pathogenic missense variant (NM_000264.5, c.1514G>A, p.G509D) and a germline nonsense *NF1* (NF1) pathogenic variant (NM_001042492.3, c.334C>T, p.Q112*). The proband has two sons (Figure 1d). Her elder son, aged 34 years, has inherited both *PTCH1* and *NF1* pathogenic variants and has exhibited jaw cysts, BCCs, neurofibromas and café au lait patches to date. Her younger son, who inherited the *NF1* pathogenic variant alone, has developed neurofibromas, café au lait patches and a plexiform neurofibroma of the neck. At age 32 years he also developed an aggressive grade 3 brain stem glioma, resistant to radical radiotherapy.

NBCCS and NF1 are each associated with pathognomonic tumours. In NBCCS,[2] BCCs and jaw cysts are typical, and, more rarely, ovarian fibromas, meningiomas, medulloblastomas and cardiac fibromas are reported. In NF1,[3] cutaneous and plexiform neurofibromas are typical and astrocytomas, pheochromocytomas, gastric carcinoid tumours, glomus tumours,[4] GISTs[5] and perineuriomas[6] may infrequently occur. Perineuriomas are rare, benign, peripheral nerve sheath tumours showing perineurial cell differentiation.[7] In NF1, just two cases of isolated, large, subcutaneous soft tissue perineuriomas have been reported.[6]

Our proband was exceptional in developing a spectrum of rare tumours including a glomus tumour that was not at a typical subungual site,[4] cutaneous perineuriomas and multiple jejunal GISTs, which may relate to the digenic inheritance of heterozygous pathogenic variants in *PTCH1* and *NF1*. We hypothesize that biallelic loss of either or both genes may be present in the various tumour cell types seen in the proband. We report this case to highlight the utility of genetic testing when patients with NBCCS or NF1 present with unusual symptoms or signs.

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S.J. Ghadiri,[1] P. Brennan,[2] E. Calonje,[3] A.J. Carmichael[1] and N. Rajan[4,5]

[1]*Department of Dermatology and* [2]*Clinical Genetics, James Cook University Hospital, Middlesbrough TS4 3BW, UK;* [3]*Department of Dermatopathology, St John's Institute of Dermatology, St Thomas' Hospital, London, SE1 7EH, UK;* [4]*Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, UK; and* [5]*Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne NE1 3BZ, UK*

Correspondence: Neil Rajan.

Email: neil.rajan@newcastle.ac.uk

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External validation of a model to predict risk of keratinocyte skin cancer after renal transplantation in a Western European cohort

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Dear Editor, Renal transplant recipients (RTRs) face an increased risk of developing keratinocyte skin cancer (KSC) due to their lifelong immunosuppressive drug use.[1] Current recommendations call for 6–12-monthly skin examinations for all RTRs.[2] To develop a more personalized screening procedure, Urwin *et al.*[3] identified the most significant KSC risk factors in an Australian sample and developed a predictive index (PI) that stratifies patients into high-, medium- and low-risk groups. We have externally validated this PI and evaluated its geographical transportability to the Netherlands.

The PI was based on data from an Australian cohort of 363 RTRs who received their renal transplants between 1970 and 2000. A PI score can be derived from the following equation, containing seven dichotomous predictors with values 1 or 0 representing the presence or absence of the predictor, respectively:

$$\begin{aligned}\text{PI} = {} & (2 \times \text{age at transplantation} \geq 50 \text{ years}) \\ & + (2 \times \text{daily ultraviolet radiation exposure} > 1 \text{ h}) \\ & + (2 \times \text{years in hot country} > 30 \text{ years}) \\ & + (3 \times \text{any pretransplant squamous cell carcinoma}) \\ & + (2 \times \text{any pretransplant KSC}) \\ & + (1 \times \text{any childhood sunburn}) + (1 \times \text{skin type I}).\end{aligned}$$

Based on the PI score a distinction was made between a high-risk group with a score ≥ 7 (screen after 6 months), a

medium-risk group with a score of 5 or 6 (screen after 2 years) and a low-risk group with a score ≤ 4 (screen after 5 years). A cumulative probability of remaining KSC free < 70% was used as a criterion for selection of the screening intervals.

In this study, the PI was validated in a cohort of patients who received a renal transplant at the Maastricht University Medical Centre in the Netherlands between 1982 and 2015. Information about the predictors was gathered through a questionnaire, while skin cancer data were obtained from a national registry. Model performance in terms of discrimination and calibration was assessed graphically with Kaplan–Meier plots, and a C-index and hazard ratios were calculated.[4]

Data on predictor variables were complete in 417 patients out of 424 responders, and 507 patients who did not complete the questionnaire were excluded. In total, 131 KSCs were observed during a median follow-up of 9.5 years (range 6 months to 36 years). Harrell's C-index was 0.71 [95% confidence interval (CI) 0.65–0.76], indicating moderate discriminative ability of the PI scores. The hazard ratios of developing KSC across the risk groups (high vs. low risk and medium vs. low risk) are 7.90 (95% CI 3.40–18.33) and 2.82 (95% CI 1.92–4.12), respectively.

Calibration was assessed by comparing the observed cumulative probabilities of remaining KSC free in each group at 6 months, 2 years, 3 years and 5 years post-transplantation in the Dutch and Australian populations (Table 1). Kaplan–Meier estimates for remaining KSC free were rather similar in the low-risk group, but estimates in the medium-risk group were substantially higher in the Dutch group than in the Australian sample. Only seven patients were stratified as high risk and the precision of the Kaplan–Meier estimates for this subgroup is very low, illustrated by the wide 95% CIs.

The lower KSC risk in the Netherlands may be due to less ultraviolet radiation exposure than in Australia.[4,5] However, KSC risk in the Netherlands has increased over time, and Table 1 also shows that in patients who received their transplant between 2011 and 2015, the KSC risk was more comparable between the Dutch and Australian cohorts. We assume that improvements in skin cancer registration combined with increased awareness account for the higher KSC incidence in later periods.[6,7]

The model was developed to provide more personalized advice on when to start skin cancer screening. Urwin *et al.* considered the time when KSC-free survival dropped below 70% as a critical threshold and therefore recommended screening the high-, medium- and low-risk groups at 6 months, 2 years and 5 years post-transplantation, respectively. This threshold is reached later in the Dutch medium-risk group, after more than 3 years and not after 2 years, even in the period 2011–2015. Which threshold is used depends on the risk one is willing to take. In every scheme, some patients will develop tumours before their first screening visit; therefore, self-screening remains crucial.

Table 1 Observed Kaplan–Meier probabilities of remaining free of keratinocyte skin cancer for the three risk groups

	Australian sample 1970–2000 (n = 351)	Dutch sample 1982–2015 (n = 417)	Dutch sample 2011–2015 (n = 134)
PI ≥ 7			
No. of patients (%)	82 (23.4)	7 (1.7)	3 (2.2)
6 months	0.80 (0.68–0.88)	0.86 (0.33–0.98)	1
2 years	0.43 (0.31–0.55)	0.29 (0.04–0.61)	0.33 (0.01–0.77)
3 years	–	0.29 (0.04–0.61)	–
5 years	0.17 (0.09–0.28)	0.14 (0.02–0.88)	–
10 years	–	–	–
PI 5–6			
No. of patients (%)	126 (35.9)	133 (31.9)	60 (44.8)
6 months	0.95 (0.88–0.98)	0.98 (0.93–0.99)	0.97 (0.87–0.99)
2 years	0.77 (0.68–0.84)	0.93 (0.86–0.96)	0.92 (0.81–0.96)
3 years	–	0.89 (0.82–0.93)	0.85 (0.73–0.92)
5 years	0.53 (0.42–0.63)	0.81 (0.72–0.86)	0.66 (0.48–0.79)
10 years	–	0.57 (0.46–0.66)	–
PI ≤ 4			
No. of patients (%)	143 (40.7)	277 (66.4)	71 (53.0)
6 months	0.99 (0.94–1.00)	0.99 (0.98–1.00)	0.96 (0.87–0.99)
2 years	0.95 (0.90–0.98)	0.97 (0.95–0.99)	0.93 (0.84–0.97)
3 years	–	0.96 (0.92–0.98)	0.90 (0.80–0.95)
5 years	0.89 (0.82–0.94)	0.92 (0.89–0.95)	0.85 (0.73–0.91)
10 years	–	0.86 (0.81–0.90)	–

The data are presented as the probability (95% confidence interval) unless stated otherwise. PI, predictive index according to Urwin *et al.*[3] A higher score indicates greater risk.

The study has some limitations. More than half of the eligible RTRs did not respond, which increases the likelihood of selection bias. Also, inaccurate recall of the presence or absence of predictors may have resulted in nondifferential misclassification of patients into risk groups and bias towards underestimation of the discriminative ability of the PI scores. The high-risk group consisted of only seven patients, which does not allow for any robust conclusions.

A larger-scale study including a cost-effectiveness analysis will be necessary to confirm the calibration and fine tune the screening intervals.

E.J. Verhage,[1] P.J. Nelemans,[2] S.M.J. vanKuijk,[3] M.H.L. Christiaans[4] and N.W.J. Kelleners-Smeets[1,5]

[1]*Department of Dermatology;* [2]*Department of Epidemiology;* [3]*Department of Clinical Epidemiology and Medical Technology Assessment;* [4]*Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, the Netherlands; and* [5]*GROW Research Institute for Oncology and Developmental Biology, Maastricht University, Maastricht, the Netherlands*
Email: emilyjverhage@gmail.com

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Bacterial and fungal microbiome characterization in patients with rosacea and healthy controls

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Dear Editor, Rosacea is a common inflammatory skin disease, but its pathophysiology is still unclear.[1] Several microorganisms, including *Cutibacterium acnes*, *Demodex* spp. and *Staphylococcus epidermidis*, have been suggested to play roles in its pathogenesis.[2] However, it is suspected that the community of microorganisms in and on the skin, rather than a single species, plays a more causative role in the disease.[2] Our study aimed to characterize and compare the skin bacterial and fungal microbiomes between patients with rosacea and healthy controls.

In this study we recruited 21 patients with erythematotelangiectatic rosacea (ETR), 15 patients with papulopustular rosacea (PPR) and 22 healthy volunteers from Peking University First Hospital. The study was approved by the institutional review boards of Peking University First Hospital (approval #2018-198). Informed written consent was obtained from all participants. Fifty women and eight men were included in the study (age range 18–64 years, mean 33.9 ± 10.9 years). There were no significant differences with respect to age and sex between groups. Topical and systemic antibiotics and antifungal medications were avoided for at least 4 weeks. No washing was permitted for 24 h prior to sample collection. Skin swabs were collected from the bilateral cheeks. DNA extraction, 16S rRNA and ITS1 amplicon sequencing and analyses were performed as described previously.[3,4] The Wilcoxon and Kruskal–Wallis tests were used to examine community differences between the groups.[5]

Disease severity was assessed based on a combination of clinical presentations and the Red images in the VISIA Complexion Analysis System (Canfield Imaging Systems, Fairfield, NJ, USA). There were 14 patients with mild disease and 22 patients with moderate-to-severe disease.

Our results revealed a significant shift in the relative abundance of certain bacterial taxa between patients with each rosacea subtype and healthy controls. *Actinobacteria* and *Firmicutes* were the dominant bacterial phyla on the cheek. Both patients with ETR and those with PPR had an increased relative abundance of *Firmicutes* and a decreased abundance of *Actinobacteria* compared with healthy controls (Figure 1a). At the genus level, *Cutibacterium* was dominant on healthy facial skin, and its relative abundance was significantly decreased in both the ETR and PPR groups (ETR vs. PPR vs. control: 27.3% vs. 23.3% vs. 62.6%, Kruskal–Wallis test, $P < 0.01$).

We also observed an increase in the relative abundance of *Staphylococcus* in patients with ETR (ETR vs. control: 23.0% vs. 7.7%, Wilcoxon test, $P < 0.05$) and an increase in *Streptococcus* in patients with PPR compared with control patients (PPR vs. control: 9.6% vs. 2.2%, Wilcoxon test, $P < 0.05$) (Figure 1b). Although the abundance of *Staphylococcus* was higher in patients with PPR (18.0%) than in controls (7.7%), the difference did not reach significance. These results indicate a potential role of *Staphylococcus* and *Streptococcus* in the pathophysiology of different subtypes of rosacea. Comparison of dominant bacterial taxa between patients with mild disease and those with moderate-to-severe disease did not show significant differences in our results.

We also examined fungal taxon alterations in patients and controls. The skin fungal microbiome on the cheek was dominated by the phyla *Basidiomycota* and *Ascomycota*, and the

