong genotypic trends (data not shown). The *MTHFR* 677TT genotype showed a similar risk to carrier status (OR=1.59, CI 0.77-3.28).

We investigated whether available serum folate measurement was associated with either SCC or genotype. There was no significant association when serum folate was added to the logistic regression model (with low folate was defined as ≤ 2.8 ng/ml, OR=0.672, CI=0.29-1.55). One study has demonstrated that homozygosity for the C677T polymorphism in the *MTHFR* gene significantly increases total homocysteine concentrations and lowers folate levels in kidney graft recipients.[21] Current studies are underway to evaluate red cell folate in this cohort.

We noted no significant association of the VDR genotypes with SCC outcome (FokI: OR=1.14, 95% CI=0.65-2.00; ApaI: OR=1.41, 95% CI=0.77-2.57).

The data presented here indicate that the *MTHFR* 677T variant contributes strongly to risk of SCC in RTR, with the increase in risk most marked among younger patients. While this is a relatively small study, the risk is sufficiently pronounced, with a lower 95% confidence limit of 1.41. Perturbation of DNA methylation patterns occurs in cancer cells and has an

important role in tumorigenesis. [22] Changes in genomic DNA methylation associated with cancer can be global DNA hypomethylation or and gene specific hyper or hypomethylation. Aberrant DNA methylation causes silencing of tumour suppressor genes. The promoters of both p14 ARF and p16 tumour suppressor genes encoded by the CDKN2A gene are often hypermethylated in SCC. E –cadherin gene, which encodes for E-Cadherin cell adhesion molecule which is important in the structural integrity of the epidermis, is one of the genes most frequently hypermethylated in SCC [23] [24.] DNA hypomethylation has been demonstrated to result in genomic instability and is a feature of melanoma, e.g melanoma antigen (MAGE) often hypomethylated in melanoma. [22] Therefore aberrant methylation has an effect on skin cancer development. The methylation status of SCC in RTR is unknown but in those individuals with *MTHFR* polymorphisms, low folate levels may predispose to abnormalities in DNA methylation and contribute to skin cancer risk.

The genetic findings detailed in this paper may have implications for the management of SCC risk in other clinical conditions. Specifically, patients treated with methotrexate may be at increased risk of SCC. Although methotrexate is not one of the immunosuppressants used in renal transplant patients it is used extensively in patients with psoriasis. This possibility is supported in a study by Paul et al where exposure to methotrexate was a significant risk factor for squamous cell carcinoma in patients with psoriasis.[25] Folic acid supplementation may ameliorate the oncogenic side effects of such treatments and may be particularly important in those with risk conferring MTHFR genotypes. Our hypothesis that the excess risk of SCC in RTR patients associated with MTHFR 677T allele carrier status could be reduced or eliminated by folic acid

supplementation requires evaluation in adequately powered prospective studies.

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Table 1: Basic Clinical Features of the Study Population Based on SCC status

		SCC	**No skin cancer**	**Total**
Total		117	250	367
Gender	Males	74 (63%)	143 (57%)	217 (59%)
	Females	43 (37%)	107 (43%)	150 (41%)
Age	>50 years	90 (77%)	82 (33%)	172 (47%)
	<= 50 years	27 (23%)	165 (67%)	192 (53%)

Time since transplant	>= 8 years	69 (59%)	97 (46%)	166 (51%)
	<8 years	47 (41%)	115 (54%)	162 (49%)
Skin Type	I	39 (33%)	52 (21%)	91 (25%)
	II	52 (44%)	109 (44%)	161 (44%)
	III	22 (19%)	63 (25%)	85 (23%)
	IV	4 (3%)	20 (8%)	24 (7%)
	V	0	4 (2%)	4 (1%)
Sun Exposure score	> 8	80 (70%)	84 (39%)	164 (50%)
	<= 8	35 (30%)	129 (61%)	164 (50%)
MTHFR_677T	T allele carrier	78 (67%)	142 (57%)	220 (60%)
	Non carrier	39 (33%)	108 (43%)	147 (40%)

Note: Percentages are based on column totals for each covariate, for patients with available data.

Table 2 Risk of Squamous Cell Carcinoma in Renal Transplant Patients

□

Exposure (Factor)	**P-value**	**Odds Ratio**2	**Lower 95% CI**	**Upper 95% CI**
Univariate Analysis				
MTHFR_677T carrier	0.0726	1.52	0.96	2.41
Multivariate Analysis				
Male gender	0.9700	0.99	0.55	1.80
Age > 50 years	<.0001	7.47	3.94	14.16
Time since transplant ≥ 8 years	<.0001	3.31	1.84	5.97

Skin Type	0.0262	1.47	1.05	2.06
Sun Exposure score > 8	0.0069	2.45	1.28	4.70
MTHFR_677T carrier	0.0019	2.54	1.41	4.56

Comparison is between patients with no history of skin cancer (N=250), and those with a diagnosis of SCC (n=117), and full information on covariates.