recently been investigated.[2] We read with interest the paper entitled 'Markers of circulating tumour cells in the peripheral blood of patients with melanoma correlate with disease recurrence and progression' by Reid *et al.*[3] preceded by the Commentary 'The challenge of developing useful blood-based biomarkers in melanoma' by Sullivan,[4] both published in a recent issue of *BJD*. Surprisingly, neither Reid *et al.* nor Sullivan cited the article that we published in the *BJD* in 2009 reporting the results of our investigation on a similar issue entitled 'Melanoma-associated markers expression in blood: MUC-18 is associated with advanced stages in melanoma patients'.[5]

We collected whole peripheral blood samples of patients with melanoma containing RNA stabilizers. In particular, we analysed, using a multimarker RT-PCR, the coexpression of a panel of five MAMs, Tyr-OH, MART-1, MAGE-3, p97 and MUC-18/MCAM, stratified according to early and advanced stages of the disease. We documented by using a logistic regression univariate analysis that the MUC-18/MCAM level is a significant independent variable among patients with advanced disease. Similarly, Reid *et al.* detected and quantified the levels of five different MAMs (MLANA, ABCB5, TGFβ2, PAX3d and MUC-18/MCAM), and found that the expression of the last was significantly more common in nonsurgically treated stage IV patients with a negative treatment outcome than in those with a positive outcome (43% vs. 9%). We are pleased to note that, among the MAMs investigated, the authors reached our results and conclusions on the possible role of MUC-18/MCAM as a suitable marker of poor patient outcome, presumably indicating an ineffective eradication of circulating melanoma cells. They confirmed, as we proposed, multimarker quantitative RT-PCR as a valuable potential technique for monitoring the disease status. It would be of interest, as a further proposal, to detect primary melanoma tissue MUC-18/MCAM expression and correlate this marker of poor outcome with the tumour thickness, peripheral blood level and follow-up of patients, in order to suggest additional tools of stratification and/or distinction for tumour progression.

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Funding sources: none.

Conflicts of interest: none declared.

No genetic support for a contribution of prostaglandins to the aetiology of androgenetic alopecia

DOI: 10.1111/bjd.12292

Madam, Androgenetic alopecia (AGA) is a common age-dependent trait, characterized by a progressive loss of hair from the scalp. The hair loss may commence during puberty and up to 80% of white men experience some degree of AGA during their lifetime.[1] Research has established that two essential aetiological factors for AGA are a genetic predisposition and the presence of androgens (male sex hormones).[1,2] A recent meta-analysis of genome-wide association studies (GWAS) has increased the number of identified loci associated with this trait at the molecular level to a total of eight.[3] However, despite these successes, a large fraction of the genetic contribution remains to be identified. One way to identify further genetic loci is to combine the resource of GWAS datasets with knowledge about specific biological factors likely to be involved in the development of disease. The focused evaluation of a limited number of candidate genes in GWAS datasets avoids the necessity for extensive correction for multiple testing, which typically limits the power for detecting genetic loci at a genome-wide level.[4] Because the presence of genetic association suggests that candidate genes are likely to operate early in the causative chain of events leading to the phenotype, this approach may also function to favour biological pathways for their importance in the development of AGA.

In their study, Garza *et al.*[5] were the first to use a global gene expression approach to identify differentially expressed genes in balding vs. nonbalding scalp from men with AGA. The authors found elevated levels of prostaglandin D_2 synthase (PTGDS) and its enzymatic product prostaglandin D2 (PGD_2) in balding vs. nonbalding scalp. Their results suggest an inhibitory effect of elevated PTGDS and PGD_2 on hair growth by premature induction of catagen, the cessation phase of the hair growth cycle. These inhibitory effects seem to be specifically mediated by interaction with the G-protein coupled receptor 44 (GPR44). Garza *et al.*[5] thus suggest an involvement of PGD_2 and its receptor GPR44 in AGA aetiology. To test for supportive

genetic evidence for a contribution of prostaglandins to AGA aetiology, we performed gene-based tests [± 50 kb of the 5′ and 3′ untranslated regions (UTRs)] for *PTGDS* and *GPR44* using VEGAS[6] on an existing GWAS dataset of 3891 early-onset AGA cases and 8915 controls reported as a meta-analysis.[3] This is the largest genetic dataset assembled to date in AGA and is a unique resource for testing specific hypotheses of a possible contribution of a gene or set of genes to the development of AGA. The gene-based analysis for *PTGDS* (44 SNPs) revealed no significant association with AGA ($P = 0{\cdot}77$). *GPR44* (58 SNPs) showed a nominally significant association ($P = 0{\cdot}03$). However, this association did not withstand correction for multiple testing when adjusting for the two genes analysed. Also, none of the investigated SNPs in *PTGDS* or *GPR44* showed a nominally significant individual association with AGA ($P > 0{\cdot}05$). To detect any variants that might affect the expression of *PTGDS* or *GPR44* via a cis-regulatory effect, we also looked for an association of SNPs 1 Mb around the transcription start and end points of the two genes (*PTGDS*: 685 SNPs; *GPR44*: 1141 SNPs). No association with AGA ($P < 0{\cdot}05$) was observed after correcting for multiple testing with $1/2 \times n$ (number of SNPs), which is appropriate for populations of European ancestry.[7] Additionally, we searched the seeQTL database, which combines information on known eQTL associations from 14 human eQTL-datasets [http://www.bios.unc.edu/research/genomic_software/seeQTL/ (last accessed 31 March 2013)], for known cis- and trans-eQTLs that influence the expression of *GPR44* or *PTGDS*. The database lists five eQTLs for *GPR44* and six eQTLs for *PTGDS* with $P < 0{\cdot}05$ that were derived from analyses in human monocytes and brain tissue. However, none of these known eQTL SNPs or their respective genotyped proxy SNPs ($r^2 > 0{\cdot}8$) showed an association with AGA of $P < 0{\cdot}05$. To test whether *GPR44* or *PTGDS* might confer an effect on AGA by epistatic interaction with known AGA loci, we used a logistic regression model implemented in INTERSNP[8] to test for both allelic [1 degree of freedom (df)] and genotypic (4 df) interactions within a published German dataset for AGA, comprising 581 cases and 617 controls.[9] We did not find any significant evidence ($P < 0{\cdot}05$) for epistasis. In summary, neither the gene-based analysis, the analysis for cis- and trans-regulatory variants, nor the interaction analysis yielded evidence for a significant contribution of genetic variation within or around *PTGDS* and *GPR44* to early-onset AGA. Therefore, our results fail to provide genetic support for a role of prostaglandins in the early causative chain of events that lead to AGA. As prostaglandins themselves are likely to be strictly regulated by additional tissue-specific and transcription factors, the effect of prostaglandins in AGA may be indirectly conferred by AGA-associated variants affecting these regulatory factors. Moreover, although we were not able to identify any AGA-associated cis- or trans-regulatory effects within existing eQTL datasets, our analyses do not rule out the existence of hair follicle tissue-specific eQTL effects on *PTGDS* or *GPR44*, as hair follicle-specific eQTLs have not been systematically investigated to date. Finally, despite having analysed the largest genetic dataset assembled to date in AGA, we still may have missed a very small effect because of power limitations. The nominally significant finding of the gene-based analysis for *GPR44* may be a candidate in this respect. However, a much larger dataset than the present one will be necessary to provide robust evidence for such a small effect. In addition, it will be interesting to observe how complementary analyses and methods will contribute to a better understanding of the mechanisms that lead to the interesting differences in prostaglandin expression between balding and nonbalding hair follicles observed by Garza *et al.*[5]

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Appendix

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Funding sources: The study was supported by the Life & Brain GmbH and the Alfried Krupp von Bohlen und Halbach-Stiftung.

Conflicts of interest: none declared.

Eyebrow alopecia: centrofacial trichoblastomatosis

DOI: 10.1111/bjd.12294

Madam, We report the case of a 21-year-old while woman who presented to our clinic complaining about progressive, bilateral eyebrow loss that had been developing for the previous 18 months. Even though the patient was otherwise asymptomatic, she admitted a cosmetic concern. Her past medical history and that of her family were both unremarkable. She had been diagnosed with folliculitis by her general practitioner, having been placed on minocycline 100 mg daily during 3 months to no avail.

On physical examination, skin-coloured papules on both medial thirds of her eyebrows could be observed. These papules tended to coalesce, forming an ill-delimited plaque with loss of the accompanying hair (Fig. 1a). Similar lesions could also be found on the glabellar area, resulting in a feeling of infiltration of these areas. The rest of her skin and mucosal examination showed no abnormalities, the hairs of her scalp, eyelashes and rest of the body being spared.

A complete work-up for eyebrow loss, including blood cell count, general biochemistry, thyroid profile and autoimmunity profile, was normal. On a subsequent follow-up visit, further lesions had appeared on the patient's nasal dorsum and both nasolabial folds (Fig. 1b,c).

Skin biopsy specimens of the medial third of the eyebrow, nasal dorsum as well as the nasolabial fold were obtained. The histopathology of all revealed the presence of aggregates of follicular germinative cells along with small infundibular cysts, which were surrounded by a densely cellular stroma. These findings were consistent with trichoblatoma (Fig. 2). A genetic study based on mutations of the gene *CYLD1* was then performed in order to rule out Brooke–Spiegler syndrome (BSS, OMIM #605041) and multiple familial trichoepitheliomas (MFT, OMIM #601606), which was negative.

Eventually, treatment with imiquimod 5% cream applied five times a week for 6 weeks was started, but it did not result in improvement of the patient's condition. Therapy with ablative laser was not considered due to its unavailability in our reference area.

Alopecia of the eyebrow is an infrequent presenting complaint and is scarcely referred in the literature. This eyebrow loss may be the consequence of a variety of conditions, such as dermatoses (atopic, Hertoge's sign or seborrhoeic dermatitis), endocrinopathies (being a cutaneous manifestation of hypothyroidism), infectious diseases (Hansen disease or secondary syphilis), trauma (including self-inflicted, such as trichotillomania or trichotemnomania), hereditary disorders (which in the neonate may be the expression of numerous genodermatoses such as ectodermal dysplasia) and malignancies. Regarding the latter, basal cell (BCC) and squamous cell carcinomas are highly frequent. Surgical excision of the tumour stops progression of the hair loss.[1]

Trichoblastoma is an adnexal neoplasm with follicular differentiation, which usually appears between the ages of 20 and 80 years, but without a sex preference. It may develop in any area of the body, except for those lacking pilous follicles. Trichoblastoma often presents as a solitary, skin-coloured nodule, no larger than 2 cm in diameter, although cases of multiple trichoblastomas/trichoepitheliomas have been described.[2] Trichoepithelioma is actually considered a variant of trichoblastoma, because this term includes widely benign proliferations with follicular germinative differentiation. Terms such as trichogerminoma, trichoblastic fibroma, desmoplastic tricho-