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ATP-binding cassette subfamily A-1 (ABCA1) levels are increased in the aqueous humour of proliferative diabetic retinopathy patients

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Editor,

The adenosine triphosphate-binding cassette transporter ABCA1 (member 1 of the human transporter subfamily ABCA) is a cholesterol efflux membrane transporter and regulatory protein (Luciani et al., 1994) that regulates cellular cholesterol, angiogenesis and inflammation (unpublished data). Moreover, ABCA1 is associated with ischaemia.

Proliferative diabetic retinopathy (PDR) is characterized by damaged blood vessels due to diabetes-induced hyperglycaemia, which induces retinal ischaemia, and neovascularization due to hypoxia-induced vascular endothelial growth factor (VEGF) expression. However, the association between ABCA1 and PDR is not yet known. In this study, we investigated the association between retinal ischaemia and ABCA1.

We compared ABCA1 levels in the aqueous humour between patients with PDR and control subjects. To analyse ABCA1 expression, we selected six patients with PDR and four control subjects in this prospective study. The study protocol was approved by the Institutional Review Board of Hanyang University. Approximately 50–200 μl of aqueous humour was collected from each participant, using a 30-gauge needle for an intravitreal injection in patients with PDR and during cataract surgery in the control subjects. The aqueous humour samples were immediately stored in 1.5-ml microtubes at −80°C until further analysis. The expressions of ABCA1, hypoxia-inducible factor-1 (HIF), and VEGF and the downstream proteins of HIF were detected using Western blotting. AH samples containing equal amounts (25 μg) were used for Western blotting (Sung et al., 2012). The p-values were calculated using the Student's *t*-test for group comparisons, and $p < 0.05$ was considered statistically significant.

Four samples from four patients with cataract (mean age [SD], 72.8 [10.0] years; 4 [100%] women; mean visual acuity, 0.200) and six samples from six patients with PDR (mean age [SD], 63.8 [9.3] years; 2 [33.3%] women; mean visual acuity, 0.704) were studied. The expression levels of ABCA1, HIF-1α, HIF-1β and VEGF were significantly higher in the aqueous humour samples of patients with PDR compared to the control subjects (ABCA1, $p = 0.04$; HIF-1α, $p = 0.03$; HIF-1β, $p = 0.04$; VEGF, $p = 0.03$; Fig. 1).

In hypoxic conditions, HIF-1α and HIF-1β increase in human cancer cells. In previous studies, an increase in the number of HIFs induced by hypoxia increased the mRNA expression of ABCA1 in human macrophages by binding to the ABCA1 promoter (Ugocsai et al., 2010). The present study showed that ABCA1, HIF and VEGF, which were associated with

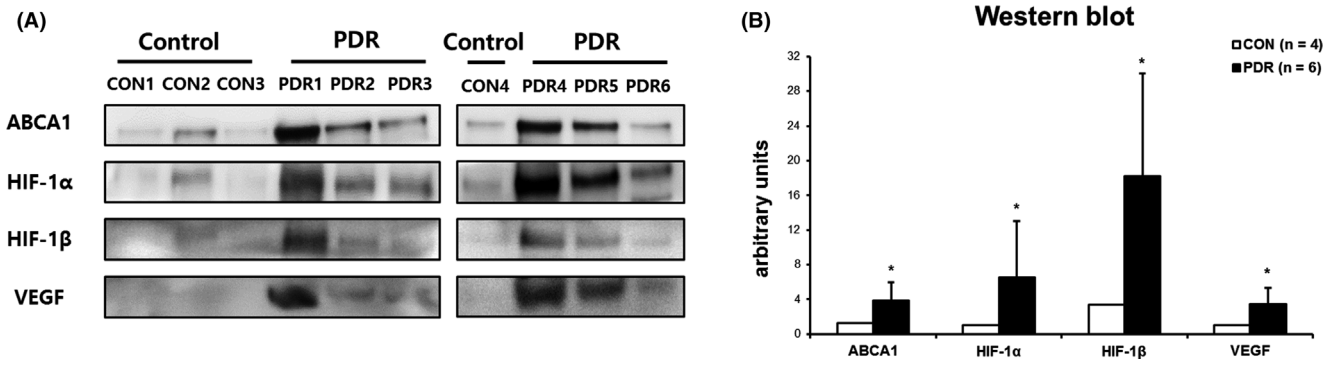

Fig. 1. Western blotting analysis of ABCA1, HIF-1α, HIF-1β and VEGF. (A) Western blot bands for ABCA1, HIF-1α, HIF-1β and VEGF in aqueous humour samples from proliferative diabetic retinopathy (PDR) patients and control (CON) subjects. Equal amounts of protein (25 μg) were used for each sample. (B) Quantification of protein levels from Western blots in CON and PDR groups. Significance was assessed using an Independent sample Student's t-test. *$p < 0.05$.

ischaemia, increased in the aqueous humour of patients with PDR.

This study did not identify the role of ABCA1 in PDR. However, the results suggested an association between ABCA1 and PDR. ABCA1 maintains endothelial nitric oxide synthase activity and regulates inflammation and angiogenesis in endothelial cells through cholesterol efflux in mice (Westerterp et al., 2016). Further, ApoA-1 binding protein, including ABCA1, regulates angiogenesis through effective cholesterol efflux (Fang et al., 2013). Thus, ABCA1 may have an association with ischaemia through angiogenesis.

In this study, we showed elevated ABCA1, HIF-1α, HIF-1β and VEGF in the aqueous humour of patients with PDR. Increases in HIF might increase the mRNA level of ABCA1 in hypoxic conditions by binding to the promoter of ABCA1. We could not determine the association between ABCA1 and neovascularization. However, for the aforementioned reasons, we propose that ABCA1 plays a role in retinal neovascularization and could be a novel target for neovascularization in PDR. Further investigations into the role of ABCA1 in retinal pathologic neovascularization are needed.

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Reasons for late diagnosis of neovascular age-related macular degeneration: a mixed-methods study

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Dear Editor,

In patients with neovascular age-related macular degeneration (nAMD), a high visual acuity (VA) at treatment onset increases the chance of maintaining good VA; however, a majority of patients has the condition for months prior to diagnosis (Ho et al. 2017). To increase the success of future interventions, it is imperative to know which delaying factors patients experience in their time to diagnosis (TTD) and possible ways to minimize these. We conducted a study investigating patients' perspective of their health-seeking behaviour in relation to delay of TTD.

This mixed-methods study was conducted using semi-structured interviews with patients having newly diagnosed nAMD. Interviews were audio recorded, transcribed and analysed using the framework method with an inductive coding approach (Gale et al. 2013). Twenty-five patients were included (mean age 77 years, 84% female). Median interview length was 10.5 min. Estimations of TTD from beginning of symptoms to diagnosis were made (Fig. 1A). Potential reasons for delay were coded into three overarching themes: factors related to patients, primary health care (PHC) and secondary health care (SHC) (Fig. 1B). Important delaying factors included patients' lack of knowledge causing a late response at symptom onset, late assignment of an appointment at the ophthalmologist (PHC) and waiting periods from referral at the ophthalmologist to the initial visit at the hospital (SHC). Improvements suggested by patients to shorten TTD comprised increased public information, regular check-ups and shorter waiting periods.

Exploring the individual self-reported timelines, the major cause of delay was related to patient response (Fig. 1A). However, several patients experienced a waiting period of 1 month from referral at the ophthalmologist to the initial visit at the hospital. A likely interaction between patient- and system-related factors was found (Fig. 1B), that is, perception of long waiting periods potentially affecting patient behaviour. Low health literacy such as missing knowledge and a delayed response to symptoms appeared to be a major risk factor for delay. The optician was reported as being the greatest facilitator in reducing TTD, while the ophthalmologist was the most important factor of delay due to the waiting periods. Supposedly, preceding lack of patient knowledge might have been a contributing factor which made patients accept this wait; however, patients with symptoms of nAMD should be examined much quicker. In the secondary healthcare system, only a few patients experienced waiting periods longer than the set objective for waiting periods (objective: 1-4 weeks), while many experienced waiting periods $\leq$ 2 weeks.

Considering delaying factors as well as improvements mentioned by patients to shorten TTD, a strong recommendation would be to improve patients' knowledge of AMD by increased public information. Furthermore, support services provided by the municipality were mentioned as a facilitator and improving these could be considered. Both suggestions have also been recommended in other studies (Sim et al. 2017; Parfitt et al. 2019). Lastly, a screening programme could be explored. However, one study investigating screening programmes at the ophthalmologist, did not find it to be