

Genetic interaction between placental growth factor and vascular endothelial growth factor A in psoriasis

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Summary

Background. Expression of vascular endothelial growth factor A (VEGFA) is increased in chronic inflammatory skin diseases, including psoriasis, and loci for two *VEGFA* single nucleotide polymorphisms are associated with early-onset psoriasis (presenting before the age of 40 years). Studies have suggested that expression of placenta growth factor (PGF) is also upregulated in cutaneous inflammation and that VEGFA-mediated angiogenesis may be dependent on the simultaneous presence of PGF within the skin.

Aim. To elucidate the biological importance of PGF in psoriasis.

Methods. We investigated whether two commonly occurring *PGF* polymorphisms were associated with early-onset psoriasis and the genetic interaction between *VEGFA* and *PGF* in psoriasis.

Results. We observed a significant ($P = 0.04$) association between rs2268614 TT and rs2268615 AA genotypes of *PGF* and early-onset psoriasis. In addition, genetic complement, comprising the *PGF* rs2268615 AA genotype and the *VEGFA* -460 (rs833061) T allele, was significantly associated with the development of early-onset psoriasis ($P < 0.03$). We identified that the *VEGFA* genotype influences *PGF* expression ($P = 0.001$) and that mean plasma levels of *PGF* are lower in patients with severe psoriasis compared with those with mild-moderate disease ($P = 0.04$).

Conclusion. Our observed genetic interaction between *PGF* and *VEGFA* appears relevant to psoriasis, a disease with an angiogenic basis, and may influence development of an antiangiogenic approach to treatment.

Introduction

The vascular endothelial growth factor A (VEGFA) family of proteins are key regulators of angiogenesis,

defined as the sprouting of new capillaries from pre-existing vessels, which occurs during embryogenesis, carcinogenesis and tissue repair,¹ and is a significant contributor to the pathogenesis of psoriasis.²

VEGFA expression by epidermal keratinocytes and endothelial cell expression of VEGFA receptors (VEGFRs) are upregulated in psoriasis.³ Our group provided the first genetic evidence, based on the analysis of single nucleotide polymorphisms (SNPs) of the *VEGFA* gene in patients with psoriasis and in healthy individuals, for *VEGFA* as a modifier gene in psoriasis.⁴ Having observed that individual VEGFA expression levels were under genetic control, we demonstrated that high VEGFA-producing genotypes in patients with psoriasis had a significant association with severe disease.⁵

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The *PGF* gene, located at position q24–q31 on chromosome 14,⁶ contains a number of linkage disequilibrium blocks, and SNPs that tag each haplotype have been identified.⁷ A significant association between the haplotype-tagging SNPs rs2286614 and rs2268615 and neovascular age-related macular degeneration has been reported in two Chinese cohorts, suggesting a role for *PGF* as a susceptibility gene in a disease with a putative angiogenic basis.⁸ Furthermore, the rs2268614 SNP has been associated with plasma levels of PGF in two independent European populations.⁹ Little is known about what regulates *PGF* gene expression, although several studies have implicated VEGFA as a key regulatory factor.¹⁰

Placental growth factor (PGF) is structurally similar to VEGFA, and is a key regulator of pathological angiogenesis, modulating the effects of the VEGFA/VEGFR-2 axis¹¹ through angiogenic signals transmitted through VEGFR-1.¹² PGF also has direct effects on endothelial cells, inducing its own signalling and modulating VEGFA-driven angiogenesis.^{11,12} In different biological contexts, PGF either facilitates angiogenesis by displacement of VEGFA from VEGFR1, thereby enhancing VEGFA availability for VEGFR2 binding,¹³ or it inhibits VEGFA-induced angiogenesis by formation of PGF–VEGFA heterodimers, which are functionally less active than VEGFA homodimers.¹⁴ Expression of PGF is upregulated in acute cutaneous inflammation, and VEGFA-mediated angiogenesis appears to be dependent on the simultaneous presence of PGF within the skin.¹⁵

We hypothesized that PGF was important in cutaneous inflammation and that there was a degree of interdependence of PGF and VEGFA in patients with psoriasis. To interrogate this we investigated whether two commonly occurring *PGF* SNPs were associated with early-onset psoriasis, and whether there was any genetic interaction between the *VEGFA* and *PGF* SNPs in patients with psoriasis.

Methods

All procedures performed in studies involving human participants were in accordance with the ethics standards of The University of Manchester and the national research ethics committee (09/H1011/43/19115) and with the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all individual participants included in the study.

Patients with psoriasis

In total, 227 patients (125 male, 102 female) with early-onset psoriasis (presenting before the age of

40 years) were recruited from the Psoriasis Clinic at Salford Royal Hospital, Manchester, UK. Patients with localized nail psoriasis or inflammatory arthropathy, or who were pregnant or breast-feeding were excluded from the study. Severe psoriasis was defined as the Psoriasis Area and Severity Index (PASI) \geq 12. Demographics for the study group are illustrated in Table 1.

Population controls

For the healthy control (HC) group, 168 people with no history of inflammatory skin disease or inflammatory arthritis were recruited from volunteers, blood donors and control subjects from the Norfolk Arthritis Register, a community-based study that aims to recruit all patients with early inflammatory arthritis together with HCs in the region covered by the former Norwich Health Authority, Norfolk, UK.¹⁶

DNA extraction from blood

Venous blood was collected into EDTA blood tubes, and genomic DNA was isolated (QIAamp DNA Blood Midi Kit; catalogue number 51185; QIAGEN Ltd UK, Crawley, West Sussex, UK).

Genotyping of the placental growth factor single nucleotide polymorphisms rs2268614 and rs2268615

We were most interested in the two most commonly occurring SNPs within the *PGF* gene, rs2268614 and the rs2268615, owing to their previously described association with plasma levels of PGF⁹ and the association with genetic susceptibility to angiogenesis-driven disease.⁸ Genotyping was successfully performed for

Table 1 Demographic details of the subjects in the study.

	PGF study group (n = 227)
Sex, n (%)	
Male	125 (55.1)
Female	102 (44.9)
Age, years	
Median \pm SD	38 \pm 14.1
Range	18–80
Age at psoriasis onset, years	
Median \pm SD	18 \pm 9.6
Range	0–39
Psoriasis severity, n (%)	
Mild–moderate (PASI < \pm 12)	105 (53)
Severe disease (PASI \geq \pm 12)	93 (47)

PASI, Psoriasis Area and Severity Index.

the rs2268614 and rs2268615 SNPs in 99% and 100% of samples, respectively, using SNP genotyping assays [Taqman Assay-on-Demand™; Applied Biosystems Ltd, Warrington, Cheshire, UK; cat. no. 4351379; assays: C_2195634_1_ (rs2268614) and C_2195635_1_ (rs2268615)] in accordance with the manufacturer's instructions. Genotype frequencies for both patients with psoriasis and control subjects conformed to Hardy–Weinberg equilibrium (HWE) expectations.

Genotyping of the vascular endothelial growth factor A single nucleotide polymorphisms –460 (rs833061) and +405 (rs2010963)

To investigate possible genetic epistasis between VEGFA and PGF, genotyping for the VEGFA –460 (rs833061) and +405 (rs2010963) SNPs were performed using a genotyping assay [Assay-by-Design™; Applied Biosystems; cat. no. 4351379; assays: C_1647381_10 (rs833061) and C_8311614_10 (rs2010963)], following the manufacturer's instructions. We successfully genotyped 99.7% of samples. Genotype frequencies for both patients with psoriasis and HCs conformed to HWE expectations.

Determination of plasma levels of placental growth factor

Plasma levels of PGF were measured using an ELISA development kit (Duoset; R&D Systems, Abingdon, UK; cat. no. DY264; minimum detection limit 5 pg/mL) in accordance with the manufacturer's instructions. Briefly, plasma samples and standards were added in assay diluent to precoated wells and incubated at room temperature. After a standard washing step, anti-VEGFA was added to each well and incubated at room temperature. After further washing, substrate solution was added to each well followed by a stop solution, and the plate was then read on a microplate reader (ThermoMax; Molecular Devices, Sunnyvale, CA, USA) at 450 nm. Pooled plasma samples gave an interassay coefficient of variation of 10.5%. PGF standards were used to ascertain interplate variability.

Determination of plasma levels of vascular endothelial growth factor A

Plasma levels of VEGFA were measured using a specific ELISA (Quantikine; R&D Systems; cat. no. DVE00; minimum detection limit 9 pg/mL) in accordance with

the manufacturer's instructions, as described in the previous section. VEGFA standards were used to ascertain interplate variability.

Statistical analysis

Quality control (QC) inclusion thresholds for both individual sample and SNP genotyping missing data rates were set at > 90%. Genetic associations were tested with Fisher exact test. An a priori analysis decision was made to not make multiple comparisons, and therefore adjustment for multiple testing was not required. The association of each SNP with case–control status was tested using logistic regression. Statistical interaction between PGF and VEGFA was tested for each pairwise combination of SNPs by fitting an interaction term to the model.¹⁷ Regression analysis was performed using R software (v3.3.3). Univariate analysis of variance was used to determine genetic influences on PGF protein expression in patients with psoriasis. Unpaired Student *t*-test was used to compare circulating levels of PGF in patients with severe and mild–moderate psoriasis. Normality testing of patient demographic factors suggested that age and age of onset of psoriasis were not normally distributed and data were therefore expressed as median ± SD. PGF levels were normally distributed and data were therefore expressed as mean ± SEM. For all analyses, $P < 0.05$ was considered statistically significant and was based on two-sided hypothesis tests.

Results

Early-onset chronic plaque psoriasis is associated with single nucleotide polymorphisms in the placental growth factor gene

Patients with psoriasis ($n = 226$) (Table 1) had a significantly increased frequency of the rs2268614 TT genotype of PGF ($P = 0.03$, OR = 1.86, 95% CI 1.07–3.35) (Table 2) compared with HCs ($n = 166$).

Patients with psoriasis ($n = 227$) also had a significantly increased frequency of the rs2268615 AA genotype of PGF ($P = 0.03$, OR = 1.81, 95% CI 1.05–3.22) (Table 2) compared with HCs ($n = 168$).

This study had 80% power to detect an OR of 1.5 for patients with psoriasis compared with HCs for the PGF SNPs with 5% Type I error.¹⁸ The correlation between the genotype frequencies for both PGF SNPs was high ($r^2 = 0.93$), suggesting linkage disequilibrium.

Table 2 Single point analysis for each of the *PGF* and *VEGFA* polymorphisms within a population of patients with early-onset chronic plaque psoriasis and healthy controls.^a

Gene	SNP rs ID	Risk	Statistical analysis ^b		
			<i>P</i>	OR	CI
<i>VEGFA</i>	rs833661	T	0.66	0.89	0.52–1.5
<i>VEGFA</i>	rs2010963	C	0.19	0.76	0.50–1.14
<i>PGF</i>	rs2268614	TT	0.03	1.86	1.07–3.35
<i>PGF</i>	rs2268615	AA	0.03	1.81	1.05–3.22

^a*PGF* rs2268614 and rs2268615; *VEGFA* rs833661 and rs2010963. ^bFisher exact test.

Psoriasis susceptibility is associated with genetic epistasis between placental growth factor and vascular endothelial growth factor A

A genetic interaction between *PGF* and *VEGFA* was investigated using a recessive model for each SNP.¹⁷ Logistic regression analysis identified a significant genetic interaction between the *PGF* rs2268615 AA genotype and the *VEGFA* –460 (rs833061) T allele ($P = 0.03$, OR 5.05, 95% CI 1.19: 22.34; Table 3). Subjects with the *PGF* rs2268615 AA genotype and the *VEGFA* –460 (rs833061) T allele had significantly increased risk of having psoriasis compared to subjects without the *PGF* rs2268615 AA genotype and the *VEGFA* –460 (rs833061) T allele (Fisher exact test, $P < 0.01$).

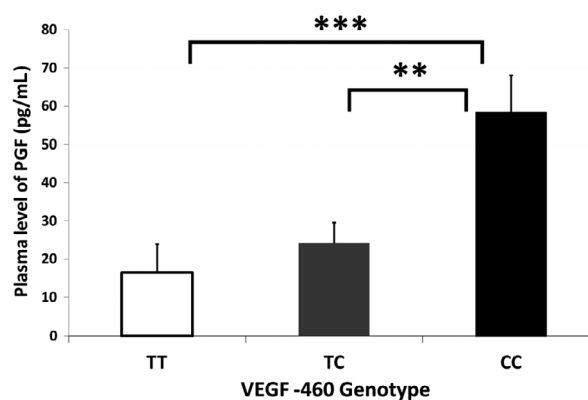
Vascular endothelial growth factor A genotype influences placental growth factor expression in patients with psoriasis

We investigated whether *VEGFA* genotype influenced PGF expression. Patients with psoriasis and the *VEGFA*

Table 3 Interaction analysis for each pairwise combination of *PGF* and *VEGFA* single nucleotide polymorphisms within a population of patients with early-onset chronic plaque psoriasis ($n = 226$) and healthy controls ($n = 162$).

Interaction		Statistical analysis ^a		
<i>VEGFA</i>	<i>PGF</i>	<i>P</i>	OR	CI
rs833661	rs2268615	0.03	5.05	1.19–22.34
rs2010963	rs2268615	0.31	1.83	0.56–5.80
rs833661	rs2268614	0.07	3.90	0.87–17.79
rs2010963	rs2268614	0.50	1.50	0.44–4.88

^aStatistical interaction between *PGF* and *VEGFA* was tested for each pairwise combination of SNPs by fitting an interaction term to the model, with regression analysis was performed in *R* software (v3.3.3). Carriage of the *VEGFA* –460 T allele and presence of the rs2268615 *PGF* AA genotype were significantly associated with the development of early-onset chronic plaque psoriasis ($P = 0.03$, OR = 5.05).

**Figure 1** The vascular endothelial growth factor gene (*VEGFA*) –460 genotype influences placental growth factor (PGF) expression in patients with early-onset chronic plaque psoriasis. Patients with psoriasis ($n = 189$) and the *VEGFA* –460 TT genotype ($n = 53$) had significantly lower plasma levels of PGF than patients with psoriasis with the CC genotype ($n = 32$) at the –460 locus. *** $P = 0.001$; ** $P = 0.002$. Statistical analysis: univariate analysis of variance.

–460 (rs833061) TT genotype had significantly ($P = 0.001$) lower plasma levels of PGF (mean ± SEM 16.53 ± 7.4 pg/mL) than patients with psoriasis with the CC genotype (58.39 ± 9.6 pg/mL) at the –460 (rs833061) locus (Fig. 1). Patients who were heterozygote at the –460 *VEGFA* locus had an intermediate plasma level of PGF (24.28 ± 5.3 pg/mL) suggesting an allele dosing effect.

Patients with psoriasis and the *VEGFA* +405 (rs2010963) CC genotype had significantly ($P = 0.04$) lower plasma levels of PGF (mean ± SEM 15.46 ± 11.0 pg/mL) than those with the GG genotype (41.70 ± 6.4 pg/mL) at the +405 locus (Fig. 2). Patients who were heterozygote at the +405 (rs2010963) *VEGFA* locus had an intermediate plasma level of PGF (19.98 ± 5.8 pg/mL) suggesting an allele dosing effect.

Patients with psoriasis and the *PGF* rs2268614 TT genotype had lower plasma levels of PGF (mean ± SEM 21.68 ± 9.05 pg/mL) than patients with TC or CC genotypes at the rs2268614 locus (TC: 33.21 ± 6.21 pg/mL; CC: 26.18 ± 7.18 pg/mL), which was not statistically significant ($P = 0.38$). There was no significant difference between plasma levels of PGF in patients with psoriasis and the *PGF* rs2268615 AA genotype (21.68 ± 9.05 pg/mL) and patients with either the AC or CC genotypes at the rs2268615 locus (AC: 33.21 ± 6.21 pg/mL; CC: 26.18 ± 7.18 pg/mL; $P = 0.38$). Plasma levels of PGF did not correlate with plasma levels of *VEGFA* (Pearson r correlation).

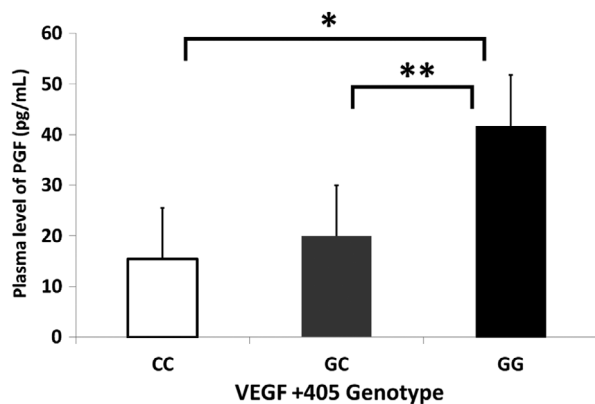


Figure 2 The vascular endothelial growth factor gene (*VEGFA*) +405 genotype influences PGF expression in patients with early-onset chronic plaque psoriasis. Patients with psoriasis ($n = 189$) and the *VEGFA* +405 CC genotype ($n = 25$) had significantly lower plasma levels of PGF than patients with the GG ($n = 74$) genotype at the +405 locus. ** $P = 0.01$; * $P = 0.04$. Statistical analysis: univariate analysis of variance.

Plasma levels of placental growth factor are significantly lower in patients with severe psoriasis

Plasma levels of PGF were significantly lower in patients with severe psoriasis (mean \pm SEM 20.77 \pm 4.6 pg/mL; $n = 93$) compared with those with mild-moderate disease (36.77 \pm 6.4 pg/mL; $P = 0.04$; $n = 105$) (Fig. 3).

Discussion

Psoriasis is a polygenic disease, and it is possible that there are several genotypically different but phenotypically similar forms of chronic plaque psoriasis.¹⁹ In the current study, we identified genetic associations with two *PGF* SNPs and early-onset psoriasis, in addition to evidence for a genetic interaction between *PGF* and *VEGFA* SNPs and psoriasis susceptibility. To our knowledge, this study is only the third to describe a genetic association between SNPs in the *PGF* gene and any disease pathology. We have demonstrated in previous studies that *VEGFA* genotype can determine production of VEGFA in both healthy individuals and in patients with psoriasis.^{4,5} In the current study we found that *VEGFA* genotype, which we have described previously as a key determinant of VEGFA protein expression, can also influence circulating levels of PGF in patients with psoriasis.

Our results demonstrate that patients with psoriasis who are genetically high VEGFA producers have low circulating levels of PGF and that low VEGFA

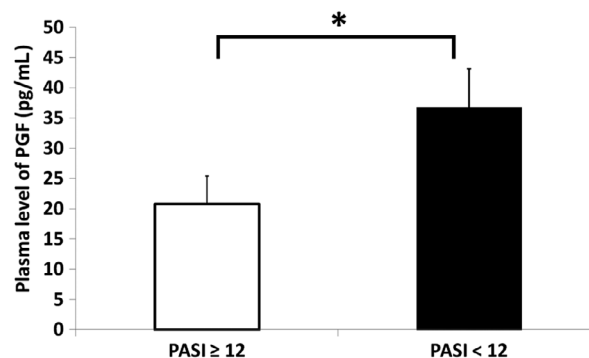


Figure 3 Plasma levels of placental growth factor (PGF) were significantly lower in patients with severe early-onset chronic plaque psoriasis. The mean plasma levels of PGF were significantly lower in patients with severe psoriasis [Psoriasis Area and Severity Index (PASI ≥ 12 ; $n = 93$)] compared with patients with mild-moderate disease (PASI < 12 ; $n = 105$). * $P = 0.04$. Statistical analysis: unpaired Student *t*-test.

producers have high circulating levels of PGF. Although further work is needed to elucidate the mechanism for our observation that *VEGFA* genotype influences PGF expression, based on our previous work we hypothesize that *VEGFA* genotype determines expression of VEGFA, which in turn regulates PGF production. Thus the relative production of VEGFA and PGF, influenced by *VEGFA* genotype, affects cutaneous angiogenic activity (Fig. 4). We have previously described that *VEGFA* genotype distinguishes two groups of patients with psoriasis, high VEGFA producer and low VEGFA producers, on the basis of VEGFA production by peripheral blood mononuclear cells.⁵ Taken together, these results suggest that in patients with severe psoriasis, who we have previously shown to have an association with the high VEGFA-producing *VEGFA* genotypes,^{4,5} the stimulatory signal for angiogenic activity will be strong (high plasma levels of VEGFA with low levels of PGF) (Fig. 4). By contrast, in individuals with mild-moderate disease who are genetically 'low VEGFA producers', high levels of PGF may result in preferential formation of PGF-VEGFA heterodimers and PGF-PGF homodimers, which function to inhibit VEGFA-induced angiogenesis (Fig. 4).

Recent work describing a significant association between the *PGF* rs2286614 and rs2268615 SNPs and neovascular age-related macular degeneration in two Chinese populations⁸ and a key role for rs2268614 *PGF* SNP in the regulation of PGF expression, possibly as a consequence of the SNP being located at the binding sequence of the GA-binding

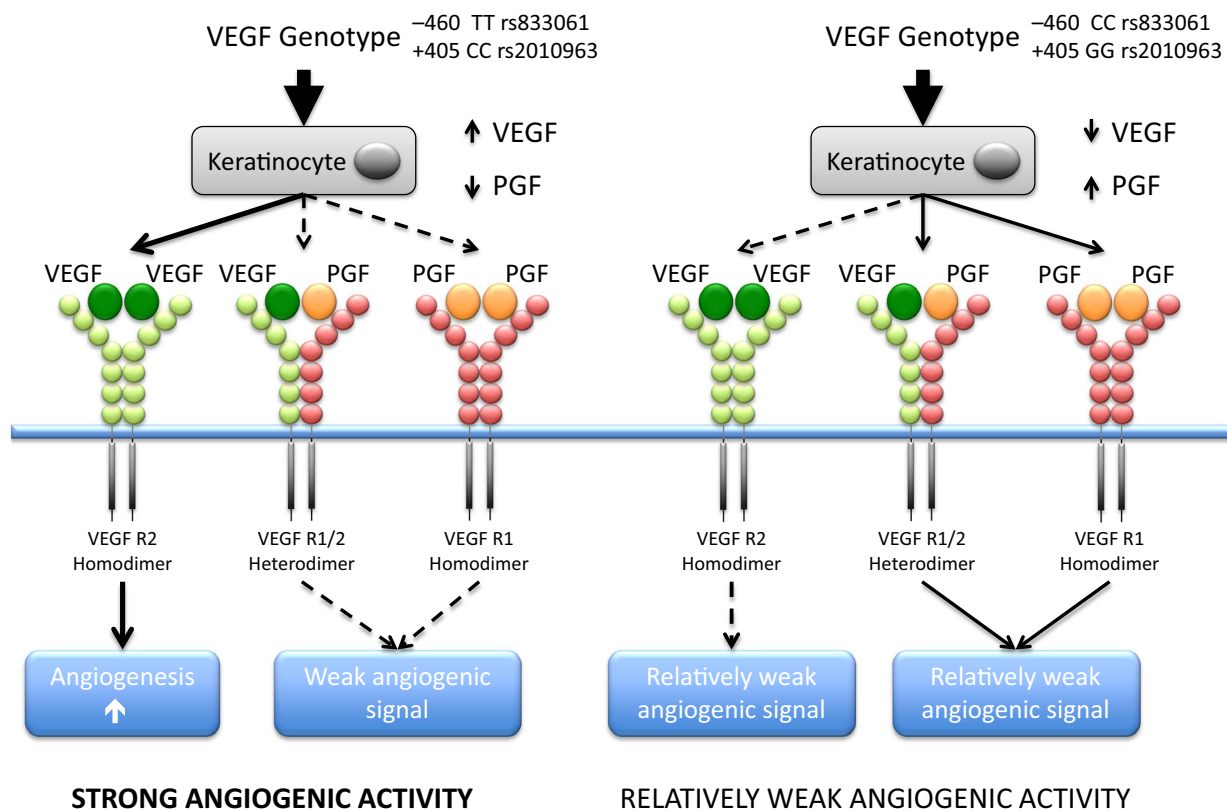


Figure 4 The relative production of vascular endothelial growth factor (VEGFA) and placental growth factor (PGF) is influenced by *VEGFA* genotype and this may affect cutaneous angiogenic activity. When VEGFA is produced in greater abundance than PGF (left panel) then the proportion of VEGFA homodimers will be large and the stimulatory signal for angiogenic activity mediated through VEGFR-2 will be strong. By contrast, if PGF production is greater than that of VEGFA (right panel) there is preferential formation of PGF-VEGFA heterodimers and PGF-PGF homodimers, which are functionally less active than VEGFA homodimers. This results in a relatively weak angiogenic signal and in this context PGF functions to inhibit VEGFA-induced angiogenesis.

factors,⁹ suggest a potential key role for *PGF* in diseases with a significant angiogenic component.

Our study has several limitations, including its relatively small sample size. However, it does replicate findings reported in other population cohorts, while describing these for the first time in psoriasis. A strong correlation between plasma levels of PGF and age, sex and smoking status has been observed.⁹ Information on smoking status and other lifestyle factors was not available for a portion of our study participants, which restricted analysis of how these factors could modify genetic regulation of PGF expression in patients with psoriasis. However, analysis of *PGF* polymorphisms and relevant demographic and lifestyle factors could inform future work examining the risk of psoriasis and PGF plasma levels.

Our findings challenge the development of antiangiogenic treatment strategies for patients with psoriasis, particularly as circulating levels of PGF can

increase during anti-VEGFA therapy for cancer, a possible mechanism of anti-VEGFA drug resistance in patients with cancer. However, aflibercept (also known as VEGFA Trap), a fusion protein with binding domains for native VEGFA receptors VEGFR-1 and VEGFR-2, may be an agent of particular interest in psoriasis, as it irreversibly binds to circulating VEGFA and PGF, and was shown to significantly improve the clinical and histological features of psoriasis-like lesions in a *VEGFA*-transgenic mouse model.²⁰

Conclusion

Defining the angiogenic signature in patients with psoriasis through measurement of circulating angiogenic factors, including VEGFA and PGF, might serve as predictive biomarker of both psoriasis severity and the likelihood of response to antiangiogenic treatments.

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