

# Decreased peroxisome proliferator-activated receptor $\gamma$ level and signalling in sebaceous glands of patients with acne vulgaris

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## Summary

Little is known about the altered lipid metabolism-related transcriptional events in sebaceous glands of patients with acne vulgaris. Peroxisome proliferator-activated receptor (PPAR) $\gamma$ , a lipid-activated transcription factor, is implicated in differentiation and lipid metabolism of sebocytes. We have observed that PPAR $\gamma$  and its target genes, *ADRP* (adipose differentiation related protein) and *PGAR* (PPAR $\gamma$  angioprotein related protein) are expressed at lower levels in sebocytes from patients with acne than in those from healthy controls (HCs). Furthermore, endogenous PPAR $\gamma$  activator lipids such as arachidonic acid-derived keto-metabolites (e.g. 5KETE, 12KETE) are increased in acne-involved and nonacne involved skin of patients with acne, compared to skin from healthy individuals. Our findings highlight the possible anti-inflammatory role of endogenous ligand-activated PPAR $\gamma$  signaling in human sebocyte biology and suggest that modulating PPAR $\gamma$ - expression and thereby signaling might be a promising strategy for the clinical management of acne vulgaris.

## Introduction

Peroxisome proliferator-activated receptor (PPAR) $\gamma$  is a lipid-activated nuclear hormone receptor that plays a role in sebaceous lipogenesis, differentiation and inflammation.<sup>1–3</sup> Sebaceous glands (SG) express PPAR $\gamma$ , and various eicosanoids can activate this transcriptional factor *in vitro*.<sup>2,3</sup> This results in raised lipid production, as shown in SZ95 sebocytes.<sup>3</sup> However, it is not known what role PPAR $\gamma$  expression, endogenous PPAR $\gamma$  ligands and PPAR $\gamma$ -mediated signaling play in acne-involved sebaceous glands.

To address these issues, focusing on expression levels of PPAR $\gamma$  in acne vulgaris, we systematically examined

the expression pattern of this transcription factor in sebocytes using immunohistochemistry. We also assessed *in situ* laser-micro dissected SGs from fresh frozen human skin tissue biopsies and determined mRNA expression levels of the PPAR $\gamma$  gene and its target genes, adipose differentiation related protein (*ADRP*) and PPAR $\gamma$  angioprotein related protein (*PGAR*). In addition, we measured eicosanoid levels in SGs from the skin of patients with acne and of healthy controls (HCs). Patients with acne had low PPAR $\gamma$  expression and signalling activity, and increased levels of various eicosanoids with PPAR $\gamma$ -activation potential. Therefore, it is reasonable to speculate that specific PPAR $\gamma$ -activating eicosanoids might have important roles in acne pathomechanism.

## Methods

The study was approved by the research ethics committee of University of Debrecen, and adhered to the guidelines of the Declaration of Helsinki. All patients provided informed consent.

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## Skin biopsies

Patients were examined according to the Global Acne Grading System Score (GAGS) to evaluate the severity of acne vulgaris.<sup>4</sup> Patients selected for biopsy had GAGS grade 1 (mild) acne; in total, nine patients with acne vulgaris provided biopsy tissue. The biopsies were taken from the upper back; both from acneic papules on acne-involved skin (AS) and from nonacne-involved skin (NAS). Skin biopsies were also taken from five age-matched HCs as reference (Fig. 1a,b).

## Isolation of eicosanoids

Previously we found that administration of various PPAR $\gamma$ -activating eicosanoids significantly increased the expression levels of *PGAR* and *ADRP*.<sup>3</sup> Selected PPAR $\gamma$ -activating eicosanoids may play an important role in the pathogenesis of acne vulgaris.<sup>1,2</sup> In the present study, we determined the level of various PPAR $\gamma$ -activating eicosanoids in skin biopsies from patients. For quantitative determination of various eicosanoids and docosanoids we used analytical sample preparation and determination based on an established high performance liquid chromatography–tandem mass spectrometry method used for retinoid quantification,<sup>5</sup> which was recently used in a published report on eicosanoid and docosanoid analysis for organs and serum analysis.<sup>6</sup> Owing to the limited amount of tissue available, we pooled three skin samples from HCs, and compared these with three pooled AS acne and three pooled NAS acne samples from three patients. Two sets of data were analysed.

## Results

### Peroxisome proliferator-activated receptor $\gamma$ and its target genes are expressed *in situ* both in normal sebaceous glands, and in the acne-involved and nonacne-involved sebaceous glands of patients with acne

Immunolabelling identified PPAR $\gamma$  protein expression predominantly in the nuclei of normal SGs obtained from HCs (3+), but expression was lower in SG cells of patients with acne, both in AS and NAS samples as well.

SGs were isolated *in situ* from the cryosection samples by laser-microdissection,<sup>3</sup> and mRNA level of PPAR $\gamma$  was examined from the collected cells using real-time quantitative PCR (qPCR). The expression of PPAR $\gamma$  mRNA was higher in SGs from HCs than in AS or NAS samples (Fig. 1c).

It is generally accepted that PPAR $\gamma$  activity is indicated by mRNA expression of its target genes such as *ADRP* or *PGAR*, as established in other cell types.<sup>7</sup> We also found that laser-microdissected SG samples from HCs expressed *ADRP* and *PGAR* mRNA at higher levels than SGs in AS or NAS samples from patients with acne (Fig. 1c). These data suggest that PPAR $\gamma$  and its target genes are present in healthy SGs of patients with acne and also in SGs of age-matched HCs.

### Various eicosanoids are present and increased in the skin of patients with acne

The level of PPAR $\gamma$ -activating eicosanoids was lowest in healthy skin samples, while NAS acne skin samples had higher levels, and AS acne samples had the highest level of eicosanoids (Fig. 1d). This suggests that the level of PPAR $\gamma$ -activating eicosanoids is sufficient or even excessive in the SGs of patients with acne, maybe due to the low level of PPAR $\gamma$  and reduced feedback regulation in SG samples from patients with acne.

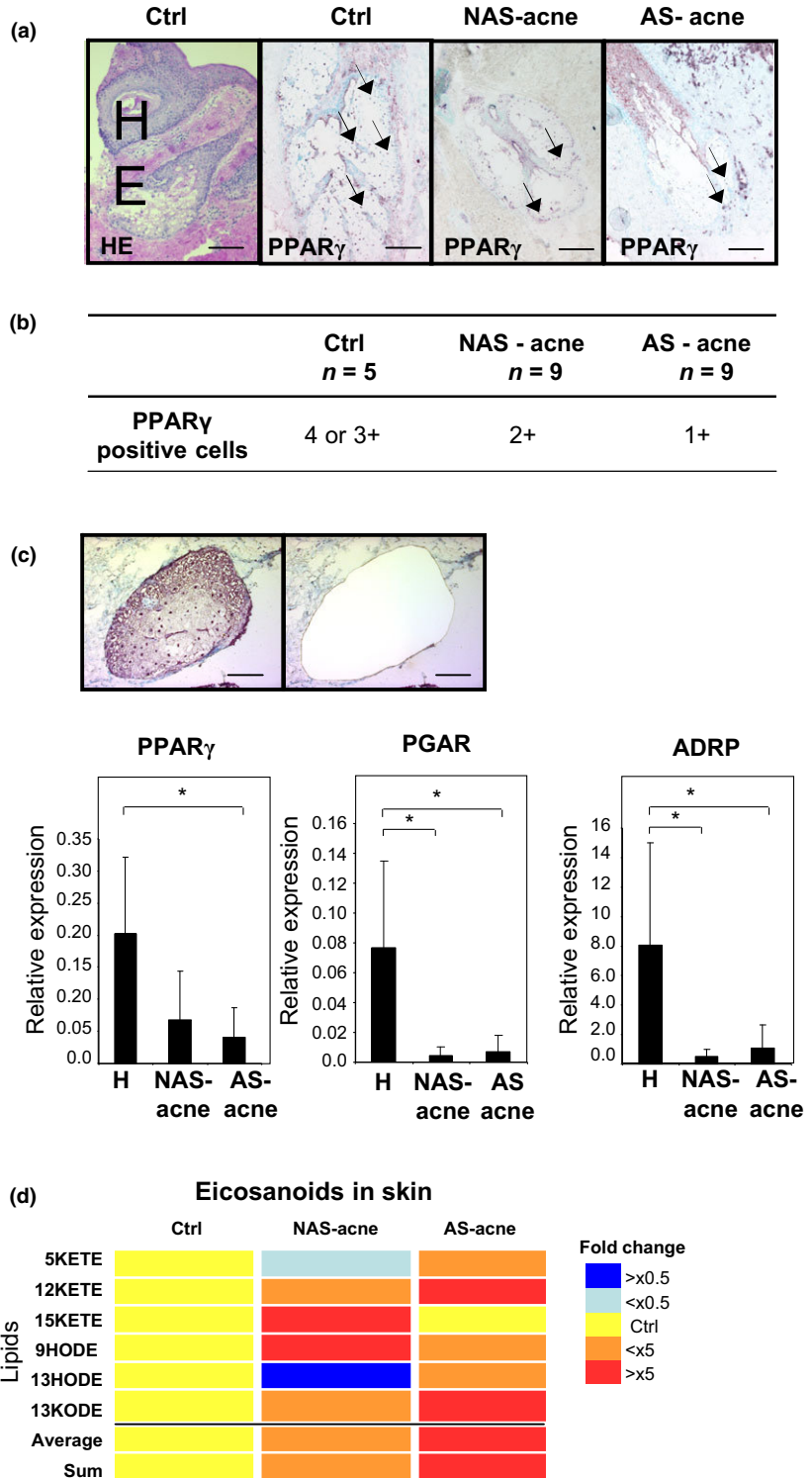
## Discussion

We found that PPAR $\gamma$  and its target genes *ADRP* and *PGAR* were expressed at lower levels in sebocytes of AS from patients with can compared with those from healthy individuals. Interestingly, the receptor and its targets were detected at low levels in NAS SGs from patients with acne. These results appear to be consistent with the established anti-inflammatory role of PPAR $\gamma$ <sup>8</sup> and indicate that the inflammatory milieu is likely to contribute to reduced receptor levels and signalling.

Previously we showed that selected PPAR $\gamma$ -activating eicosanoids were able to initiate PPAR $\gamma$  signalling in SZ95 sebocytes.<sup>3</sup> We demonstrated that the same compounds are present in acne-affected skin and that their levels are increased. Therefore, it is tempting to speculate that this might be part of feedback regulation. However, other lipids or cytokines might contribute to regulation as well.<sup>1</sup> Nevertheless, our findings suggest that PPAR $\gamma$  might play a protective role in normal human sebocytes against excessive lipid accumulation and induction of inflammatory responses. In the skin of patients with acne, PPAR $\gamma$  expression was lower or even absent in inflamed and NAS sebaceous glands; this appears to be a major feature and may be the reason behind dysregulated lipid homeostasis and inflammation. Alestas *et al.* reported that increased level of the eicosanoids prostaglandin E3 and leucotriene (LT)B4 were

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**Figure 1** (a,b,c) Comparative protein and mRNA expression patterns of peroxisome proliferator-activated receptor (PPAR) $\gamma$  in human normal sebaceous glands (SGs) from healthy individuals (Ctrl) ( $n = 5$ ), and acne-involved (AS) SGs ( $n = 9$ ) and nonacne involved (NAS) SGs ( $n = 9$ ) from acne patients. (a,b) Immunohistochemistry with tyramin amplification-based immunostaining counterstained with methyl-green or Meyers haematoxylin, then evaluated semi-quantitatively for % of PPAR $\gamma$ -positive (black arrow) sebocytes of SGs. -, negative; 1+, 0–20%; 2+, 20–50%; 3+, 50–75%; 4+, 75–100%. (c) Laser-capture microdissection of SG cells, with mRNA expression determined by reverse transcription quantitative PCR for PPAR $\gamma$ , and PPAR $\gamma$  angioprotein related protein (PGAR) and adipose differentiation related protein (ADRP). Both protein and mRNA expression of these molecules were decreased in AS and NAS samples of compared with Ctrl samples. Data were analysed using a two-tailed unpaired *t*-test, and  $*P < 0.05$  values were regarded as significant. In addition, statistical differences were further verified using one-way ANOVA with Bonferroni and Dunnett *post hoc* tests, resulting in similar results. In real-time quantitative PCR experiments, the mean and SD were calculated for both the normalized and the normalizer values for all experiments. The results were normalized to the human cyclophyllin gene. Data shown are mean  $\pm$  SD of five or nine independent determinations performed in triplicate.  $P < 0.05$  vs control. (d) High performance liquid chromatography–tandem mass spectrometry (HPLC MS-MS) analysis of eicosanoids in whole skin samples: Ctrl, AS and NAS. Eicosanoids were elevated in NAS and even higher in AS, compared with controls. Three samples were pooled and measured. Heat map of concentrations; colour bar defines concentration fold change. Two sets of samples were measured in triplicate, data shown are the average result of one set of samples.



detected in arachidonic acid (AA)-treated SZ95 sebocytes, and enzymes that regulate production of these eicosanoids (LTA4 hydrolase, 5-lipoxygenase and cyclooxygenase-2), were increased in SGs of AS skin of

patients with mild or moderate acne, but not in SGs from NAS skin or in SGs from HCs.

These results suggest that the enzymatic machinery for functional leucotriene and prostaglandin pathways

are active in sebocytes, and that the enzymes that regulate these pathways are increased in AS SGs. It is possible that PPAR $\gamma$  is a key molecule in negative feedback regulation as an anti-inflammatory molecule and that when PPAR $\gamma$  level and activity is low, the negative feedback reaction is turned off, and the enzyme machinery of inflammation, such as the leucotriene and prostaglandin pathways, is not inhibited. It is possible also that under these conditions, production of PPAR $\gamma$  ligand eicosanoids is not inhibited, thus the level of these eicosanoids are also raised compared with healthy SGs. This would be a classic hormonal regulation. Furthermore, elevation of PPAR $\gamma$  ligand eicosanoids in NAS skin was also observed in the current study. Based on these data, it could be speculated that PPAR $\gamma$  is not a secondary molecule that is downregulated due to inflammation, but rather that it is already low prior to inflammation, which suggests that downregulation of PPAR $\gamma$  has a causative role in the initiation of the autoinflammatory reaction in acne vulgaris. Confirming this theory, it is known that topical application of the PPAR $\gamma$  agonist azelaic acid (AA) produces anti-inflammatory effects in acne vulgaris.<sup>9</sup> In addition, AA induces PPAR $\gamma$  mRNA expression and transcriptional activity in human sebocytes,<sup>10</sup> which also supports the possible anti-inflammatory role of PPAR $\gamma$  in sebocytes.

It has also been reported that PPAR $\gamma$  activation increases lipogenesis.<sup>2,3</sup> However, these measurements were carried out in human immortalized sebocyte cultures (Seb-1, SZ95), and the primary cells were obtained from healthy sebaceous glands, which express high levels of PPAR $\gamma$ . Thus, it is possible that if PPAR $\gamma$  is at a high level, its activation increases lipid production in healthy SGs. In the present study, we observed that PPAR $\gamma$  is low in SGs in both AS and NAS skin of patients with acne. Therefore, it seems likely that when PPAR $\gamma$  is missing, the inflammatory regulation of SG fails, and autoinflammatory reactions are turned on. In addition lipid production in acne SGs is higher than in healthy SG, which suggests that in AS SG the primary effect of PPAR $\gamma$  might be regulation of inflammation, and maybe also in maintaining the balance of lipid production, but not simply the level of lipids.

Our data clearly show that PPAR $\gamma$  levels and signalling activity are decreased in sebocytes from patients with acne. This suggests that PPAR $\gamma$  might be a clinical target in affected sebocytes in the management of acne vulgaris.

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## Learning points

- PPAR $\gamma$  protein is a transcription factor that is involved in regulation of lipid metabolism of sebocytes.
- PPAR $\gamma$  protein and mRNA levels are high in SGs of skin from healthy individuals, but low in SGs of patients with acne.
- PPAR $\gamma$  activity is indicated by the mRNA expression of its target genes such as *ADRP* and *PGAR* in sebocytes and in other cell types.
- *ADRP* and *PGAR* levels –referring to PPAR $\gamma$  activity – are low in SGs of patients with acne, compared with normal SG.
- PPAR $\gamma$  activator ligand eicosanoids are presented at high levels in the skin of patients with acne.
- Our data suggest that in normal human sebocytes in healthy individuals, PPAR $\gamma$  might play a protective role against excessive lipid accumulation and inflammatory responses, which makes this molecule a possible therapeutic target in acne vulgaris.

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