

# BRAIN COMMUNICATIONS

## Effect of smoking on macular function and retinal structure in retinitis pigmentosa

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Retinitis pigmentosa is an inherited neurodegenerative disease of the retina. We investigated smoking as a modifiable environmental factor for the progression of this currently untreatable disease. Clinical data, smoking history, macular function and morphology including visual acuity, visual field sensitivity, ellipsoid zone width and central retinal thickness were investigated. Association between pack × years and these parameters were evaluated using generalized estimating equation models to adjust confounding factors such as age and sex. A total of 410 patients with retinitis pigmentosa ( $\geq 20$  years old; 209 female) were included, 164 had a smoking history. Patients without smoking history revealed a better visual acuity than smokers (0.39 versus 0.57,  $P = 0.001$ ). The pack × years index was associated with worse visual acuity and thinner central retinal thickness after adjusting for age and sex ( $P = 0.0047$  and  $0.0099$ , respectively). Visual field and ellipsoid zone width showed a non-significant decline with increasing pack × years. This study indicates an association of smoking with worse macular function and structural integrity in retinitis pigmentosa patients, and hence a potential detrimental effect of smoking on the disease course.

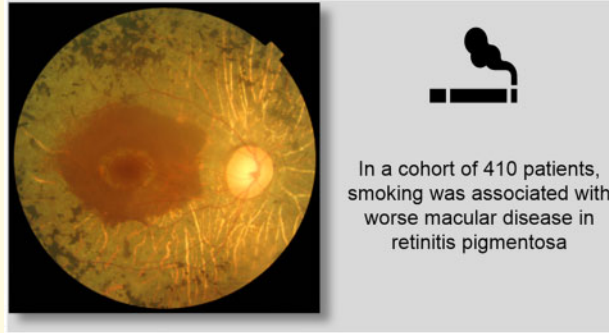
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**Keywords:** environmental factors; neurodegeneration; visual acuity; retinitis pigmentosa; tobacco

**Abbreviations:** CRT = central retinal thickness; GEE = generalized estimating equation

## Graphical Abstract



## Introduction

Retinitis pigmentosa refers to a group of inherited neurodegenerative diseases affecting the retina (Hartong *et al.*, 2006). Retinitis pigmentosa typically starts with night blindness in young age, progresses to visual field defects and leads to legal blindness in the majority of patients. In the working-age population, retinitis pigmentosa is a leading cause of severe visual impairment and blindness with currently limited treatment options (Scholl *et al.*, 2016; Verbakel *et al.*, 2018).

The age of first symptom and the disease progression are highly variable in retinitis pigmentosa even if patients share the same or a similar mutational background (Mathijssen *et al.*, 2017), indicating an essential role for additional genetic and/or environmental disease modifiers. This is supported by animal models in which anti-oxidant (Komeima *et al.*, 2006), docosahexanoic acid (German *et al.*, 2006), environmental enrichment (Barone *et al.*, 2012) or mutations in a second gene may affect the course of retinal degeneration (Samardzija *et al.*, 2006). In fact, clinical trials were conducted to investigate beneficial effects of medical treatment (exogenous disease modifiers), and some indicated significant benefit (Sacchetti *et al.*, 2015).

Smoking is amongst the most prevalent and important environmental factors influencing human health. Smoking is associated with various types of cancer, cardiovascular, pulmonary diseases and accounts for millions of deaths worldwide (Mathers and Loncar, 2006). Smoking may also induce or increase the risk for many eye conditions, including highly prevalent diseases such as cataract or age-related macular degeneration, and rare genetic disorders such as Leber hereditary optic neuropathy (Nita and Grzybowski, 2017). In addition, smoking has been associated with various neurodegenerative diseases including Alzheimer's disease (Larsson *et al.*, 2017).

It has been shown that oxidative stress is associated with cone photoreceptor cell death (Campochiaro and Mir, 2018). Thus, we hypothesized that smoking which exacerbates oxidative stress may also affect the clinical course of retinitis pigmentosa, in particular at a disease stage when the cone-rich macular area is also affected. Consequently, we investigated possible associations between smoking, macular functional and structural integrity in patients with retinitis pigmentosa.

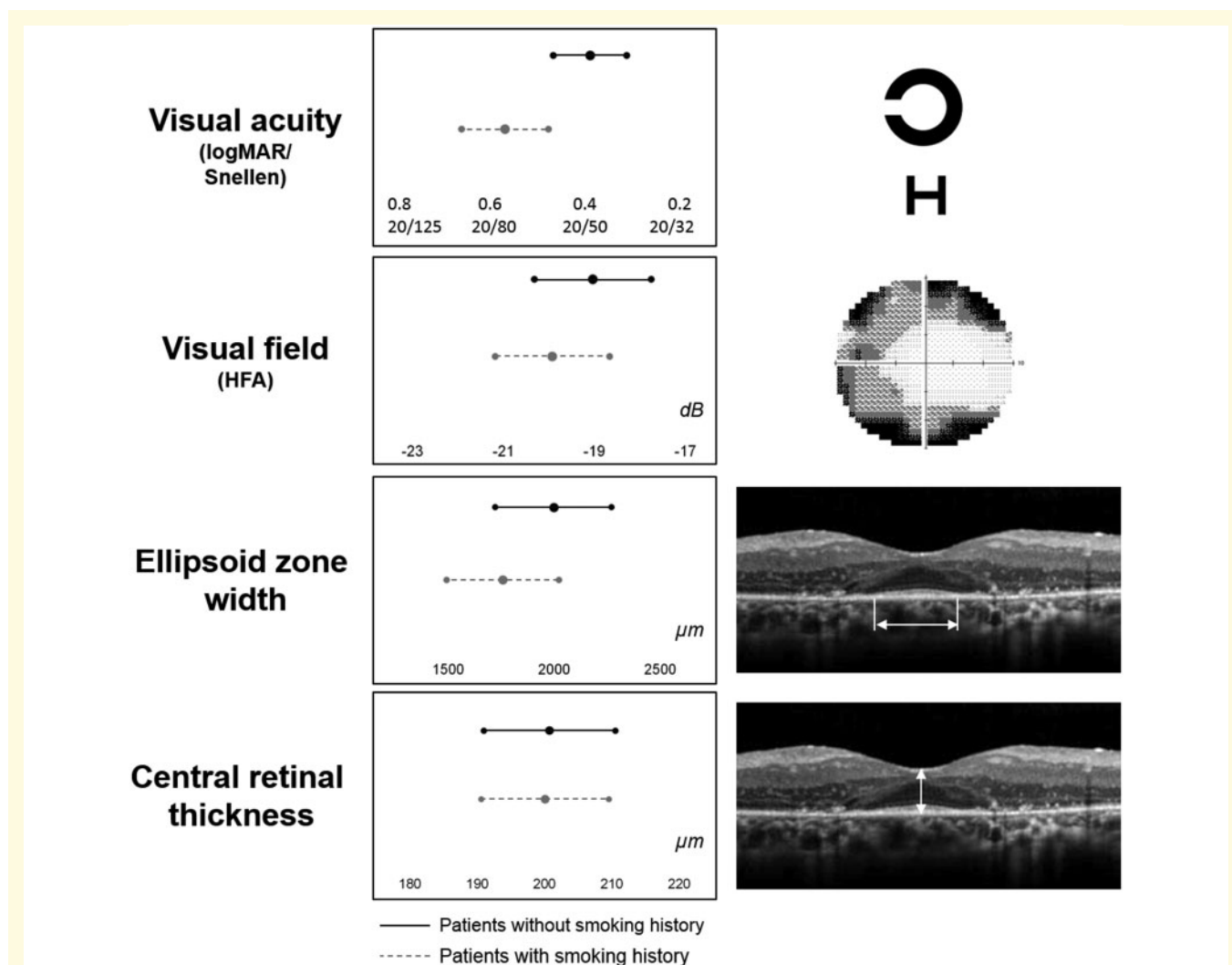
## Materials and methods

### Patients

This retrospective study was approved by the local ethics committees in Kyoto, Japan and Bonn, Germany. The study protocols adhered to the tenets of the Declaration of Helsinki. Part of the patients also underwent genetic testing with written informed consent.

Patient data were collected from 2012 to 2017. The diagnosis of retinitis pigmentosa was based on the presence of night blindness, visual field defects and characteristic retinitis pigmentosa fundus appearance. Non-recordable or reduced amplitude on electroretinography confirmed the clinical diagnosis unless patients refused or were unable to perform this examination. Genetic testing including next-generation sequencing was performed as previously reported (Oishi *et al.*, 2014; Birtel *et al.*, 2018).

Inclusion criteria for this study were age above 20 years, detailed smoking history and absence of visually significant cataract (Emery-Little grade  $\geq 3$ ; posterior subcapsular opacity). Patients with other ocular conditions that may affect the outcome measures, such as corneal opacities, retinal vein occlusions, history of major ocular trauma and history of retinal surgery, were excluded. Posterior vitreous adhesions, epiretinal membranes without significant retinal distortion and minor cystoid spaces in the retina were not



**Figure 1 Comparison of ever-smoker and non-smoker in patients with retinitis pigmentosa.** Measurements are shown as mean and 95% confidence intervals. Measured items are illustrated in the right column. Smokers tended to have worse vision and retinal integrity.

excluded because they are common findings in retinitis pigmentosa and an exclusion would critically undermine the applicability of our findings.

### Clinical examination and image acquisition

The primary outcome measure was best corrected visual acuity, measured with Landolt C chart or Snellen chart that was converted to logarithm of minimum angle of resolution (logMAR).

Further parameters included, average central retinal thickness (CRT, inner surface of the internal limiting membrane to outer surface of the retinal pigment epithelium) and width of the ellipsoid zone, which represents structural photoreceptor integrity. These measurements were performed manually on horizontal and vertical optical coherence tomography scans through the fovea

(Spectralis HRA + OCT, Heidelberg Engineering, Heidelberg, Germany) (Fig. 1). In addition, mean deviation by visual field testing, measured with Humphrey visual field analyser (Carl Zeiss Meditec, Dublin, CA, USA) using SITA-standard 10-2 programme, was analysed. Smoking was measured by packs (20 cigarettes) consumed per day  $\times$  years. All previous mentioned examinations were performed when smoking history was evaluated. Electronic cigarettes were not included.

### Sample size calculation and statistical methods

More than 5 pack  $\times$  years of smoking were regarded as significant smoking history (Moore *et al.*, 2010). We considered that more than 0.1 logarithm of minimum angle of resolution unit of difference between those with and without significant smoking history can be clinically

significant. On the assumption of 0.5 Unit standard deviation and that smoking will never have beneficial effect (one-sided comparison), sample size required to detect 0.1 unit difference with statistical power 80% and alpha error 5% was calculated as 197 for each group.

## Statistical analysis

Because the data showed deviation from normal distribution with Kolmogorov–Smirnov test, clinical characteristics of smokers and non-smokers were compared using Mann–Whitney test. Chi-square test was used to compare distribution of sex. Patients were categorized based on pack  $\times$  year: Category 0, no smoking history; Category 1, pack  $\times$  year  $>0$  and  $\leq 15$ ; Category 2, pack  $\times$  year  $>15$  and  $\leq 30$ ; Category 3, pack  $\times$  year  $>30$ . Clinical parameters were examined among groups using Dunnett test. The associations between logarithm of minimum angle of resolution and pack  $\times$  year, as well as the retinal structure and pack  $\times$  year, were further analysed; age- and gender-adjusted effect sizes were estimated for right and left eyes combined, using generalized estimating equation (GEE) models to account for the correlation between eyes (Hanley et al., 2003). Pack  $\times$  year was treated as continuous variable. These statistical analyses were conducted using R software (Version 3.4.1). For GEE, the CRAN package ‘gee’ was employed.

## Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

**Table 1** Causative genes and number of patients in the present study

<i>C2orf71</i>	1	<i>PDE6B</i>	7
<i>CEP78</i>	1	<i>PRCD</i>	1
<i>CEP290</i>	1	<i>PRPF6</i>	1
<i>CNGA1</i>	7	<i>PRPF8</i>	3
<i>CNGB1</i>	3	<i>PRPF31</i>	8
<i>CRB1</i>	1	<i>PRPH2</i>	4
<i>CRX</i>	3	<i>RDH12</i>	2
<i>EYS</i>	62	<i>RHO</i>	9
<i>FAF161A</i>	1	<i>RP1</i>	4
<i>GUCY2D</i>	1	<i>RP1L1</i>	6
<i>IFT140</i>	1	<i>RP2</i>	2
<i>IMPG2</i>	2	<i>RPE65</i>	1
<i>MAK</i>	1	<i>RPGR</i>	14
<i>MERTK</i>	1	<i>RPGRIP1</i>	1
<i>MFSDB</i>	1	<i>SAG</i>	1
<i>MYO7A</i>	1	<i>SNRNP200</i>	4
<i>NR2E3</i>	3	<i>TOPORS</i>	2
<i>NRL</i>	1	<i>TULP1</i>	1
<i>PDE6A</i>	2	<i>USH2A</i>	18

## Results

This study included 410 retinitis pigmentosa patients (201 men and 209 women) with a mean age of 53.6 years (95% confidence interval 52.2–55.1). Thirteen eyes were excluded because of retinal detachment, macular hole, major trauma, corneal opacity, acute angle closure glaucoma, phthisis bulbi and enucleation. Thus, the study cohort consisted of 807 eyes. Genetic testing was performed in 366 patients and identified causative genes in 184 patients (50.3%). Causative genes are provided in Table 1. Visual field testing was available from 324 patients (648 eyes).

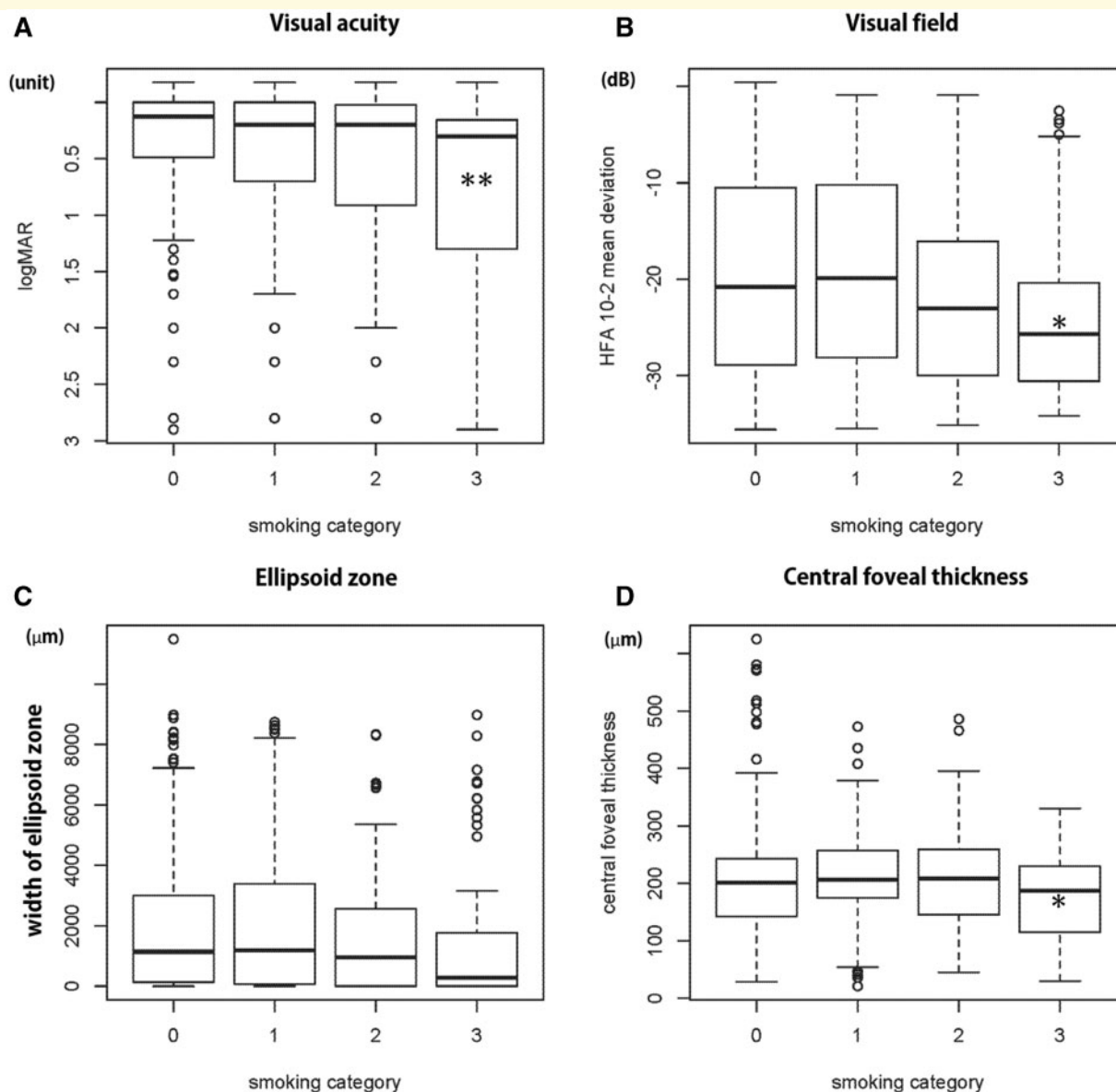
Totally, 164 patients had a history of smoking, with mean pack  $\times$  years of 19.7 (95% confidence interval: 17.8–21.6). Age was not significantly different between smokers and non-smokers (53.2 versus 54.3 years old,  $P=0.295$ ). Male patients were more likely to have a smoking history than female (117/201, 58.2% versus 47/209, 22.5%,  $P=1.92 \times 10^{-13}$ ). Sixteen patients carried mutations in X-linked genes (*RP2* and *RPGR*), and nine of them were smoker (56.3%).

Smokers, when compared to non-smokers, had significantly worse visual acuity (logMAR mean  $\pm$  standard deviation,  $0.57 \pm 0.78$  versus  $0.39 \pm 0.66$ ;  $P=4.53 \times 10^{-4}$ ; Snellen equivalent of the means: 20/74 versus 20/49) and shorter width of the ellipsoid zone ( $1758 \pm 2215 \mu\text{m}$  versus  $2000 \pm 2286 \mu\text{m}$ ;  $P=0.019$ ). Visual field testing was performed in 324 patients of whom 195 were non-smokers and 129 smokers, and revealed the same trend despite non-significant  $P$ -values ( $-19.9 \pm 10.5$  dB versus  $-19.0 \pm 10.7$  dB,  $P=0.30$ ). Central retinal thickness measures were not different ( $200.1 \pm 79.2$  versus  $200.8 \pm 81.7 \mu\text{m}$ ,  $P=0.53$ ) (Fig. 1).

Visual acuity, visual field sensitivity and CRT deteriorated with increasing pack  $\times$  years (Fig. 2). Particularly, Category 3 patients (pack  $\times$  year  $>30$ ) showed worse VA ( $P=1.14 \times 10^{-4}$ ), worse visual field ( $P=4.50 \times 10^{-2}$ ) and smaller CRT ( $P=4.33 \times 10^{-2}$ ) compared with Category 0 (no smoking history). This association was confirmed after an adjustment for age, gender and bilateral eyes using GEE ( $P=0.0047$  for visual acuity and 0.0099 for CRT, respectively). Visual field sensitivity and ellipsoid zone also declined as the pack  $\times$  year increases, though it was not statistically significant after adjustment with GEE (Table 2).

## Discussion

In this study, visual acuity in patients with retinitis pigmentosa was worse amongst smokers compared with non-smokers. As age and sex (patients with X-linked retinitis pigmentosa tend to have worse phenotype) can be confounding factors, we performed GEE to adjust for these parameters; however, pack  $\times$  year was independently associated with worse visual acuity. This is the first



**Figure 2 Relationship between increasing pack–years and retinal function/morphology.** Retinitis pigmentosa patients were categorized based on pack × year: Category 0, no smoking history; Category 1, pack × year > 0 and ≤ 15; Category 2, pack × year > 15 and ≤ 30; Category 3, pack × year > 30. Visual acuity (A), visual field sensitivity (B), width of ellipsoid zone (C) and central foveal thickness (D) tended to decline as the pack × year increases. HFA, Humphrey visual field analyser. \* $P < 0.05$  and \*\* $P < 1 \times 10^{-4}$  compared to category 0 after Dunnett correction.

clinical evidence that smoking, as a modifiable environmental factor, may have a negative effect on the disease course of retinitis pigmentosa.

Structural changes of the retina were more advanced in smokers who had a shorter EZ width and thinner CRT compared with non-smokers. A consistent trend was observed in each analysis; however,  $P$ -values were not consistently significant probably due to the large variation within groups. Previously, tobacco-associated retinal changes were also observed in otherwise healthy subjects (Yang *et al.*, 2019). However, the change was observed

as thinning of retinal nerve fibre layer and ganglion cell layer. We consider that the outer retinal changes seen in the present study are specific to patients with retinitis pigmentosa.

The potentially detrimental effect of smoking on retinal integrity could be explained by diverse mechanisms: First, tobacco contains numerous oxidative components (Smith and Hansch, 2000) and reduces macular pigment making the retina more susceptible to oxidative stress (Hammond *et al.*, 1996). Oxidative stress was associated with cone cell death



**Table 2** Result of generalized estimating equations

	Estimate	Robust SE	Robust z	P-value
Visual acuity				
Age	0.01	1.73E-3	6.82	8.70E-12
Sex	-0.06	0.05	-1.19	0.23
Pack × year	0.01	1.85E-3	2.83	4.69E-3
Visual field				
Age	-0.12	0.03	-4.61	3.97E6
Sex	2.73	0.84	3.25	1.17E-3
Pack × year	-0.02	0.03	-0.69	0.49
Ellipsoid zone width				
Age	-11.6	5.5	-2.1	0.04
Sex	669.2	172.2	3.9	1.02E-4
Pack × year	-3.1	6.0	-0.5	0.60
CRT				
Age	-0.4	0.2	-2.0	4.95E-2
Sex	-8.6	7.4	-1.2	0.24
Pack × year	-0.6	0.2	-2.6	9.92E-3

CRT, central retinal thickness; SE, standard error; visual acuity, measured with logarithm of minimum angle of resolution; visual field, measured with Humphrey visual field analyser 10-2 mean deviation.

(Campochiaro and Mir, 2018) and may explain macular functional deficit seen in the present study. Second, smoking may cause chronic inflammation; smoking directly affects immune cells, increases proinflammatory cytokine and exacerbates local and systemic inflammation (Lee et al., 2012). Previously, it has been shown that smoking-induced chronic inflammation plays a role in age-related macular degeneration (Anderson et al., 2002). These mechanisms may accelerate photoreceptor cell death although the present study design cannot state which one is involved in the findings.

A limitation of this study is the cross-sectional design that can only show an association but no causal relationship. The study design is a compromise, as randomized studies are essentially impossible and longitudinal observational study may include ethical concerns if patients are not encouraged to stop smoking. An alternative explanation for a worse disease course in smoking retinitis pigmentosa patients would be that those with a more severe disease course may start smoking as a coping strategy. However, a previous report showed that retinitis pigmentosa patients are less likely be smokers compared with controls indicating that those who care about their disease condition tend to refrain from potentially harmful habit (An et al., 2014). Inclusion of both eyes might have brought potential bias despite we adjusted the effect with GEE. Lastly, we could not investigate the effect of specific gene mutations because most genes account for only a few patients which would not allow meaningful comparisons. Instead, we included a considerable number of retinitis pigmentosa patients, making the conclusions more generalizable.

In summary, the presented data suggest potential detrimental effects of smoking on retinal integrity in retinitis pigmentosa. Additional studies with larger sample or animal experiments are warranted to confirm these initial findings.

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## Competing interests

The authors report no competing interests.

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