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

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Androgenetic alopecia (AGA) is a common age-dependent trait, characterised by a progressive loss of hair from the scalp. The hair loss may commence during puberty and up to 80% of Caucasian men experience some degree of AGA throughout 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]. Despite these successes, however, 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*. (Sci Transl Med 2012; 4:126ra34, published online 21 March 2012)[5] were the first to use a global gene expression approach to identify differentially expressed genes in balding versus non-balding 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 versus non-balding 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 (+/- 50kb of the 5' and 3' UTRs) for *PTGDS* and *GPR44* using VEGAS[6] on an existing GWAS dataset of 3,891 early-onset AGA cases and 8,915 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 = 0.77). *GPR44* (58 SNPs) showed a nominally significant association (P = 0.03)~~;~~. H~~h~~owever, 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>0.05). 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 1Mb around the transcription start and end points of the two genes (*PTGDS*: 685 SNPs; *GPR44*: 1,141 SNPs). No association with AGA ($P < 0.05$) 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 data base, which combines information on known eQTL associations from 14 human eQTL-datasets (http://www.bios.unc.edu/research/genomic_software/seeQTL/), 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 $P < 0.05$ that were derived from analyses in human monocytes and brain tissue. However, none of these known eQTL-SNPs or their respective genotyped proxySNPs ($r^2 > 0.8$) showed an association with AGA of $P < 0.05$. 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 ($P < 0.05$) 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. Much larger than the present dataset, however, 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 non-balding hair follicles observed by Garza *et al.*

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