APPENDIX A

Detailed SCES-2 Methodology

Blood pressure and anthropometry

BP measurements were taken with an automatic BP monitor (Dinamap model Pro Series DP110X-RW, 100V2; GE Medical Systems Information Technologies Inc., Milwaukee, USA). Two BP readings were taken, 5 minutes apart and a third reading was taken if the difference between the first 2 readings was greater than 10mmHg (systolic) and or 5mmHg (diastolic). Height of the participant was measured in centimetres using a wall-mounted measuring tape. Weight in kilograms was measured using a digital weighing scale (SECA, model 782 2321009; Vogel & Halke, Hamburg, Germany). Body mass index of each participant was then calculated.

Blood and urine sample collection

Nonfasting blood (37.5ml) samples were drawn for biochemical tests including HbA1c, lipid profile, serum creatinine, full blood count and blood glucose. 10ml of blood was used for biochemical testing and the remaining 27.5ml was used for DNA extraction, future research and analysis. A 20 ml urine sample was collected for urine dipstick analysis and the rest was stored for future analysis and research.

Visual acuity and refraction

Presenting distance visual acuity (VA) with current optical correction (spectacles or contact lenses, if any) was measured monocularly and binocularly using a logarithm of the minimum angle of resolution (LogMAR) ETDRS numerical charts (Lighthouse International, New York, USA) at a distance of 4 meters in standardized lighting conditions. If no numbers were able to be read at 4m, the participant was moved to 3m, 2m, or 1m subsequently. If no numbers were identified on the chart, counting fingers, hand motion and light perception was

assessed in that order. Binocular near VA was performed using the numerical near visual acuity ETDRS Chart “2000” (Precision Vision, La Salle, IL, USA) at 40cm.

Spectacle correction, if applicable was measured by auto lensometer (NIDEK, LM990). Objective refraction and keratometry were measured using an auto-refractor machine (Canon RK-5 Auto Ref-Keratometer, Canon Inc. Ltd).

Subjective refraction was determined by a trained and certified study optometrist when entering VA was worse than 0.3 LogMAR. Autorefraction readings or habitual spectacle correction readings were used as the starting point for refinement of sphere, cylinder and axis until best corrected visual acuity (BCVA) was achieved. Near refractive correction was determined in order to obtain best corrected near VA. BCVA was assessed monocularly and binocularly, and was recorded in LogMAR notation using the same test protocol as presenting VA.

Iris photography

An undilated iris photo was taken using a slit-lamp camera (Topcon DC-3 camera, Tokyo, Japan) while the illumination beam completely filled the iris from an illumination angle of 45 degrees using 16X magnification. Focus was on the whole iris so that surface characteristics (Fuch's crypts, contraction furrows, colour) could be identified.

Anterior segment optical coherence tomography (OCT)

Anterior segment OCT was performed using Visante OCT (Carl Zeiss Meditec, Jena, Germany), Swept-source OCT (SS-OCT, CASIA, SS-1000, Tomey Corporation, Nagoya, Japan) and spectral-domain OCT device (Cirrus High Definition (HD-OCT), Carl Zeiss Meditec, Dublin, California) to assess the anterior chamber angle depth.

Slit lamp examination

A slit lamp examination (Haag-Streit model BQ-900; Haag-Streit, Koeniz, Switzerland) was performed by the study ophthalmologist before and after pupil dilation. The examination was performed to identify any abnormalities in eye lids, conjunctiva, cornea, iris and angles before dilation.

Intraocular pressure assessment

Intraocular pressure (IOP) assessment was taken before dilation using a Goldmann Applanation Tonometer (Haag-Streit, Switzerland). If the first IOP reading was higher than 21 mmHg, a second reading was taken, the average between the first and second reading was taken as the final measurement.

Examinations done to determine glaucoma and glaucoma suspects status

In addition to the previously mentioned procedures, glaucoma patients and glaucoma suspects (defined in **Supplementary table 1**) underwent two additional tests prior to dilation: gonioscopy and a Humphrey visual field (HVF) test. Gonioscopy was performed at the slit lamp to examine the anterior chamber and angles of the eye using a Goldmann two-mirror contact lens. The Spaeth[1] and Scheie[2] classification systems, as described in detail in the Tanjong Pagar Survey[3], were used to evaluate superior, inferior, temporal and nasal angles. For visual field testing, the 24-2 Swedish Interactive Threshold Algorithms-Fast (SITA-FAST) static visual field test was performed. The threshold-related visual field examination was performed with near refractive correction using the Humphrey Visual Analyzer II (model 750, Carl Zeiss Meditec, Switzerland). The test was deemed unreliable when the following was reported by the instrument: fixation losses >20%, false positives >33% or false negatives >33%. The rest was deemed abnormal when the glaucoma hemifield test (GHT) reported by the instrument stated a borderline or abnormal test. Visual field tests were repeated if the results were unreliable or abnormal.

Additionally, HVF was also performed on non-glaucoma participants who completed a visual field test at the baseline SCES-1 visit for progression evaluation among non-glaucoma eyes.

Pupil dilation

All participants were dilated with tropicamide 1% and phenylephrine hydrochloride 2.5% drops, repeated twice when necessary. Phenylephrine was omitted in participants with systemic hypertension or coronary heart disease. Participants with a known allergy to mydriatic eye drops, those with closed angles or with an IOP greater than 21mmHg, were not dilated.

Retinal photography

A retinal camera (CR-1 Mark-II Nonmydriatic Digital Retinal Camera, Canon, Japan) was used to obtain minimum one-optic-nerve-centred and one-macular-centred photo for each eye. The photographs will be graded for age-related macular degeneration, retinal microvascular characteristics, including focal arteriolar narrowing, arterio-venous nicking, retinopathy, and other retinal characteristics. In addition, generalized arteriolar narrowing will be quantified using the computer-based Singapore "I" Vessel Assessment (SIVA) software to measure retinal vascular caliber.

Posterior segment OCT scans

Spectralis OCT (Heidelberg Engineering, Germany) was performed for choroidal layer, optic nerve head and lamina cribosa evaluations. Cirrus HD-OCT (Carl Zeiss Meditec, Germany) was performed for macular, optic nerve head, and retinal nerve fibre layer (RNFL) thickness measurements. These scans allow for the quantitative analysis of retinal layers such as peripapillary RNFL thickness, full macular thickness, ganglion cell-inner plexiform (GCIPL) layer thickness and drusen volume.

OCT angiography (OCT-A) (RTVue XR 100 Avanti®, Optovue® Inc., Fremont, CA, USA) was performed to characterize vasculature in various retinal layers (namely superficial, deep, outer, choroidal, optic nerve head region and radial peripapillary capillaries areas), providing improved visualization of microcirculation by tracking the intrinsic motion of blood cells in static vessels through a motion contrast approach[4]. In order to achieve this, the machine dampens axial bulk motion in retinal blood vessels and takes repeated OCT B-scans to represent only erythrocyte movement[5]. This non-invasive approach allows flow density to be measured in a matter of seconds of the desired retinal regions (macular/and or optic nerve head region seen in **Supplementary Figure 2 and 3**).

Post-dilation slit lamp examination

The assessment of cataracts was based on the Lens Opacities Classification System III (LOCS III). The optic disc was assessed through a 78D dioptre lens at 10X magnification based on the protocol from SCES[6]. The vertical cup to disc ratio was measured by an eyepiece graticule etched in 0.1mm units.

Pachymetry

Pachymetry (Advent, Mentor O&O, Norwell, MA, USA) was used to measure the central corneal thickness. The test was only indicated for subjects who had any form of ocular surgery after SCES-1, but before SCES-2. A drop of local anaesthesia (Gutt Alcaine 0.5%) was applied into the subject's eye before commencement of the test. Five readings were captured and documented.

Data quality assurance and control

All involved research personnel were research coordinators conducting procedures were trained and required to pass validation tests to ensure competency and compliance on standard operating procedures.

Data were filled in forms and entered onto a specially designed database and cross-checked by at least one other data entry personnel. All data were password protected using the password-protected electronic case report form (e-CRF) via research electronic data capture software (REDCap). This e-CRF was sent to the data management team for further inputs, quality check and data cleaning. Imaging data was stored digitally and backed up monthly. Additionally, data was frequently checked for quality and outliers were further identified and evaluated for any potential error.

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